



UNIVERSITAT DE
BARCELONA

**Modern-analog studies and high-resolution
paleoenvironmental reconstruction of the last 500 years
using the varved sediments of the Mediterranean
Lake Montcortès (Central Pyrenees)**

M^a Carmen Trapote Forné



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(Central Pyrenees)**

**M^aCarmen Trapote Forné
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TESI DOCTORAL



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Programa de Doctorat en Ecologia Fonamental i Aplicada

Modern-analog studies and high-resolution paleoenvironmental reconstruction of the last 500 years using the varved sediments of the Mediterranean Lake Montcortès (Central Pyrenees)

Memòria presentada per

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per optar al grau de Doctor per la Universitat de Barcelona

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Al meus pares, sens dubte.

Als meus germans. Als tetes.

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Agraïments

Doncs al final aquí estic, escrivint els agraïments...Encara tardaré uns dies a pair-ho. Recordo passar pel costat dels meus companys ja doctors i pensar que no podia ser, que com s'ho havien fet? Si no hi ha manera!!! Si sempre falta alguna cosa, si mai queda acabat, si els dies es converteixen en setmanes i les setmanes en mesos i els mesos en....i recordo que sempre rebia la mateixa resposta, donava igual qui fos : "Un dia no saps com i s'acaba, de cop tens una tesi i no saps ben bé com ha passat". Doncs si, tenien raó.

Es molta a la gent a la que van dedicades aquestes línies i es poca la memòria útil que queda després d'haver espremut el cervell per donar lloc al que ara mateix teniu entre les vostres mans...tot i així espero no deixar-me a ningú. Dit això procedeixo a:

Agrair als meus dos directors de Tesis, Teresa Vegas i Valentí Rull. A la Teresa per donar-me l'oportunitat de formar part del projecte MONT-500 en el que s'adscriu la meva beca. Per encoratjar-me a tirar-ho endavant sense por, per apostar per mi, per deixar-me clar que no estem sols, per el saber fer, per la seva constància, pel seu dinamisme i per les seves injeccions d'energia que en té infinita, per tots i tothom. Al Valentí, per transmetre'm la seva calma (molt necessari), per ensenyar-me a simplificar les coses, per ensenyar-me a estructurar idees, a connectar i sistematitzar, per rebre'm amb la mateixa pregunta una i altra vegada sense trastocar-se. A tots dos pel seu criteri científic, pels seus consells i per estar 24/365.

A la meva família. Als meus pares. Sense ells això no hagués estat possible, de segur. Gràcies pel seu suport incondicional, per la seva comprensió, per la seva tenacitat , pels seu bons consells, per ser un exemple a seguir, per estar sempre allà, per no dubtar ni un moment, per moure cel i terra. En resum, gràcies per ser com sou. Als meus germans, pel seu suport, pels riures, per l'aixopluc, pels bons moments, per ser un referent, per comptar amb mi i per deixar que jo compti amb ells, i sobretot! per regalar-me, en el transcurs d'aquesta tesi, dos sols que m'il·luminen la vida, ♥ els meus tetes petits ♥ .

Als amics. Si em preguntessin que és el que més m'ha agradat de la tesi; què és el que m'enduc de tot plegat? sense vacil·lar: la gent amb la que m'hi he trobat. Companys de l'Almera, m'he sentit com a casa. Pili y Eve compañeras de batalla!! buena compañía para lo bueno y para lo malo, viva la resonancia, las risas, la desesperación, la frustración...viva!Encarni, ayyyy Encarniiii!!!! Gracias por tus consejos científicos, pero sobre todo por los no científicos, gracias por abrirme la puerta a tu mundo a y a tus murcianic@s bonic@s!. Laura B, nadie me entiende como tu! Cosa de seises! Marc, holi!, tu li has posat color a tot això 😊. Armand, l'últim en arribar, i què fariem sense les discussions per qui va a buscar el cafè?, posant salsa a la vida. Biete, Angel, Jorge, Benavente, Enric, Lucia, Alberto, ... ALMEJAS! Gracias!. Guio! No sabes cuánto me he acordado de ti! "...no hay mañana..." siempre has estado ahí, gracias a ti y a Chemita. A la Eli a la Sandra i a la Núria, companyes, amigues i un gran equip amb el que sortir al camp i torbar-nos-les de tots els colors. Sense vosaltres no hagués estat possible. Begoña (Yenis), ya tu sabes...te lo digo con memes mejor. Noe, gracias, por ser como eres y punto. Antonias sin palabras, nada que no sepáis.

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Informe dels directors

La Dra. Teresa Vegas Vilarrúbia, Professora Agregada del Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals de la Universitat de Barcelona, i el Dr. Valentí Rull, Investigador Científic de l'Institut de Ciències de la Terra Jaume Almera (CSIC), directors de la Tesi Doctoral elaborada per la candidata M^aCarmen Trapote Forné, i que porta per títol "Modern-analog studies and high-resolution paleoenvironmental reconstruction of the last 500 years using the varved sediments of the Mediterranean Lake Montcortès (Central Pyrenees)"

INFORMEN

Que els treballs de recerca portats a terme per M^aCarmen Trapote Forné com a part de la seva formació pre-doctoral i inclosos en la seva Tesi Doctoral han donat lloc a quatre capítols, dels quals tres corresponen a articles ja publicats i que s'inclouen al final de la tesi. El darrer capítol es presenta en format article i serà enviat properament a una revista d'àmbit internacional. A continuació es detalla la llista d'articles publicats, el seu corresponent índex d'impacte (segons la ISI Web of Knowledge) així com el quartil a que corresponen, segons el tema, i la contribució de la doctoranda a cadascun d'ells..

- 1- Trapote, M. C., Vegas-Vilarrúbia, T., López, P., Puche, E., Gomà, J., Buchaca, T., Cñellas-Boltà, N., Safont, E., Corella, J.P., Rull, V. (2018). Modern sedimentary analogues and integrated monitoring to understand varve formation in the Mediterranean Lake Montcortès (Central Pyrenees, Spain). *Palaeogeography, Palaeoclimatology, Palaeoecology*, 496, 292-304.

L' índex d'impacte (IFI) de la revista *Palaeogeography, Palaeoclimatology, Palaeoecology*, l'any 2018 va ser de 2.616. Aquesta revista està situada en el primer quartil de la categoria "*paleontology*" i ocupa el lloc 4è d'un total de 57 revistes considerades en aquesta categoria.

- 2- Rull, V., Trapote, M. C., Safont, E., Cañellas-Boltà, N., Pérez-Zanón, N., Sigrò, J., Buchaca, T., Vegas-Vilarrúbia, T. (2017). Seasonal patterns of pollen sedimentation in Lake Montcortès (Central Pyrenees) and potential applications to high-resolution paleoecology: a 2-year pilot study. *Journal of paleolimnology*, 57, 95-108.

L' índex d'impacte de la revista *Journal of Paleolimnology* l'any 2017 va ser de 2.168.

Aquesta revista està situada en el primer quartil de la categoria "Limnology" i ocupa el lloc 5è de un total de 20 revistes considerades en aquesta categoria.

- 3- Trapote, M. C., Rull, V., Giralt, S., Corella, J. P., Montoya, E., Vegas-Vilarrúbia, T. (2018). High-resolution (sub-decadal) pollen analysis of varved sediments from Lake Montcortès (southern Pyrenean flank): A fine-tuned record of landscape dynamics and human impact during the last 500 years. *Review of Palaeobotany and Palynology*, 259, 207-222.

L'índex d'impacte de la revista *Review of Paleobotany and Palynology* l'any 2018 va ser de 1.674. Aquesta revista està situada en el segon quartil de la categoria "plant sciences". Aquesta revista ocupa el lloc 102è de un total de 228 revistes considerades en aquesta categoria.

Alhora CERTIFIQUEN

Que M^aCarmen Trapote Forné ha participat activament en el desenvolupament del treball de recerca associat a cadascun d'aquests articles així com en la seva elaboració.

En concret, la seva participació en els articles ha estat la següent:

- Participació en el plantejament dels objectius de cadascun dels treballs.
- Coordinació de les campanyes de camp i mostres a L' Estany de Montcortès
- Tractaments de mostres al laboratori.
- Obtenció, anàlisi de dades i interpretació de dades
- Redacció dels articles 1 i 3 i seguiment del procés de revisió dels mateixos. Participació activa

en el procés de redacció i revisió de l'article 2.

Finalment, certifiquem que cap dels coautors dels articles abans esmentats, i que formen part de la Tesi Doctoral de M^a Carmen Trapote Forné, ha utilitzat ni té previst utilitzar, implícita ni explícitament, aquests treballs per a l'elaboració d'una altra Tesi Doctoral.

Per les raons anteriors, considerem que la candidata a Doctora, M^a Carmen Trapote, s'ha guanyat el dret de defensar la seva tesi doctoral davant els membres del Tribunal de Doctorat, conformat per membres especialitzats en la matèria.

Barcelona, 25 de Juny de 2019

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Abstract

Varved lake sediments also known as annually laminated sediments, are natural paleoenvironmental archives containing high-resolution proxy data and precise chronologies. They are one of the few natural archives that can provide enough time resolution (seasonal/annual) to bridge the temporal gap between past and present environmental data to ensure the continuity between climatic /ecological data and paleoclimatic /paleoecological data. However, it is not easily to manage due to the scarcity of this kind of archives and the lack of modern analog studies required for inferring reliable its seasonal signal

This thesis focuses on the study of modern sedimentary analogs of a Pyrenean lake with varved sediments and into reconstruct the last 500 years of environmental change at high temporal resolution (sub-decadal). It is aimed to provide a tool for improving paleoecological reconstructions and to contribute to bridge the temporal gap between ecology and paleoecology by providing long-term high-resolution and continuous paleoenvironmental data. To do this, we performed a two-year of monthly limnological and sedimentological monitoring at lake Montcortès (Central Pyrenees) with special regard for biological and biological induced proxies (calcite, diatoms and pollen). We reconstructed the last 500 years of lake-catchment system environmental history and its interactions by using fossil pollen and pigments as environmental indicators

Data obtained during the modern analog study (2013-2015) revealed a strong seasonal trend for all studied proxies. Changes in calcite, pollen and diatoms were highly depending on seasonal succession of lacustrine and terrestrial life forms that, in turn, were modulated by environmental variables. There appeared clear dissimilarities in terms of timing and seasonal signal recorded in the three proxies between years that have been potentially related with changes in temperature and precipitation indicating sediment sensitivity to inter-annual variations. Pollen has been revealed as a most reliable indicator to track seasonality on the sediment record, being the one maintaining the same seasonal signal between years. While periods of major calcite precipitation can fluctuate within spring, summer and fall and diatoms may suffer breakage and dissolution depending of water conditions, which would truncate the final sedimentary signal.

With pollen data in combination with independent evidence from historical sources we have documented in detail most important factors responsible for landscape modulation in Lake Montcortès during the last 500 years. Such factors were mainly human related namely cropping, livestock breeding, and hemp retting. Sedimentary pigment data in combination with pollen data showed that changes in land use greatly influenced aquatic photosynthetic community indicating lake-catchment connectivity. After 1850 CE, coinciding with the beginning of industrialization, vegetation and aquatic community showed a clear point of change although with opposed inferred signals. While vegetation changes indicate land abandonment and less human pressure in the area, aquatic community indicated a trend towards eutrophication. Such change on aquatic community could be an effect of non-point nutrient sources from historical legacies of intense land use joined to atmospheric deposition derived of the industrialization process. Overall, for both, catchment and lake, the main signal inferred during the last 500 years was related with human-pressure even during harsher climate conditions (LIA). The only climate related signal inferred were heavy rainfall episodes occurred during the last of half 19th century, indicated by both proxies. However, there is still many uncertainties and open questions to solve probably related with climatic and natural forcing not accounted for directly in this work.

The results obtained in this thesis by combination of modern analog studies and high-resolution paleo environmental provide valuable long-term continuous data to contribute to understand current ecological changes and the past environmental history as part of a time continuum.

Resum

Els sediments lacustres varvats, també coneguts com sediments laminats anualment, són arxius paleoambientals naturals que contenen informació ambiental a molt alta resolució temporal i amb els quals es poden obtenir cronologies precises. Són un dels pocs arxius naturals que poden proporcionar una resolució temporal suficient (estacional /anual) per tal de tancar la bretxa que existeix entre les dades ambientals obtingudes de estudis paleoambientals, és a dir del passat, i les dades obtingudes de l'estudi de l'ambient en el present. Per tant, les dades obtingudes a partir de l'estudi d'aquest tipus d'arxiu, asseguren la continuïtat entre dades climàtiques / ecològiques i dades paleoclimàtiques / paleoecològiques. No obstant això, no és fàcil aconseguir aquest tipus de dades degut a l'escassetat de llacs amb sediments varvats i a la manca d'estudis d'anàlegs moderns els quals són necessaris per inferir de manera precisa la senyal continguda en aquest tipus de sediments.

Aquesta tesi es centra en l'estudi dels anàlegs sedimentaris moderns d'un llac pirinenc amb sediments varvats i en la reconstrucció, a alta resolució temporal (sub-decadal), dels últims 500 anys de canvi ambiental. L'objectiu és proporcionar una eina per a millorar les reconstruccions paleoecològiques i contribuir a superar la bretxa temporal entre l'ecologia i la paleoecologia proporcionant dades paleoambientals contínues i d'alta resolució. Per a fer-ho, es va realitzar un monitoreig limnològic i sedimentològic mensual durant dos anys al llac de Montcortès (Pirineus centrals), enfocat principalment, a l'estudi d'indicadors biològics o biològicament induïts (calcita, diatomees i pol·len). També s'han reconstruït els últims 500 anys d'història ambiental de la conca i del llac i de com tots dos sistemes han interaccionat. Per a fer-ho s'han utilitzat pol·len i pigments fòssils com a principals indicadors ambientals.

Les dades obtingudes durant l'estudi d'anàlegs moderns (anys 2013-2015) van revelar l'existència d'una forta tendència estacional per part de tots els indicadors estudiats. Els canvis estacionals observats en la calcita, el pol·len i les diatomees estan estretament lligats amb la successió estacional del cicle de vida dels organismes lacustres i terrestres que, al seu torn, es veuen modulats per canvis en les variables ambientals. S'han observat diferències en tots tres indicadors estudiats en termes de temporalitat i senyal estacional que han estat potencialment relacionades amb canvis de temperatura i precipitació. Aquest fet ens indica la sensibilitat del sediment a enregistrar les variacions interanuals. El pol·len s'ha revelat com l'indicador més fiable alhora d'identificar les diferents estacions en futurs estudis sedimentaris donat que és l'únic que ha mantingut el mateix senyal estacional entre tots dos anys, mentre que els períodes de major precipitació poden tenir lloc entre primavera, estiu i tardor i les diatomees poden patir trencaments i dissolució depenent de les condicions de l'aigua, fet que pot esbiaixar el senyal sedimentari final.

La combinació de dades pol·líniques i juntament amb documentació històrica, ens ha permès documentar detalladament els factors més importants responsables dels canvis en el paisatge durant els darrers 500 anys. Aquests factors són principalment d'origen antròpic essent bàsicament cultiu, cria de bestiar i activitats relacionades amb el cànem. L'estudi de les dades de pigments fotosintètics de les comunitats aquàtiques en combinació amb dades pol·líniques ens indiquen que els canvis en l'ús del sòl han estat els major responsables dels canvis ocorreguts en els productors primaris del llac indicant la connectivitat existent entre la conca i el llac i com els processos ocorreguts a la conca poden afectar als processos propis del llac. A partir de 1850 CE en endavant, coincidint amb l'inici de la industrialització, la vegetació i la comunitat aquàtica canvien sincrònicament tot i que la senyal que se n'infereix és oposada. Mentre que els canvis de vegetació indiquen baixa pressió antròpica, la comunitat aquàtica indica una tendència creixent cap a l'eutrofització. Aquest canvi en la comunitat aquàtica deure's a l'entrada de nutrients provinents de fons difuses com a

conseqüència de l'ús intensiu de sòls en temps passats i a la deposició atmosfèrica de nutrients al llac efecte de la industrialització. En general, tant per la conca com pel llac, el principal senyal inferit durant els darrers 500 anys ha estat relacionat amb activitats humanes fins i tot durant la Petita Edat del Gel on les condicions climàtiques haurien estat més dures. En relació al clima, l'únic senyal inferit han estat episodis de pluges fortes que es van produir durant l'última meitat del segle XIX i han estat enregistrats per tots dos indicadors. No obstant això, encara hi ha moltes incerteses i preguntes obertes a resoldre, probablement relacionats amb factors d'origen climàtic i natural que no han estat adreçats de manera directa en aquest treball.

Els resultats obtinguts en aquesta tesi a partir de la combinació d'estudis d'anàlegs sedimentaris moderns i les reconstruccions paleoecològiques d'alta resolució proporcionen series de dades llargues i contínues que ens ajuden a entendre els canvis ecològics actuals i la història ambiental del passat com a part d'un continu de temps.

Thesis outline

This thesis is presented as a compendium of publications. It is composed of 7 chapters and two annexes distributed as follows: an introduction, four chapters comprising the main core of the dissertation, a general discussion, the main conclusions and two annexes containing supplementary material (of chapter 2) and the already published chapters, respectively. All chapters have been written in English and references follows the journal's style. All journals are cited in the Journal Citation Reports. At the beginning of each chapter, the status of the related paper is indicated.

Chapter 1. **Introduction.** A general overview of the main issues related to the purposes of each chapter is provided. This chapter includes the description of the study area and the main objectives.

Chapter 2. **Modern sedimentary analogues and integrated monitoring to understand varve formation in the Mediterranean Lake Montcortès.** These chapters assess main factors related with calcite precipitation on Lake Montcortès related with environmental variables and the lake's limnological cycle. It shows the results if an exhaustive lake monitoring carried out during two consecutive years and the composition of the collected sedimentary material corresponding to the same period. Modern analogs for calcite and diatoms as environmental indicators are provided at monthly and seasonal resolution.

Chapter 3. **Seasonal patterns of pollen sedimentation in Lake Montcortès (Central Pyrenees) and potential applications to high-resolution paleoecology: a two years pilot study.** In this chapter focus in to identify seasonal pollen sedimentation patterns and how the relate with environmental variables and lake's limnological cycle. Modern analogs for pollen in terms of amounts and composition are provided at seasonal resolution

Chapter 4. **High-resolution (sub-decadal) pollen analysis of varved sediments from Lake Montcortès (southern Pyrenean flank): A fine-tuned record of landscape dynamics and human impact during the last 500 years.** This chapter asses past dynamics on vegetation and landscape around lake Montcortès at sub-decadal resolution during the last 500 years covering the Little Ice Age and the onset of the global warming. It is a multi-proxy approach based on pollen, charcoal particles and Non-pollen

Palynomorphs (NPPs) as environmental indicators combined with historical documentary data. A detailed study of past human-landscape dynamics is provided.

Chapter 5. **Response of aquatic photosynthetic community to 500 years of anthropogenic catchment disturbance: A high-resolution (sub-decadal) pigment analysis.** This chapter combines fossil pigment data (as indicators of in-lake changes) with pollen and NPP data (as indicators of catchment change) to understand past lake dynamics in relation to its surrounding environment and to discern the main factor driving photosynthetic community change. Statistical analyses have been used to better understand aquatic community change and to quantify variance explained by the environmental factors assessed in this work.

Chapter 6. **General discussion.** The main results presented in the prescribing chapters are discussed altogether. Possible directions are also given in this section.

Chapter 7. **Conclusions.** The main conclusions of this thesis are listed based on the conclusions of each chapter and grouped according to the objectives described in the introduction chapter.

Annex 1. Contains supplementary material of chapter 2.

Annex 2. Publications. Contains the offprint of chapters that have been already published

CHAPTER 1

General introduction and objectives.

Soundtrack: *"my! my! time flies!"* - Enya

1.1-Lake sediments, paleoecology and proxy data

Lakes comprises a small fraction of Earth's land surface, approximately 1 %, and contain a tiny volume, less than 0.02 %, of the Earth's water from the hydrosphere (Wetzel 2001, Cohen 2003). Apart of being a vital source of water and food, lakes are a key source of information on earth history over the past. They serve as natural archives of local and regional environmental history. Their sediments trap and preserve over time limnological, biological, geochemical, climatic and anthropogenic information of processes occurring in the water body, in the catchment area and also at global scale (Fig.1.1) (Cohen, 2003 Smol et al., 2002; Veski et al., 2005). Furthermore, on most of the cases, natural conditions of lake bottom environments ensure sediment content preservation through time with low rates of degradation and reworked material. Therefore, lake deposits provide either information-rich and long duration earth's history archives (Cohen, 2003).

Paleoecology, defined as "the ecology of the past", is the discipline that combines biological, geochemical and molecular information from natural archives (i.e. lake sediments) to reconstruct ecological and evolutionary systems deep into the past (Birks and Birks 1980; Rull 2014). This information is extracted from indirect indicators or proxies that record environmental information. There is a large list of potential proxies including: terrigenous, chemical and biogenic compounds, cosmogenic and volcanic particles, and fossils that originated outside of the lake- like pollen- or within the lake -like diatoms- (see Figure 1.1 for more examples). Just looking in these examples we can have an idea of the great potential to be explored on lake sediments and the range of questions that can be examined within these archives.

While most of lake sediments appear to be massive, without any structure that can be assigned to reflect an internal chronology beyond the trend of sediments getting progressively younger towards the top (Zolitschka et al., 2015), in some specific environments and conditions, sediments, can accumulate and be preserved as succession of laminae representing a seasonal cycle (Fig.1.2). This specific type of sediments is called 'varved sediments' and can be found in a wide spatial distribution and for many time windows; nevertheless, they are not easily found (Brauer 2004). The first annually laminated record was documented in 1862 by the Swedish Geological Survey in one of the first geological maps of Sweden that described rhythmically deposited clays. But, it wasn't until the beginning of 20th century that the Swedish geologist Gerard De Geer coined the term 'geochronology' and pioneered the use of unit 'year' in geological sciences using varve observation and counting as a dating tool in geology (De Geer, 1908, Zolitschka et al., 2015).

Initially, the term ‘varved sediments’ was used synonymously for annually laminated proglacial lake deposits, but today, the term varve is used for all kinds of laminations that are deposited in a seasonal pattern (Zolitscka et al. 2015) and can be found from polar to tropical regions on both lacustrine and marine environments (Ojala et al., 2012; Zolitska et al., 2015). However, the conditions required for varve formation and preservation are complex, variable and site specific although two major factors must be fulfilled: (1) strong seasonal forcing, that is often driven by annual climatic variability and, (2) lack of perturbation after deposition i.e. bioturbation or water turbulence in sediment-water interface (Ojala et al., 2000; Brauer, 2004). All types of varves contain at least two or more seasonal laminae with some distinctly contrasting features such color, composition, texture, structure and /or thickness.

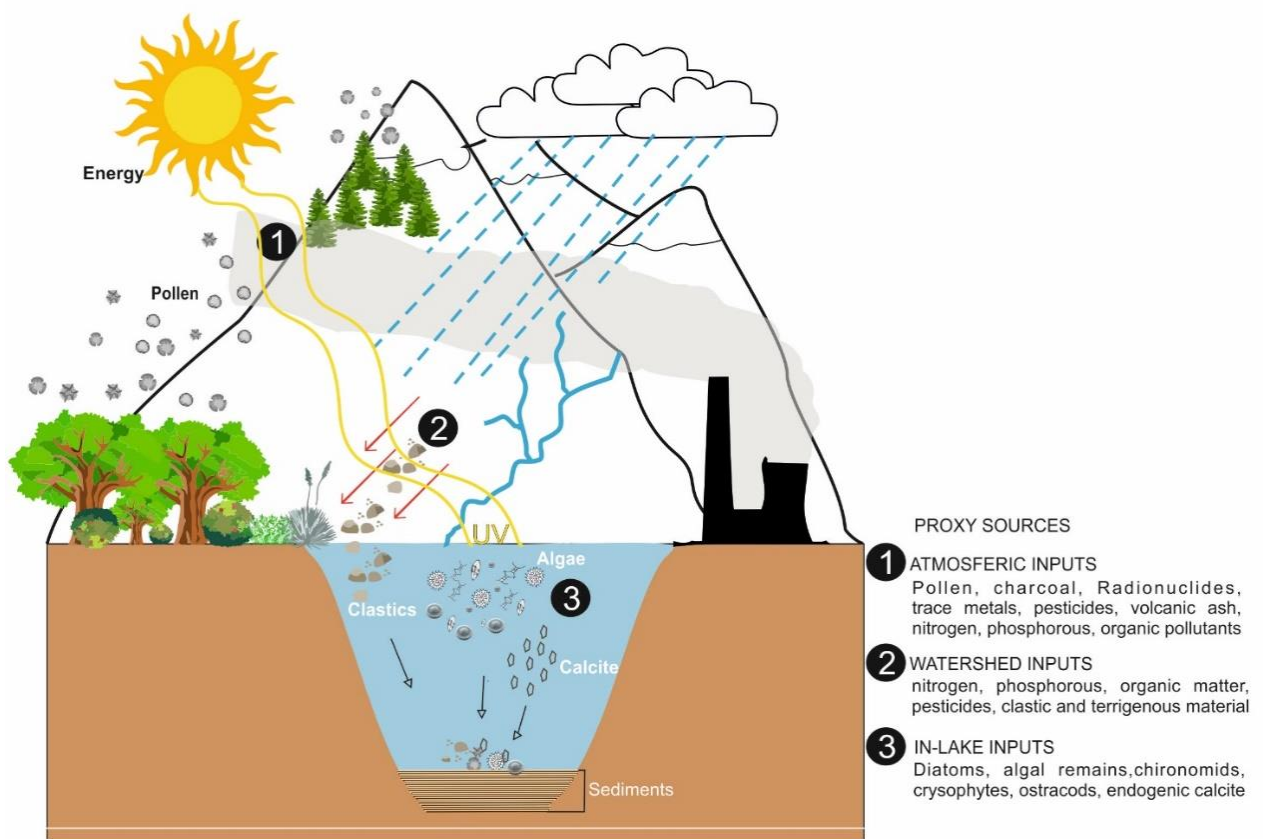


Figure.1.1- Simplified scheme of allochthonous and autochthonous sources of potential proxies. Adapted from (Cohen 2003 and Brauer 2004)

1.2-Varved sediments

1.2.1. Varve types

Based on their composition and genesis, three main varve types can be distinguished: clastic, endogenic (also called evaporitic) and biogenic varves, nonetheless, a mixture of them composes the more common type, and rarely, 'pure' varves types are found (Zolitska et al., 2015). Clastic varves predominates on polar and alpine regions under cold climatic conditions where sediment transfer is closely related with the annual freeze-thaw cycle and snow/glacier melt. Clastic varves are typically composed of coarse-grained lamination succeeded by a fine-grained upper lamina as result of deposition process controlled by density gradient of the clastic material entering to the lake. Typically, the coarse-grained laminae represents summer season, while the fine-grained laminae represents winter season (Ojala et al., 2000; Lamoureux 1999). On the other hand, endogenic and biogenic varves are more typical in temperate climate zones. Endogenic varves are formed by physically induced precipitation of minerals, i.e. manganese or iron precipitates formed in the water column during water column mixing (typically during autumn/spring), or calcite precipitation resulting of altered water pH due to high water evaporation under arid conditions (Zolitska et al., 2015). While for clastic and evaporitic varves the physical process plays a major role on their formation, on biogenic varves, biological processes are the main driver of varve formation. These types of varves reflect the cycle of annual lake productivity and processes involved are more complex (Tylman et al., 2012; Bonk et al., 2015). Increases of nutrients inputs to the lake during spring from snow melting and/or rainfall triggers algal blooms at a time of rising temperatures that favors phytoplankton growth. Therefore, a biogenic varve is composed by organic laminae containing mostly planktonic remains, amorphous organic matter and silicic skeletons (i.e. diatoms) representing spring and summer, which is followed by a final lamina consisting of plant detritus and minerogenic components representative of runoff occurred during autumn and winter (Zolitska et al., 2015; Brauer 2014). On karstic lakes, hard water lakes, or lakes basins situated in carbonaceous bedrock, biological activity can induce calcite precipitation during algal blooms because of CO₂ water depletion trigger changes on carbonate solubility product. This kind of biogenic varves are also known as 'calcite varves' and are composed by a couplet of light (carbonate) and dark (organic material and detritus) sublayers that are formed in spring/summer and autumn/winter respectively (see Figure 1.2 for schematic biogenic varve representation) (Zolitska et al., 2015; Brauer, 2004).

1.2.1. Varve chronology

The seasonal/annual nature of varved sediments permits to establish an accurate chronology by consecutive layer counting which means yearly resolved (Tiljander et al., 2002; Francus et al., 2009). It can be built by anchoring the topmost varve as representing the year of coring and counting back through time. Even with an unknown date for the starting point, a robust chronology can be established to provide relative time control with a floating chronology, similar as occurs with tree rings on dendrochronology (Fig.1.2) (Brauer 2004; Ojala et al 2012). So, varve chronologies avoid intrinsic errors of the more commonly used radiometric techniques of dating that mainly derive from the variable rates of cosmogenic isotopic production through time, however varve counting also has an intrinsic error (see Zolitska et al., 2015 for more detail). Nonetheless, radiometric dating is often applied as one of the available methodologies to confirm the annual character of finely laminated sediments (Ojala et al 2012). Hence, varved sediments provide both, independently datable evidence of environmental change and high-resolution time control that allow to explore both short- and long-lasting environmental events (seasonal to centennial).

1.3- Why varved sediments?

Many studies and reports have evidenced that current climate is changing at unprecedented rates largely as a result of human activities (IPCC 2013, 2018; Mann et al., 2009). Human-induced warming reached approximately 1°C above pre-industrial levels in 2017 which estimates 0.2 °C increase per decade (Allen et al., 2019) which is a greater value than the estimated during the last 1,000 years (IPCC 2013). Following these rapid changes, many organisms, populations and ecological communities will not be able to respond to the speed of mentioned rates (Walther et al., 2002; Davis and Shaw 2001). On the other hand, although some ecological responses can occur at decadal to secular time scales, others, for instance, ecological successions and extinctions occur at larger time scales than centuries (Rull 2014). Climate instrumental records are too short to resolve the full range of decadal to multidecadal and centennial scale of natural and human induced climate variability. The only way to obtain climatic and ecological data before instrumental records is by means of paleoecological and paleoclimatological studies. Hence, high-resolution paleoenvironmental information is strongly required for the fully assessment and prediction of the global climate change and its consequences (Rull 2010 and 2014). Paleoecology and paleoclimatology can provide the needed time span at the suitable time amplitude (seasonal to centennial) only if the adequate archive is used (Fig.1.2) (Rull 2010 and 2014). Varved sediments offer seasonal

to annual resolution that can contribute to produce continuous, homogeneous, and coherent long-term environmental and climatic records that would ensure the continuity between climatic /ecological data and paleoclimatic /paleoecological data (Rull 2014).

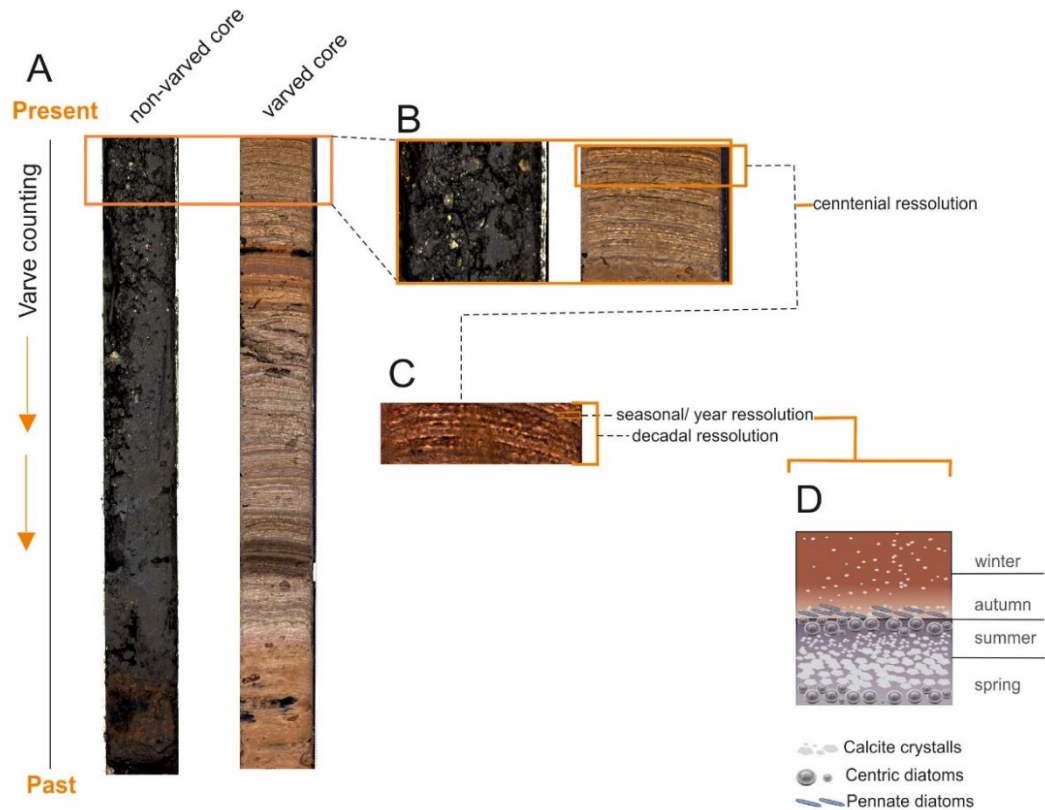


Figure.1.2- Comparison between a non-varved and a varved record (a). Zoom to varved zone and corresponding time resolution (b,c). Simplified model of calcic biogenic varve formation with calcite layer (d).

1.3.1- Last millennium

In this sense, one of the most interesting periods to study global climate instabilities and their consequences is the last millennium. During the last 1,000 years, three main climatic changes at relative short periods of time have been registered on the northern hemisphere. They are the Medieval Climate Anomaly (MCA ~ 750-1250 CE), the Little Ice Age (LIA ~ 1250-1850 CE) and the recent rising on global temperature or global warming (~ 1850 CE-to present day) (Mann et al., 2009). Furthermore, historic human-population shifts have been strongly affected by climate changes, as social stability and success depended largely on favorable climatic conditions (Tinner et al., 2003). So, the study of the last millennia can provide information about rapid climate changes and its consequences and allow to establish data continuity with the available instrumental climatic data to compare and to be used to modeling future climatic scenarios (Anderson et al., 2006). Therefore, the last millennium

appears as suitable and interesting period not only for the study of climate-environment relationships but also, for climate-human-environment relationships. Besides, more near the past, more and better-preserved historical documentation that help to build accurate past scenarios. Furthermore, the similarity between extant ecological communities and those living during the last millennium make them comparable by analogy, which can be used as a tool to infer past ecological changes (Rull 2014).

1.4-Modern analogs

Paleoecology is based on the principle of uniformitarianism, which states that laws of nature are constant across time and space (Simpson 1970). A simplified form of this statement would be “the present is the key of the past” (Tomkeieff 1962). This means that natural process operating in the past are the same as those operating on the present, though rates and intensity might have varied on time (Rull 2010). Therefore, the information extracted from the study of the present-day environment linked to present proxy variations can be used to make inferences about the past (Birks and Birks 1980). Hence, if a suitable modern analogue is found for a fossil sample, the past environment for that sample can be inferred from the environmental conditions under which the modern (analogous) sample was deposited (Birks, 1995; Birks et al., 2012). This can be applied on varved sediments not only for sediment content proxy data, but also, to understand varves itself as a proxy by: (1) verifying the annual nature of varves and the needed environment for its formation and preservation and, (2) relating its composition (organic matter, calcite, pollen, diatoms...) and their seasonal variations with present-day environmental variables. As varve formation and deposition is a highly variable and site-specific process; studies of varve modern analogs must be carried out within the varved lake itself, not each sediment record, even in the same region, necessarily reflects environmental changes in the same way in the sedimentary signal. Therefore, ideally, before the study of the varved sediment record itself, it is necessary to understand the local processes of deposition rather than searching signals that have been inferred from other records to interpret the sedimentary signal (Brauer 2004).

1.5-Mediterranean region and changing climate

Several studies and reports have identified the Mediterranean area as one of the world’s regions most vulnerable to the Climate Global Warming (Christensen et al., 2007; Lionello et al., 2012, 2014; IPCC 2013, Mariotti et al., 2015). Projected changes include a tendency for dryer conditions by a decrease in mean precipitation and increase in surface temperature with increased precipitation variability and longer periods of drought (Giorgi

and Lionello 2008; Mariotti et al., 2015). Spain is one of the areas included on these projections where is estimated as one of the hot spots of climate change. A tendency of temperature increase of 0.4 °C/decade in winter and 0.7 °C/decade in summer is projected for the 21st century with a difference only of 0.1°C/decade between the more and less favorable scenario including a projected significant reduction in total annual rainfall in all cases (Moreno et al., 2005). Therefore, the provision of high-resolution and accurate paleoenvironmental and paleoclimatic data is very valuable to predict forthcoming climate shifts and how it will affect natural ecosystems, natural resources and the socio-economic system to design appropriate management and adaptation measures to changing climate (Anderson et al., 2006).

1.6-Study site

Karstic lakes occur in carbonate and evaporite bedrock by means of dissolution and collapse bedrock that create depressions suitable for lake development. Bedrock dissolution leads to the generation of funnel-shaped dolines with steep margins which have high depth/water surface ratios (Palmquist, 1979; Cvijic, 1981). The conical shape of this type of lakes favors water stratification and lead to marked gradients and abrupt changes through water depth of, among others, dissolved salts, temperature, light penetration and oxygen concentration (Renault and Gierlowski-Kordesch, 2010). Such conditions contribute to create a dynamic depositional environment prone to allow varve forming and preservation. Although karstic lakes occupy less than 1 % of the global lake area, they are particularly abundant in North and Central America, southern China, and the Mediterranean basin (Cohen, 2003), including the Iberian Peninsula. To the date, five extant Karstic and varved lakes have been found and studied in the Iberian Peninsula: La Cruz (Romero-Viana et al., 2008), Zoñar (Martín- Puertas et al., 2009), Banyoles (Morellón et al., 2015), Arreo (Corella et al., 2011a) and Montcortès (Corella et al., 2011b; 2012) (Fig.1.3).

Lake Montcortès is one of the deepest karstic lakes in the Iberian Peninsula. It is located in the Pre-Pyrenean Range. The lake's catchment area is small and lies on Oligocene conglomerates and Triassic rocks mainly comprising carbonates, evaporites, claystones and shales. The lake is almost circular in shape and has no permanent inlet and an ephemeral outlet stream located on the north shore that controls maximum lake levels (Fig.1.3) (Camps et al., 1976). It has been considered meromictic although some holomictic events have been documented (Camps et al., 1976; Modamio et al., 1988). Lake water is alkaline and oligotrophic (Camps et al., 1976; Modamio et al., 1988; URS, 2010).

The lake is located in a transitional climatic area between the Mediterranean lowlands and the Middle Montane Belt within the sub-Mediterranean bioclimatic domain (Vigo and Ninot, 1987). Therefore, the lake is very sensitive to climate changes. Total annual mean precipitation is 668.5 mm (reference period 1961-1990), with February being the driest month and May being the wettest. Annual average air temperature is 12.8°C, with maximum and minimum mean temperatures of 23.3 (July) and 2.9 °C (January), respectively (Fig.1.3).

Three major forest formations occur at the lake region: (1) Evergreen oak forest; (2) Deciduous oak forest and (3) Conifer forest. (Mercadé et al., 2013). A dense littoral vegetation belt and hay meadows, pastures and cereal crops surround the lake for cattle feeding, mainly cows and horses (Fig.1.3). Besides farming, since 1970's the most important human activity around the lake and in the area is rural tourism. Nowadays, the area is sparsely populated with only 26 inhabitants belonging to Montcortès town itself (Idescat 2015).

In 2013, an international multidisciplinary scientific team formed by geologists, biologists, paleoecologists and paleoclimatologists started a project funded by the Ministry of Economy and Competivity (project MONT-500; reference CGL2012-33665; PI: Teresa Vegas-Vilarrúbia). They travelled to Lake Montcortès to obtain several sedimentary sequences from the deepest part of the lake for paleoecological and paleoclimatological purposes and to install devices for lake Monitoring. Ten cores were retrieved and 3 years of monthly field campaigns and monitoring were carried out.

1.7-Paleoecological studies in lake Montcortès

In terms of paleoecology, Lake Montcortès remained unknown until the last 8 years. This is not surprising because most of the paleoecology carried out on the area (Pyrenees) was focused on high-mountain lakes, which are considered less affected by human activities and therefore, better places for studying climatic and ecological signals without human distortion (Catalan et al., 2017).

There are ten published paleo-studies of Lake Montcortès from 2011 to nowadays (not included published chapters belonging to the present thesis). Among the ten existing paleo-studies, six of them are mainly based on varves itself and non-biological indicators as main proxies to reconstruct paleoclimate, paleolimnological conditions and atmospheric deposition (Corella et al., 2011, 2012, 2014, 2016, 2017; Vegas Vilarrúbia et al., 2018), although the most recent ones also include selected fossil pigments for paleolimnological

assessment. All of them reach sub-decadal resolution. On the other hand, the remaining five are mainly based on biological proxies: pollen (Rull et al 2011; Rull and Vegas-Vilarrúbia 2014 and 2015) diatoms (Scussolini et al., 2011) and non-pollen palynomorphs (Montoya et al., 2018). These works reconstruct landscape and in-lake changes as well as human activities around the lake during the last millennia and they do not attain sub-decadal resolution for biological indicators. Further, previous studies of the lake's limnological cycle monitoring exist (Camps et al., 1976; Modamio et al., 1988 Cristina et al., 2001) and their duration do not exceed one year to account for inter-annual variability and none of them included sedimentological monitoring to connect environmental and limnological variables with sedimentary signal.

A common feature of all works mentioned above is that, despite the different proxies and time-resolution explored, all of them reveal the existence of a complex interplay between climate and human activities that sometimes interacted in a synergistic way. This feature, together with the varved nature of the sediments and the large amount of information already available makes Montcortès lake an attractive place to investigate climate-human-environment relationships.



Figure.1.3- Location of the five varved and karstic lakes within the Iberian Peninsula (a). Map of Lake Montcortès (square) relative to Montcortès town (ellipse) (b). Long-term climatic data of the area (reference period 1961-1990) (c). Panoramic view of Lake Montcortès (d). Horses and cows pasturing around Lake Montcortès (e, f, g). Scientific team preparing material for coring (h). One of the retrieved cores (white laminations can be appreciated) (i). Scientific team during one of the lake's monitoring campaigns (j). Source: Photos took by the scientific team and fieldwork staff.

This PhD thesis has two overriding goals. On one hand, we aimed to provide modern sedimentary analogs for better interpreting Montcortès sedimentary record by understanding the factors affecting formation, transport and deposition patterns of the different fractions composing varves in Lake Montcortès. As the interpretative power of high-resolution paleoenvironmental reconstructions relies on the availability of modern analogs with the same temporal resolution of the sedimentary record, within this objective, we focused on to obtain modern analogs at seasonal resolution. On the other hand, we aimed at performing a high-resolution multi-proxy paleoenvironmental reconstruction covering the Little Ice Age and the onset of rising temperatures under the global warming conditions to nowadays using biological indicators. In this sense, we focused on three main aspects: i) To reconstruct changes occurring at the catchment area mostly related with vegetation and land-use ii) to reconstruct changes occurring in the water body related to the aquatic photosynthetic community, and how both relate each other and, iii) to attempt to disentangle potential causes of changes and their origin (climatic or anthropogenic). To achieve these objectives, two years of limnological and sedimentological monthly monitoring were performed and analysis of subfossil pollen, non-pollen palynomorphs (NPPs), charcoal particles and photosynthetic pigments extracted from lake's Montcortès varved sediments were done at sub-decadal resolution on a continuous sampling.

The results of this thesis are divided into four independent chapters that correspond to three already published articles (Chapter 2, 3 and 4) and one chapter ready for journal submission (chapter 5). Each chapter address specific objectives defined as following:

- In Chapter 2: The main purpose is to explore the link between varve formation, composition and preservation, with environmental variables through the study of Lake Montcortès limnological cycle, at monthly and seasonal resolution. Within this specific objective, emphasis is placed on to understand processes related to calcite precipitation.
- In Chapter 3: This part aims to identify seasonal pollen sedimentation patterns in terms of amounts and composition and how they relate with environmental and limnological variables during two years of lake monitoring to provide a tool to better interpret past records of the same lake.
- Chapter 4: The main purpose is to carry out a detailed, high-resolution and time-continuous paleoenvironmental reconstruction of vegetation and landscape dynamics to investigate vegetation history, land-use and human impact around Lake Montcortès.

- Chapter 5: This chapter aims to describe changes on photosynthetic community of Lake Montcortès for the last 500 years, and to quantify relationships between water community and processes occurring in the catchment to determine the role of landscape and human-related activities in mediating lake response to environmental change.

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CHAPTER 2

Modern sedimentary analogues and integrated monitoring to understand varve formation in the Mediterranean Lake Montcortès (Central Pyrenees, Spain).

Soundtrack: "Crystalized"- The XX

Original publication (*Appendix2 in the supplementary material*):

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Abstract

Varved sediments provide unique opportunities to carry out high-resolution paleoclimatic and paleoenvironmental reconstructions with accurate time control. To better interpret the sediment record it is necessary to understand the physical, chemical and biological factors that influence varve formation and preservation. We explored the link between the annual limnological cycle and current varve deposition in the oligotrophic hard-water Lake Montcortès (Central Pyrenees). The varves of this lake consist of couplets of dark organic and light calcareous laminae. A two-year limnological monitoring (10/2013-10/2015) combined with a sediment trap study were conducted at monthly resolution. Limnological and sedimentological measurements were compared with meteorological data. Although the lake was considered meromictic in the first limnological studies, we documented total mixing of the water column both winters. In spite of this, long periods of stratification and hypolimnetic anoxia create suitable conditions for varve formation and preservation. Sediment deposition followed a clear seasonal pattern related to biological processes in the euphotic zone. During summer and fall, calcite precipitation was favored by high calcite saturation indices and enhanced primary production that promoted relatively high pH values as a result of CO₂ uptaking. There was considerable variability in the amount of calcite deposition between years, which was linked to seasonal temperature differences. In addition, calcite crystal sizes and diatom fluxes showed seasonal patterns related to calcite saturation index and changes in water stratification, which in turn were also related to temperature variability. Seasonal sedimentation patterns were strongly linked to primary producers and especially sensitive to temperature shifts. It results in a clear seasonal signal and varve formation. We compared our results with previous sedimentological interpretations of the varved record of this lake. This study improves the interpretation of Lake Montcortès sediment record extending back several millennia.

Keywords: Biogenic varves, Seasonal resolution, Calcite precipitation, Sediment traps, Modern analogues, Mediterranean region.

2.1- Introduction

Lake sediments are one of the most valuable environmental archives used for paleoenvironmental reconstructions. The sediments store past environmental changes by recording the influences of a variety of processes in the lake water body as well as in the catchment area. Among the different types, varved sediments are especially suitable for this purpose because they allow the performance of high-resolution (annual, sub-decadal) paleoenvironmental and paleoclimatic reconstructions with accurate time control (Saarnisto 1986; Ojala et al., 2013). Formation and preservation of varves require specific conditions. Annually laminated sediment formation is favored in places with strong seasonal contrast and requires a variable flux of components from multiple autochthonous and allochthonous sources to the sediment (Zolitschka et al., 2015). These sediments are preserved only in the absence of post-depositional reworking and sediment mixing. This condition is more common to happen in deeper lakes because they tend to be stratified and favor oxygen consumption in bottom waters. Then, prolonged (meromictic) or seasonal (monomictic/dimictic) suboxic to anoxic conditions in the hypolimnion are needed to prevent bioturbation, the most important mechanism of sediment mixing in lakes (Zolitschka et al., 2015).

Based on their composition and genesis, three main varve types (and their mixtures) can be distinguished: clastic, endogenic, and biogenic (Zolitschka et al., 2015). Deposition of clastic and endogenic varves depends mainly on seasonal runoff from the catchment and chemical precipitation of minerals from the water column (Zolitschka et al., 2015). However the mechanisms involved in biogenic varve formation are much more complex because they include bio-geochemical processes. Processes responsible of varve formation are highly variable and site-specific. Therefore, a good understanding of the local processes that promote particle flux dynamics and seasonal changes in sediment fluxes in each individual site and for a particular record is crucial to better interpret the sediment signal (Leeman and Niessen 1994; Brauer, 2004).

The understanding of depositional conditions must be recognized as a prerequisite for a reliable varve chronology and appropriate interpretation of multi-proxy records to fully exploit the potential of varved sediments. For this reason, modern analogue studies are highly valuable and needed. Sediment trap studies in combination with limnological and meteorological monitoring are the best way to obtain feasible modern analogs. This approach not only allows identification of the distinct pathways involved in varve formation

but also determination of the period of varve deposition to assess the seasonal signal (Rodrigo et al., 1993; Miracle et al., 2000; Tylmann et al., 2012; Bonk et al., 2015).

While most published varved records are from northern and central Europe (Ojala et al., 2012), varved sequences have also been found in southern Europe and in the Mediterranean region, e.g. in the Iberian Peninsula (IP). This region is particularly attractive due to its sensitivity and vulnerability to climate change. In fact, during the last decades, temperatures have risen faster than the global average in the Mediterranean regions (Lionello et al., 2014). Also, model projections agree that future warming and drying in this area will be higher than during last century (Mariotti et al., 2015).

To date, six lakes with varved sediments have been studied in the IP, including a pliocene paleolake (Muñoz et al., 2002), and 5 extant karstic lakes: La Cruz (Romero-Viana et al., 2008), Zoñar (Martín-Puertas et al., 2009), Banyoles (Morellón et al., 2015), Arreo (Corella et al., 2011a) and Montcortès. This latter lake is the subject of our study and shows the longest continuous varved record retrieved thus encompassing three millennia of well established varve chronology (Corella et al., 2011b; 2016). Montcortès varves appear as couplets of light calcite and brownish organic layers that are believed to have deposited in spring/summer and fall/winter, respectively, as it occurs in biogenic varves typical of lakes located in carbonate bedrock (Corella et al., 2012). Due to the absence of modern analogue studies, these authors suggested potential mechanisms to explain varve formation based on the available literature for other similar hard-water lakes (Brauer, 2004).

Actually, among the five prevailing varved lakes in the IP mentioned above, only Lake La Cruz has been studied and monitored to obtain modern analogues to suitably interpret the sediment record (Miracle et al., 2000; Romero et al., 2006, Romero-Viana et al., 2010). In spite of the growing research interest that varved sediments have recently attracted (Ojala et al., 2012) and although some modern analogue studies are already available -mainly from north-central Europe (Tylman et al., 2012; Ojala et al., 2013; Bonk et al., 2015)- more monitoring data is needed to properly interpret the sediment signal, especially for Mediterranean lakes.

With this study, we aim to better understand the link between varve formation and the annual limnological cycle at Lake Montcortès and provide modern analogues to better explore the potential for high resolution paleoecological and paleoclimatic reconstruction of its sedimentary record. We carried out i) *in situ* measurements of physicochemical parameters in the water column and ii) measurements of sedimented material using

sediment traps at monthly resolution. Special attention was paid to processes related to calcite precipitation and to understand how sediment composition varies seasonally and inter-annually related to environmental variables. It is expected that these results help to test previous hypotheses regarding varve formation in Lake Montcortès (Corella et al., 2011b; 2012).

2.1.1- Study site

Lake Montcortès is a relatively small and deep karstic lake located in the Pre-Pyrenean Range (NE Spain) in the Pallars Sobirà region (42° 19' N; 0° 59' E) at 1,029 m a.s.l. (Fig. 2.1). The lake's catchment area is small (watershed surface area ~1.39 km²). It lies on Oligocene conglomerates and Triassic rocks mainly comprising carbonates, evaporites, claystones and shales. The lake is mainly fed by groundwater and runoff (Corella et al., 2011b). It has no permanent inlet and only two ephemeral streams located on the southern area of the watershed drain the lake. Water losses are due to evaporation and drainage from an outlet located on the northern shore that controls maximum lake levels (Corella et al., 2014). The lake is roughly circular, with a surface area of 0.14 km² and a maximum water depth of 30 m. Although Lake Montcortès has been considered meromictic (Camps et al., 1976), there is evidence of a holomictic event that occurred during the winter of 1978–79 (Modamio et al., 1988). Available data indicate that the lake water is alkaline and oligotrophic (Camps et al., 1976; Modamio et al., 1988; URS 2010). According to the nearest meteorological station, la Pobla de Segur (Fig. 2.1A), total annual mean precipitation is 668.5 mm, with February being the driest month and May being the wettest. Annual average air temperature is 12.8°C, with maximum and minimum mean temperatures of 23.3 (July) and 2.9°C (January), respectively. The lake is situated in a transitional climatic area between the Mediterranean lowlands and the Middle Montane Belt within the sub-Mediterranean bioclimatic domain (Vigo and Ninot 1987). Therefore, the lake is very sensitive to climate changes. The surrounding vegetation is basically forest formations of evergreen and deciduous oak trees (*Quercus rotundifolia* and *Q. pubescens*) and conifer forests of *Pinus nigra* subsp. *salzmannii* (Mercadé et al., 2013). The lake's nearest surroundings are dominated by herbaceous vegetation types represented by pastures (for cattle and horses), hay meadows and crops of cereal and alfalfa (Rull et al., 2011; Mercadé et al., 2013). The lake's shoreline presents a steep talus and a littoral vegetation belt dominated by hygrophite communities of *Phragmites australis* accompanied by *Juncus* sp., *Scirpus* sp., *Typha* sp. and *Sparganium* sp. (Mercadé et al., 2013).

Lake Montcortès is located at the Baix Pallars municipality which has a total population of 350 habitants, with only 26 belonging to the town of Montcortès itself (Idescat 2015) Land use is limited to cereal crops and livestock pastures (Fig. 2.1B). The lake has historically been an important water resource for numerous surrounding villages and farmhouses with a long history of human occupation (Scussolini et al., 2011; Rull et al., 2011).

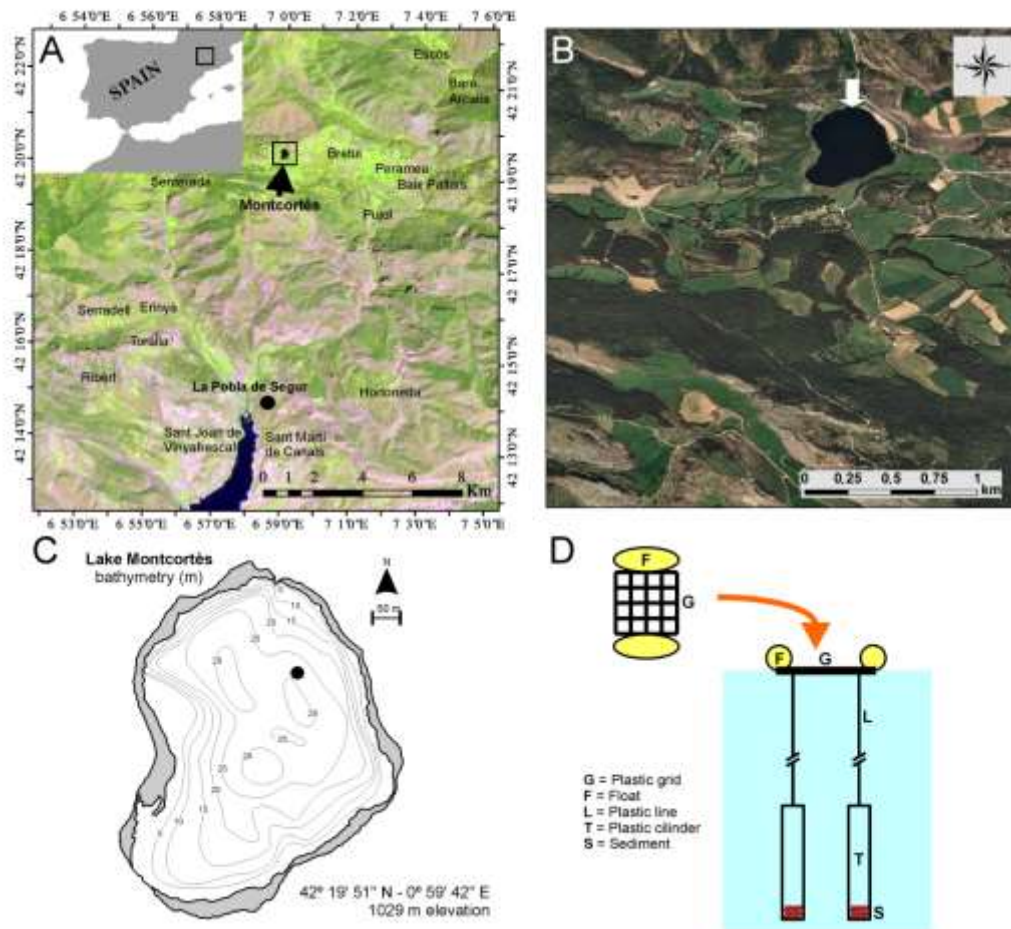


Figure.2.1- Study site: A. Geographical location within the Iberian Peninsula and regional map showing Lake Montcortès encircled and with an arrow and the meteorological station (black point) (source: Courtesy of U.S. Geological Survey). B. Aerial photograph of Lake Montcortès (white arrow) and surrounding area. C. Bathymetric map of Lake Montcortès and location of sampling point (black point). D. Field arrangement of sedimentary traps.

2.2- Methods

2.2.1- Limnological monitoring

Physical and chemical variables were monitored in the water column, and samples were collected monthly from October 2013 to October 2015. Profiles at 1 m depth intervals were obtained from the surface to the bottom of the water column for temperature (T), dissolved oxygen (DO), electric conductivity (EC) and pH using a multi-parameter water quality probe (Hydrolab DS5). Water transparency was determined using a Secchi disc. Light penetration was measured using a Photosynthetic Active Radiation (PAR) sensor. Water samples for chemical analyses and phytoplankton identification and counting were collected at three different depths coinciding with the epilimnion (~0.5 m), metalimnion (thermocline; from ~ 5 to 17m) and hypolimnion (~20 m). Phytoplankton samples were stored in amber glass bottles and fixed with concentrated Lugol's iodine solution. Identification and counting was carried out under inverted microscope at 400x magnification following the Uthermöhl method (Uthermöhl, 1931). Cellular biovolume determinations were carried out according to Wetzel and Likens (1991).

Total alkalinity was analyzed using standard titration methods for freshwater samples. Total nitrogen (TN) and total phosphorus (TP) were analyzed using alkaline persulfate oxidation, whilst soluble reactive phosphorous (SRP) was analyzed by the molybdate ascorbic method following Grasshoff et al. (1983). Cation concentrations of calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}) and potassium (K^{+}) were determined at the Scientific and Technological Center of the University of Barcelona (CCiTUB) using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES) with a Perkin Elmer Optima 8300 spectrometer under standard conditions. The calcite saturation index (Ω) was calculated according to equation 1:

$$\Omega = [\text{Ca}^{2+}] \times [\text{CO}_3^{2-}] / K_{\text{cal}} \quad (1)$$

where $[\text{Ca}^{2+}]$ and $[\text{CO}_3^{2-}]$ are the molar concentrations of calcium and carbonate ions, and K_{cal} is the solubility product of calcite. Here, we use $\log_{10}(\Omega)$ to express the calcite saturation index (SI).

Values of $[\text{CO}_3^{2-}]$ were computed from alkalinity and pH with the CO2SYS_XLS v 2.1 program (Pierrot et al., 2006).

2.2.2- Sampling and analyses of sediment trap material

Particulate matter settling through the water column was collected using sediment traps. Two cylindrical opaque PVC traps, 8.5 cm in diameter and with a 9:1 aspect ratio (Bloesh and Burns 1980) were used. They were fastened to a floating platform (Fig. 2.1D) and placed at 20 m water depth at 5 m above the sediment surface. One trap was emptied quarterly at the end of each season for seasonal diatom and calcite crystal analysis. Trapped material for diatom analysis was preserved in formaldehyde (4%). The second trap was emptied every month for trapped material characterization and quantification. The collected material was transferred into a plastic container and stored at 4 °C prior to subsampling. To determine sediment weight and composition, sample aliquots were filtered through pre-ashed (500 °C) and pre-weighed GF/F filters until filter saturation and then oven-dried for 48 h at 60 °C. Filters for total suspended solids (TSS; g L⁻¹) analysis were weighed, and total mass fluxes (TMF) were calculated from equation (2):

$$\text{TMF (g m}^{-2}\text{ d}^{-1}\text{)} = \text{dry net weight (g)} / \text{active area (m}^2\text{) time (d)} \quad (2)$$

To analyze carbon and nitrogen content of TSS, filters were weighed and analyzed for total particulate carbon (TPC), total particulate organic carbon (POC) and total nitrogen (TN). Samples for POC analysis were acidified to remove inorganic carbon. TPC, POC and TN were analyzed at the CCI TUB using an elemental organic analyzer, Thermo EA 1108, working under standard conditions. Total inorganic particulate carbon (PIC) was calculated as the difference between TPC and POC. Calcite contents was calculated from PIC concentrations by multiplying by 8.33, a factor referring to the molar weight of CaCO₃. To estimate organic matter (OM) from POC content in the trapped material, we applied a widely used conversion factor for soils and sediments by multiplying POC by 1.7 (Nelson and Somers 1996; Bluszc et al., 2008). We also used the theoretical relationship defined by pure organic matter in its simplest form (CH₂O) and multiplied POC by a factor of 2.5 (Leipe et al., 2010). With this approach, we obtained a range of estimated OM that covers possible variations depending upon the type of OM present in the sample.

To confirm the presence of calcite crystals and to assess seasonal variations of their size and shape trapped material from quarterly traps was analyzed by SEM. For this purpose, samples were oxidized with H₂O₂ at 200 °C, washed and then filtered using a Whatman polycarbonate filter of 0.4 μm pore size. Calcite crystals were then identified by means of spectrograms obtained in the microanalysis and characterized for size and morphology under the scanning electron microscope (SEM) with an Energy Dispersive X-ray Spectroscopy

and the help of the *Inca250* software. A minimum of 170 and a maximum of 358 calcite crystals were counted per sample, and the maximum (I_{\max}) and minimum lengths (I_{\min}) were measured.

For diatom analysis, an aliquot of trapped material from the quarterly traps was cleaned and prepared using standard methods (Abrantes et al., 2005). At least 300 valves per sample were counted, and microspheres were added to calculate valve influx (Battarbee et al., 2001). Samples were mounted in Naphrax© and analyzed using a Polyvar light microscope at 1000x magnification. Diatom species were identified using Krammer and Lange-Bertalot (1986-2004).

Meteorological data were obtained from the meteorological station of La Pobla de Segur (Catalonian Meteorological Service) located 19 km of Lake Montcortès (Fig. 2.1A). We used these data to assess inter-annual climatic variability covering the monitoring period by calculating the deviation or anomaly of monthly precipitation and temperature values from the mean long-term reference period 1961–1990. The selected variables were mean monthly air temperature (TM), mean monthly precipitation (PPT) and wind speed (W) (reference period 1961–1990) and the calculated TM and PPT anomalies as Anom TM and Anom PPTM, respectively.

2.3- Results

2.3.1- Modern limnology and water column properties

Water column temperature profiles of Lake Montcortès showed thermal stratification during most of the year, with mean surface and bottom temperatures of 14.6 °C and 5.2 °C, respectively. During winter, the surface water cooled until it reached 4–5 °C, and the increase in water density promoted thermic homogenization of the entire water column and all physical properties (Fig. 2.2). During spring and summer warming (April to September), surface temperature rose quickly, and thermal stratification developed. The mixing zone moved to shallower depths (5–7 m); a well-defined thermocline developed between 5 and 15 m and lasted until early winter (December) (Fig. 2.2A). Maximum epilimnetic temperatures of 24.7 °C were recorded during summer 2015, while the maximum during 2014 was 22.2 °C.

Dissolved oxygen showed maximum concentrations (14–17 mg L⁻¹) between 7 to 10 m in the euphotic metalimnion from May to September around the thermocline (Fig. 2.2B) and then decreased with depth. Secchi disc ranged from ~4 to ~9 m, and the limit of the

euphotic zone was always deeper than 7 m and below the oxygen peak (Fig. 2.2B). Winter water column mixing events took place in January 2014 and 2015. When stratification began from March onward, oxygen depletion occurred, and anoxic conditions started to develop in the hypolimnion. Hypoxia ($\leq 2 \text{ mg L}^{-1}$) began in April, and anoxia (0 mg L^{-1}) was reached in June and remained until December (Fig. 2.2B). The maximum thickness of the anoxic layer was observed at the end of summer (September–October) and reached up to 15 m lake depth in 2014 and close to 17 m lake depth in 2015. This represents an anoxic layer thickness close to 10 m.

Values of pH were highest coinciding with oxygen maxima in the metalimnion. During stratification, pH values ranged from 7.1 close to the bottom to 8.7 in the epilimnion (Fig. 2.2C), coinciding with increasing phytoplankton biovolumes, and were near 7.8 during winter mixing. Total alkalinity was roughly constant through time and depth, with mean values ranging from 3 to 3.5 meq L^{-1} , indicating well-buffered waters even in the hypolimnion, where pH was lower (Fig. 2.2C). Electric conductivity (EC) did not experience significant seasonal changes along the water column, except for subtle EC minima that occurred during stratification in both years coinciding with oxygen maxima. EC anomalies, calculated as the difference between depth average and the corresponding value for each depth, were plotted to document these minima (Fig. 2.2E).

Ca^{2+} was the most abundant dissolved cation followed by Mg^{2+} (Table 1). Values of SRP and TP were very low. TN was relatively more abundant and increased notably in the hypolimnion during the stratification period, while it was depleted in epi and metalimnion (Table 1).

The succession of main phytoplankton groups followed a seasonal pattern during the entire sampling period (Table 1). Central diatoms belonging mainly to the genus *Cyclotella* peaked in early spring, taking advantage of turbulence after overturn. During summer and early fall Chlorophytes were the most abundant group, except in summer 2015 when diatoms remained dominant in the metalimnion, while in the epilimnion Cryptophytes dominated. Cryptophytes were dominant mainly during winters when all other groups decreased in abundance. Interestingly, a significant part of the planktonic diatoms and Chlorophytes was extremely small, exhibiting sizes of only $\sim 5 \mu\text{m}$.

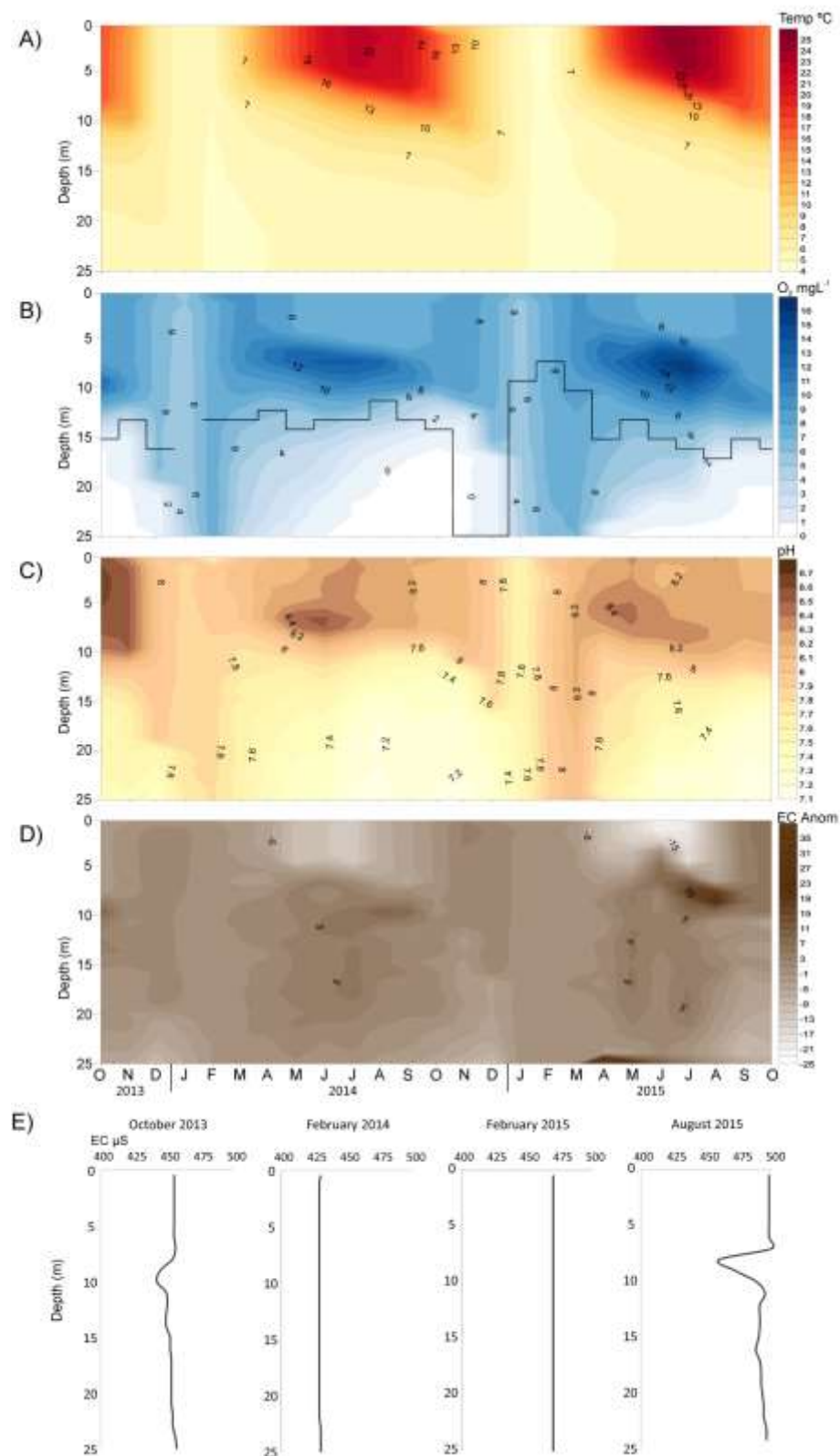


Figure.2.2- Depth profiles of A. water temperature; B. oxygen concentration with euphotic zone (solid line); C. pH; D. electric conductivity (EC) anomalies and E. four discrete electric conductivity profiles corresponding to mixing periods (February 2014 and 2015) and stratification period (October 2013 and August 2015) note the EC decreases in metalimnetic waters during stratification .

	2013		2014			2015		
Ca (mg L ⁻¹)	Fall	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
Epi ^a .	128.09	127.50	102.5	145.00	107	83.57	83.57	82.78
Meta ^b .	112.99	127.95	122.67	138.36	101.21	82.97	81.71	81.56
Hypo ^c .	125.03	144.21	130.18	118.25	103.44	82.96	81.76	84.38
Mg (mg L ⁻¹)								
Epi.	15.65	18.26	13.92	19.21	17.93	17.89	18.12	17.32
Meta.	17.04	18.29	16.02	19.16	18.82	17.76	17.8	17.18
Hypo.	17.38	19.23	16.2	19.19	18.56	17.76	17.61	17.50
K (mg L ⁻¹)								
Epi.	2.34	2.78	2.20	2.79	2.45	2.53	2.73	2.63
Meta.	2.52	2.81	2.40	2.81	2.69	2.54	2.53	2.68
Hypo.	2.60	2.88	2.60	2.78	2.69	2.54	2.53	2.63
Na (mg L ⁻¹)								
Epi.	3.04	3.66	2.26	3.63	3.31	3.24	3.60	3.63
Meta.	3.50	3.71	2.91	3.29	3.17	3.08	3.31	3.58
Hypo.	3.34	3.36	2.87	3.32	3.23	3.28	3.32	3.50
SRP (μM)								
Epi.	0.050	0.042	0.037	0.051	0.049	0.040	0.010	0.034
Meta.	0.019	0.012	0.053	0.047	0.042	0.050	0.010	0.036
Hypo.	0.071	0.036	0.047	0.047	0.049	0.057	0.011	0.050
TP(μM)								
Epi.	0.190	0.200	0.170	0.350	0.650	0.045*	0.180	0.050
Meta.	0.191	0.200	0.212	0.853	0.624	0.547*	0.511	0.130
Hypo.	0.198	0.201	0.264	0.697	0.900	0.512*	0.402	0.396
TN (μM)								
Epi.	30.28	50.63	34.37	24.19	28.06	45.35	36.84	28.65
Meta.	43.21	50.73	37.25	27.36	34.55	45.63	39.54	28.84
Hypo.	71.47	53.81	61.68	69.81	59.55	44.84	53.45	59.45
Phytoplankton succession								
Epi	Chlorophyta	Cryptophyta	Bacillariophyta	Chlorophyta	Chlorophyta	Cryptophyta	Bacillariophyta	Cryptophyta
Meta.	Chlorophyta	Cryptophyta	Bacillariophyta	Chlorophyta	Chlorophyta	Cryptophyta	Bacillariophyta	Bacillariophyta

(*) Missing sample of January; ^a Epilimnion; ^b Metalimnion; ^c Hypolimnion.

Table 2.1 – Seasonal average concentration of cations and nutrients and main phytoplankton groups.

2.3.2- Fluxes of trapped material

Total mass fluxes (TMF), calcite and POC fluxes are displayed in Figure 2.3. The collected material from sediment traps reveals important changes during the two years of sampling. TMF followed a clear seasonal pattern in which low values occurred mainly during winter and early spring and the highest values during summer and fall (Fig. 2.3A). Progressive flux increases started in early spring (April–May), coinciding with oxygen increase together with increasing fluxes of POC (Fig. 2.3B). After that, both TMF and POC started to decrease during fall with oxygen depletion. Increases in POC fluxes were roughly followed by increases in calcite deposition (Fig. 2.3B and C). Calcite is mainly deposited during summer and fall, with higher values recorded in fall 2013 and summer 2015 (Fig. 2.3C). Periods with more intense calcite deposition also coincided with higher values of calcite saturation in the epilimnion and metalimnion. The Mg-to-Ca molar ratio did not exceed 0.37 (Fig. 2.3C), and epi- and metalimnion remained permanently calcite-saturated throughout the study period (Fig. 2.3E). The highest values of calcite saturation were recorded during spring and summer (Fig. 2.3E), coinciding with rising temperature and primary production. The hypolimnion also endured saturation during most of the year but presented lower saturation values than epi- and metalimnetic waters. Overall, calcite saturation values declined in late summer and fall. During the late fall of 2014, the hypolimnion was near undersaturation for calcite, and it was only during the fall of 2015 when values fell below zero recording undersaturation values (Fig. 2.3E).

TMF, POC and calcite fluxes showed a similar behavior and are moderately correlated (TMF vs POC $r = +0.80$, $p(a) < 0.05$, $n = 25$; TMF vs PIC $r = +0.86$, $p(a) < 0.05$, $n = 25$ and PIC vs POC $r = +0.76$, $p(a) < 0.1$, $n = 25$). Total amounts of calcite deposition varied broadly between years, a fact that is evident by comparison with the data from 2014 to 2015 (Fig. 2.3C). During 2014, calcite fluxes remained below OM fluxes, while during 2013 and 2015 calcite values clearly exceeded OM during fall and from early summer to fall (Fig. 2.3D).

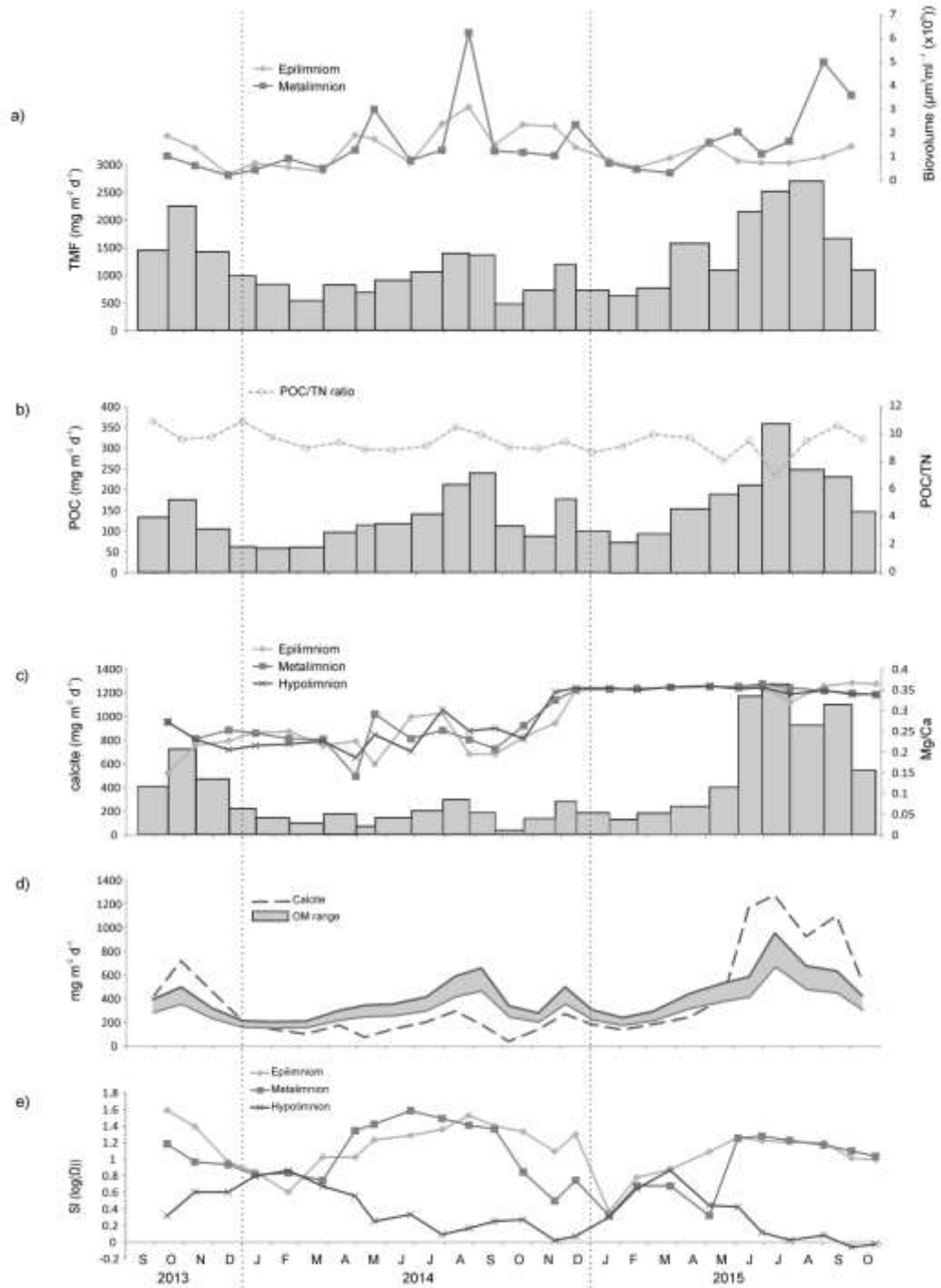


Figure.2.3- Monthly fluxes of major sediment components deposited in sediment traps during monitoring period 10/2013-10/2015: A. Total mass fluxes (TMF) with changes in phytoplankton biovolume for the epilimnion and the metalimnion; B. POC fluxes and corresponding POC/TN ratios; C. calcite fluxes with epi-, meta and hypolimnion Mg/Ca; D. calcite fluxes versus estimated range of organic matter fluxes (OM); E. Calcite saturation index (SI) for epi-, meta- and hypolimnion.

Presence of calcite crystals was confirmed in all trapped samples. A clear seasonal pattern in calcite sizes was observed (Fig. 2.4A). Larger crystals with higher I_{max} and I_{min} correspond to fall and winter in traps and tend to decrease progressively from fall to summer through the two years of the study. Most of the crystals have blocky and polyhedral habits (Fig. 2.4B to 2.4G). Some crystals appeared attached to central diatom frustules of the genus *Cyclotella* (Fig. 2.4H).

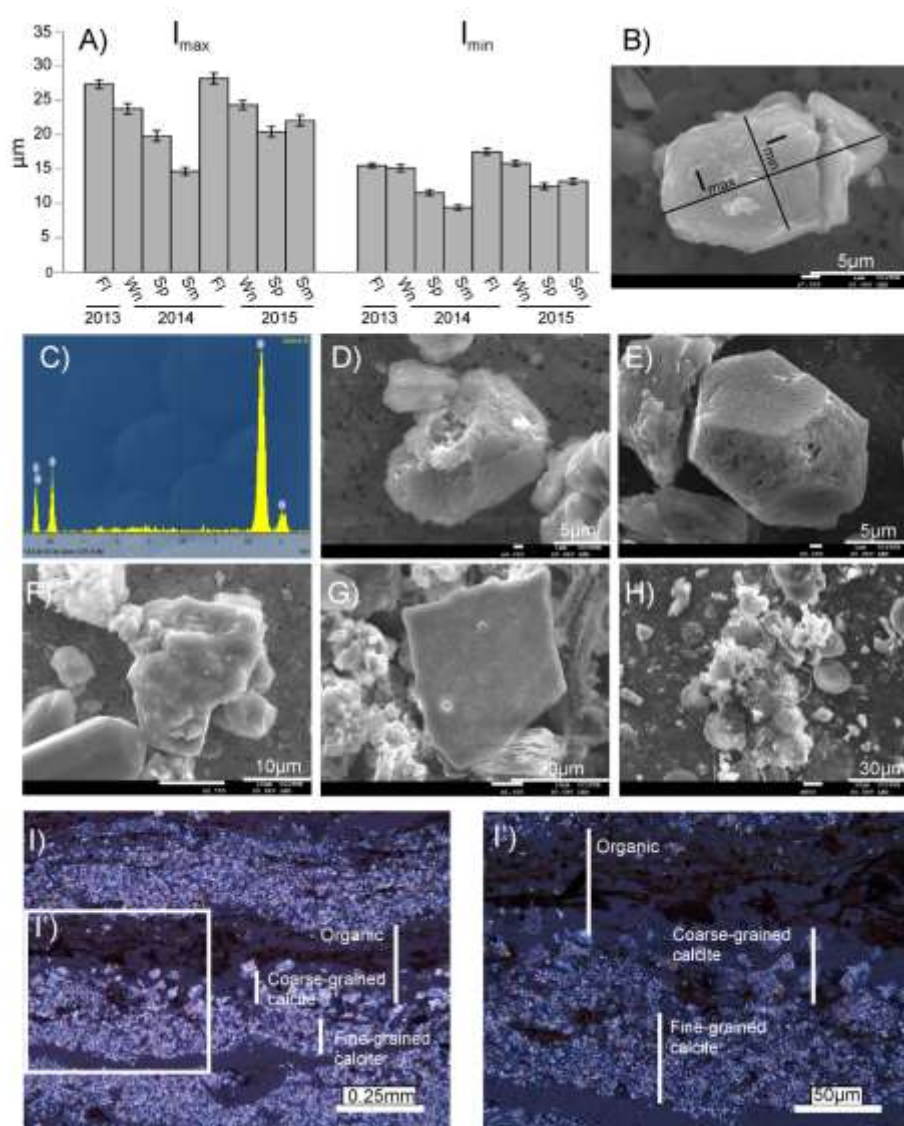


Figure.2.4- Monthly fluxes Calcite crystals found in quarterly sediment traps in Lake Montcortès: A. Mean values of I_{max} and I_{min} (μm) corresponding to Fall (F), winter (W), spring (Sp) and summer (S) and bars indicate standard deviation; B-H. SEM images of calcite crystals: B. I_{max} and I_{min} measurements; C. calcite spectogram; D-G. images of calcite crystals. Thin section microscopic image showing one of the varve structures found in Lake Montcortès sediment: I. calcite layer with fine-grained calcite crystals followed by coarse-grained calcite crystals and the organic layer; I') more magnified detail of varve structure.

2.3.3- Trapped diatoms

We analyzed diatoms from quarterly trapped sediment samples to estimate their timing and contribution to the deposited material. Higher diatom fluxes were recorded during spring and summer (Fig. 4.5A). This pattern roughly matches with the total diatom cells counted in the water column (Fig. 4.5B). Central diatoms accounted for more than 50% of the diatom composition during most of the year and reached abundances above 75% during spring and summer. Seasonal changes in relative abundances of the main species of central diatoms *Cyclotella cyclopuncta*, *C. ocellata*, *C. radiosa* and *Stephanodiscus hantzschii* are shown in Figure 4.5C. *C. cyclopuncta* generally dominated the assemblage, while *C. ocellata* was less abundant with constant proportions. *C. radiosa* showed small proportions, except during spring for both years, when it increased. The differences between the diatom content of the traps and the water column during the spring of 2015 can be explained by poor preservation of the material deposited in the corresponding trap, which was considerably damaged, diluted and hardly able to be counted.

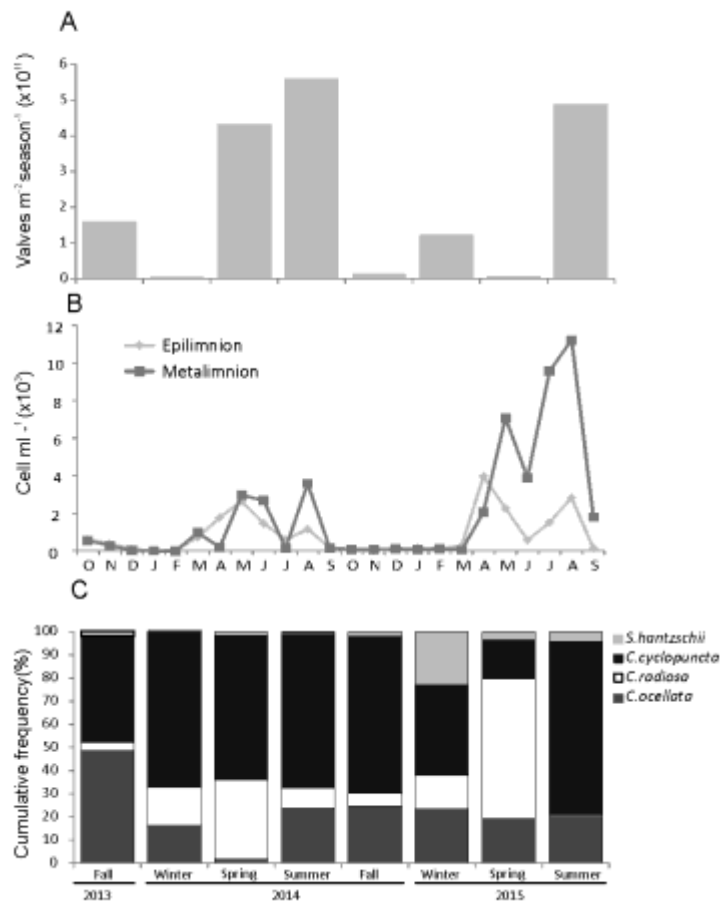


Figure.2.5- Diatom dynamics: A. fluxes of diatoms in quarterly traps; B. Diatom cell abundances in the epilimnion and metalimnion; C. relative abundances of centric diatoms

2.3.5- Meteorological conditions

Monthly mean air temperatures decreased from August–September to December–January, when temperatures remained above 4 °C. Maximum temperatures were reached during July of both years: 22 °C for July 2014 and 26.5 °C for July 2015 (Fig. 4.6A). Temperature anomalies corresponding to 2015 were generally positive and higher than anomalies in 2014, indicating a warmer year (Fig. 4.6A). Precipitation was high in late summer and fall for both years (Fig. 4.6B). During 2015, negative precipitation anomalies were persistent from December 2014 to June 2015 and positive from July to October. Comparing the same periods for 2014, December 2013 to June 2014 was wetter than in 2015 with more positive anomalies, while July to October 2015 was similar to 2014.

Monthly mean wind speeds during the studied period reached maximum values during spring and the minimum values during winter of both years and did not exceed 1.5 m s⁻¹ during the studied period (Fig. 4.6B).

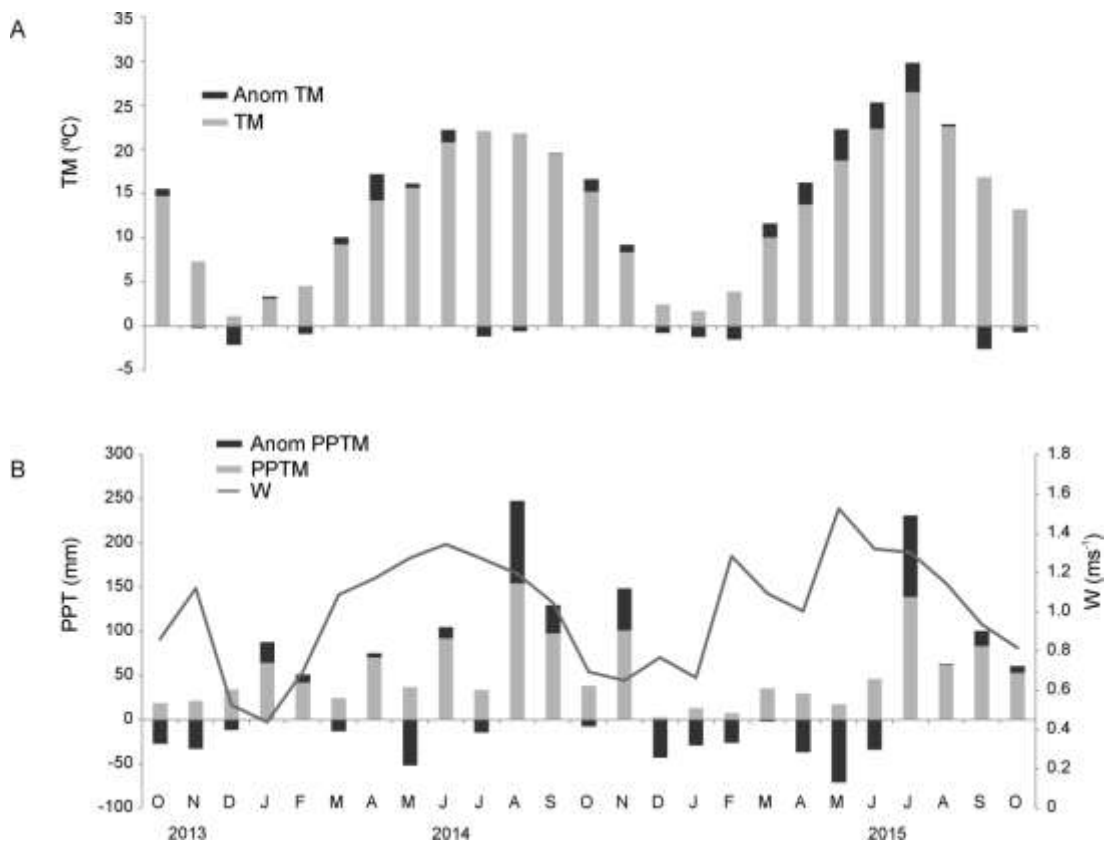


Figure.2.6- Meteorological data: A. Monthly mean air temperature (TM); B. mean precipitation (PPT) and their anomalies (black bars) along with wind speed (W) (line).

2.4- Discussion

2.4.1- Varve formation: sediment fluxes and composition during the annual cycle.

The annual pattern of particle flux dynamics and composition in Lake Montcortès was closely related to biogeochemical processes in the euphotic zone and changing meteorological conditions throughout the year. Fluxes of sediment reached maximum values in summer and fall that matched with maxima of phytoplanktonic growth, pH, and calcite and POC deposition in traps values. POC values were higher during summer and fall when calcite was mainly deposited, suggesting that the main origin of calcite crystals are autochthonous. Other sources of calcite input, such as detrital carbonates from the catchment, seem to be not significant during the studied period. Well-formed blocky and rhombohedral calcite crystals and the presence of diatom frustules attached to these crystals support the autochthonous origin of calcite (Pere Anadón pers. com.). Moreover, low values of the POC-to-TN ratio indicate the prevalence of cellulose-poor autochthonous algal organic matter over lignin and cellulose-rich allochthonous organic matter derived from vascular plants (Meyers and Teranes 2001), suggesting low input to the lake from the catchment area. Additionally, there are no discontinuities in TMF that could be attributed to external inputs, i.e., there are no TMF increases that coincide with months of higher precipitation anomalies (Fig. 4.3 and 4.6). These observations are consistent with the fact that biologically induced calcite precipitation took place in Lake Montcortès.

Calcite precipitation can be triggered by CO₂ uptake during algal photosynthesis. Primary productivity affects the carbonic-carbonate system in epilimnetic and metalimnetic waters by decreasing CO₂, increasing pH, and shifting the equilibria toward the CO₃²⁻ species (Kelts and Hsü 1978, Dittrich and Obst 2004). This biochemical mechanism leads to calcite supersaturation in the limnetic zone (Hodell et al., 1998). In addition, when photosynthesis occurs, precipitation would be enhanced by phytoplankton providing particulate surface areas for calcite-crystal growth (Dittrich et al., 2004). In fact, the epi- and metalimnetic waters of Lake Montcortès were already supersaturated with calcite when precipitation occurred at $\log \Omega \geq 1$ and with a rather low Mg/Ca molar ratio that indicates favorable environmental conditions for the formation of pure calcite or low Mg-calcite crystals (Müller et al., 1972). Calcite supersaturation values for calcite precipitation need to be $\log \Omega \geq 2$ under experimental conditions (Dittrich and Obst 2004), but it has been shown that precipitation in lakes can happen far below that threshold. Some examples are Lake Constance, where calcite precipitates at $\log \Omega \leq 1$ (Stabel et al., 1986), and the oligotrophic Lake Lucerne, where precipitation occurred when $\log \Omega$ was near 0.4 (Dittrich et al., 2004). A likely explanation for

this discrepancy is the presence of nuclei provided by phytoplankton cells, which are necessary for crystal growth (Dittrich and Obst 2004). Nucleation of calcite crystals triggered by phytoplankton and small particles of picoplankton (0.2-2 μm), such as cyanobacteria or sulfur-reducing bacteria, have been shown and documented by several studies (Dittrich and Obst 2004; Baumgartner et al., 2006). This explanation likely applies in our case, since small Chlorophyta (such as *Tetraedron minimum* and *Oocystis spp.*) and small centric diatoms ($\sim 5 \mu\text{m}$) are dominant during periods of calcite precipitation, and persistent populations of sulfur bacteria (Chromatiaceae and Chlorobiaceae) are known to thrive at the hypolimnion-metalimnion boundary of Lake Montcortès (Cristina et al., 2000). Additionally, during stratification period, the filters we used to trap deposited material were intensely stained with the unmistakable color of purple bacteria (Supplementary Fig.4.1), and their presence was confirmed by okenone and isorenieratene pigment analysis (Vegas-Villarúbia et al., 2018). Moreover, high calcite fluxes happened at the same time that negative EC anomalies were recorded. This slight decrease in EC could be a consequence of ion depletion caused by nutrient uptake and precipitation of calcite, but any simultaneous decrease in alkalinity and Ca concentration that could support the link between EC and calcite precipitation went unnoticed. This outcome may be a consequence of sampling resolution; in fact, variations in Ca and alkalinity might have happened between sampling data and thus remained unnoticed in our records. Nonetheless, decreases in EC in epilimnetic and metalimnetic water related to calcite precipitation have been observed in other hard-water lakes (Miracle et al., 2000; Bluszcz et al., 2008).

The spring and the summer of 2015 were anomalously warm, and the calcite flux was more than twice compared to the preceding year for the same seasons (Fig. 4.3C). These warmer conditions were perfectly recorded in epi- and metalimnetic water temperatures that coincided with higher values of DO as a result of enhanced primary production. Thus, the influence of atmospheric temperature increase on processes involved in calcite precipitation is evidenced by the differences in the magnitudes of deposited material between both years.

Actually, 2015 was the warmest year on the record registered in Europe (NOAA 2015) recording the second warmest summer on the record. Also, 2015, was the warmest year in Spain tied with 2011 that recorded 0.96 °C temperature anomaly (1981-2010 reference period) (AEMET 2015). Since the late 1990ies until nowadays, increases of mean lake-water temperature between 0.5 and 0.6 °C per decade have been recorded in the majority of studied European lakes (Dokulil. 2013). Besides, the average thickness of calcite sublayers of

the Montcortès record has nearly doubled since the 19th century (Corella et al., 2011b, 2012). These results suggest that variations of biogenic calcite in the sedimentary record might indicate temperature changes, at least partially. The thickness of calcareous layer in Lake Montcortès seems to be a reliable paleotemperature proxy.

2.4.2- Varve preservation: Limnological conditions

Previous studies of Lake Montcortès found persistent anoxic conditions and sulfide production on bottom waters year-round in 1975/1976 and 1997/1998 respectively (Camps et al., 1976; Cristina et al., 2000). Based on these studies and in the laminated nature of the sediment record, Lake Montcortès was considered to be meromictic (Corella et al., 2012; Valero-Garcés et al., 2014). However, Modamio et al., (1988) reported a complete oxygenation of the water column associated with a winter mixing event (1978/1979). During the two-year period of monitoring, we observed that Lake Montcortès mixed during both winters. Nevertheless, during 2016 the lake did not mix completely (unpublished data). The observed mixing events in Lake Montcortès were tightly related with a mean air-temperature decrease below the lake's surface water-temperature that caused denser surface water layers to sink, promoting the mixing of the water column and thus, homogenization of oxygen concentration throughout the water column. Meteorological forcing at the air-water interface is known to be the main determinant of the heat balance of many lakes (Edinger et al., 1968; Sweers, 1976). All this evidence suggests that Lake Montcortès has an interannually changing mixing regime and cannot be considered meromictic *sensu stricto* (Hakala 2014). This is supported by a recent study documenting a 45.3% mixing recurrence during the last 500 years (Vegas-Vilarrúbia et al., 2018). With present global warming, an expected strengthening might significantly affect nutrient and oxygen regeneration within the water column.

Although meromictic conditions are especially suitable for varve preservation, short periods of mixing and seasonally suboxic to anoxic conditions during stratification seem to be enough to prevent bioturbation and allow varve preservation, even in dimictic lakes (Ojala 2000; Hakala 2004; Zolitschka et al., 2015). In Lake Montcortès this is supported by the continuous presence of varves shown by the sediment record of the last three millennia. Other factors that may affect varve preservation are resuspension of bottom sediments through wind or density-driven water circulation and bottom currents that interrupt continuous sedimentation and cause erosional hiatuses. The latter two processes can be excluded if the lake basin is deep with a small surface area and geomorphologically protected

(O'Sullivan 1983), this is the case for Lake Montcortès (Corella et al., 2012). Furthermore, lake mixing took place when minimum wind speeds were recorder both years studied (Fig.4.6.B).

2.4.3- Comparison with previous varve interpretations

The seasonal sedimentation pattern obtained from the sediment trap study is consistent with the alternating structure of light (calcite) and dark (organic) laminae observed in the sediment record of Lake Montcortès supporting the seasonal nature of the sediment laminane. According to our study, calcium calcite layer would form mostly in summer/fall and dark organic laminae mostly during winter/spring. This is more clearly evidenced in Figure 4.3D where OM and calcite fluxes are compared. Thus, our results do not totally agree with former studies that situated the formation of the calcite layer in spring/summer, and the formation of the organic layer in fall/winter (Corella et al., 2012). We did not observe conspicuous pulses of calcite precipitation, as occurs in lakes with the presence of whiting events (Miracle et al., 2000). Nonetheless, there was a clear differentiation between calcite and OM deposition in fall 2013 and summer/fall 2015, while this was not the case during 2014. In the latter case, the depositional pattern might result in a lack of sublayer differentiation and consequently of a varve or sub-layer absence for that particular year. In fact, this is coherent with Corella et al.(2011b), as they found laminated intervals with scarce light calcite layers in the Montcortès record of the last 6,000 years. Knowing when and why such events happened is crucial when it comes to building varve chronologies to minimize the counting error. Varve fading or absence can lead to mistakes in the recognition of annual cycles in the sediments.

Calcite precipitation began to increase in late spring with higher deposition rates during summer lasting until fall. Consequently, the light laminae would represent the merged effect of temperature and primary production. Dark laminae (OM) would represent the productivity of the lake through the year. Calcite laminae differentiation would depend of the amount of calcite precipitated during summer/fall. Such correspondence should be demonstrated by analyzing the individual sediment layers covering the studied period but presently this cannot be accomplished as it is necessary to wait for diagenesis to consolidate the sedimentary deposits. So far, our results give further information about seasonal composition of sediment material and highlight the importance of modern analogue studies to properly interpret the sediment signal at a seasonal and sub-annual scale.

Calcite crystal-sizes from quarterly traps found in this study showed a seasonal pattern likely related to water temperature, being smaller when formed during spring and summer. Additionally, deposition of smaller crystals was concurrent with higher fluxes of diatoms and high SI. It is known that when calcite saturation is high, e.g. in spring and summer, crystals were formed rapidly and do not have time to grow. However, in fall and winter, water is not very supersaturated in calcite, and crystals can increase their sizes while travelling to the bottom (Kelts and Hsü 1978). Thus, smaller calcite crystals in Lake Montcortès would be interpreted as occurring during periods with elevated SI related to higher temperatures and primary production. This pattern bears some relationship with the internal calcite sub-layering present in the sediment record of Lake Montcortès (fining upward with coarser calcite crystals in the lower part of the calcite layer; coarsening upward, the converse of fining upward; and a homogeneous layer of coarse crystals (Corella et al., 2012)). The seasonal calcite-crystal distribution observed in 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 in fall (Fig. 2.4I and I'). This texture is frequently observed in the sedimentary sequence from AD ~1350 to 1850 AD and less frequently from then until present day (Corella et al., 2012). Future micro-facies analysis of the sediment record retrieved after 2015 might show the corresponding varves and sub-layering patterns of the studied period (2013-2015).

Three *Cyclotella* species coexisted over the studied period: *C. cyclopuncta*, the dominant taxon, together with *C. ocellata*, while *C. radiosa*, which is bigger than the two other species, increased in spring, being competitive during periods of high turbulence promoted by winter mixing. The growth of highly silicified species as a response to turbulence, as well as increases in small centric diatoms during stratification (e.g., *C. cyclopuncta*), have been observed in other lakes as a consequence of changes in water stratification (Rühland et al., 2015). Changes in relative abundances of *C. cyclopuncta* and *C. radiosa* in Lake Montcortès are chiefly a response to shifts in stratification of the water column. Therefore, increasing temperature would favor small *C. cyclopuncta* during intensified stratification periods. These findings provide new insights at seasonal time resolution to refine inferences made from the diatomological record in former studies working at coarser resolution. For instance, based on available information on diatom autoecology, the presence of *Cyclotella compta* (a synonym for *C. radiosa*) and *C. cyclopuncta* in the sediment record was attributed to warmer and cooler periods, respectively (Corella et al., 2012). Another study found frequencies of *C. cyclopuncta* and *C. radiosa* to vary inversely

and only appeared together in a single sample over the last 1500 years and suggested that these shifts may be a response to nutrient availability (Scussolini et al., 2011). However, present-day data reveals an alternative scenario where both taxa coexist together and differences in relative abundances respond to seasonal changes in water stability with warmer conditions favoring *C. cyclopuncta*

Both changes in diatom species abundances as well as in shapes and sizes of calcite crystals are related indirectly to temperature by means of changes in SI and water column stratification. Additionally, the occurrence of calcite precipitation and differences in calcite amounts between the years are related with temperature variability. Consequently, varve succession in Lake Montcortès is a direct product of seasonally changing environmental conditions and is especially sensitive to alterations in temperature.

The importance of modern analogue studies lies in the need of understanding the specific processes taking place in a specific place within specific features (hydrology, geomorphology, limnology, climate and anthropogenic influence) that shape the resulting sedimentary sequence and the resulting environmental signal. Then, general validity processes could not apply properly to successfully interpret the paleorecord. For example, Bonk et al. (2015) demonstrated that multiple calcite precipitation events took place in a single year related with changes in the mixing regime of lake Żabińskie (Poland) depending of climate conditions. These results were coherent with the sedimentary structure of the last 20 years where a maximum of four calcite layers were observed within a single year. The modern analogue study in this case provided a basis to establish a reliable varve chronology. Based on three years of monitoring data on seasonal particle pulses in Lake Van (Turkey), Stockhecke et al. (2012) found that the temporal and lateral variations of lake basin changes on particle accumulation and composition (lithology) were linked to atmospheric circulation patterns that controlled hydrological and meteorological conditions. Thus, they provide a basis for the reconstruction of past seasonal climate patterns. All of these monitoring studies successfully gave sense to modern processes to be compared with the fossil record for these specific places setting a baseline to reduce sources of error for future sediment studies. Anyhow, although monitoring studies are highly time consuming and costly, long-term water column and particle-flux monitoring should continue to better assess inter-annual variability and to improve future interpretations of the past.

2.5-Conclusions

Two years of sediment trap and limnological monitoring in the Lake Montcortès enabled us, for the first time for this lake, to link the limnological cycle to seasonal particle fluxes and provide modern sedimentary analogues potentially useful for improving the quality and quantity of paleoenvironmental and paleoecological information.

Varve deposition in Lake Montcortès is a direct result of seasonal limnological changes occurring in the water column and its seasonality is consistent with the annually laminated nature of the sediment record. Sedimentary patterns are closely related to biological processes occurring in the euphotic zone. There is a clear seasonal signal, useful for paleolimnological interpretation, expressed in the alternation of calcite precipitation during summer and fall and organic matter precipitation during winter and spring. Summer/fall calcite precipitation is favored by high calcite saturation indices and high pH values, in connection with enhanced primary production and the eventual capacity of phytoplankton cells to act as condensation nuclei. Inter-annual differences were expressed in terms of the amount of precipitated calcite, which is related to temperature variability. Thus, the thickness of calcite layers in the sediment could be considered as a potential paleoclimatic temperature proxy. A hypothesis that should be tested in the future. In addition, changes in calcite-crystal size and in the composition of diatom assemblages would give additional information about changes in SI and thermal stratification.

According to our results, the varves that formed during the two studied years would likely match one of the varve types previously identified in the sedimentary record of the last millennium. Microstratigraphic investigations from sediment cores retrieved after 2015 is needed to confirm the resulting sedimentary pattern corresponding to the two studied years. Relationships between environmental and meteorological conditions and characteristics of individual varves need to be tested against longer time series of environmental and meteorological data.

Although it has been demonstrated that Lake Montcortès is not strictly meromictic but with alternating holomictic and meromictic periods, the long duration of thermal stratification and hypolimnetic anoxia create suitable conditions for varve formation and preservation. Nonetheless, the limnological monitoring and the sediment trap survey should continue to account for potential inter-annual variability, which is especially meaningful in the Mediterranean area and in the context of global warming

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CHAPTER 3

Seasonal patterns of pollen sedimentation in Lake Montcortès (Central Pyrenees) and potential applications to high-resolution paleoecology: a 2-year pilot study.

Soundtrack: “*Somebody that I used to know*” - Gotye

Original publication (*Appendix2 in the supplementary material*):

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Abstract

Lakes with varved sediments are especially well suited for paleoecological study, from annual to even seasonal resolution. The interpretative power of such high-resolution paleoenvironmental reconstructions relies on the availability of modern analogs with the same temporal resolution. We studied seasonal pollen sedimentation in varved Lake Montcortès, Central Pyrenees (Spain), as a modern analog for high-resolution reconstruction of Late Holocene vegetation and landscape dynamics. Seasonal samples were obtained from sediment traps that were submerged near the maximum water depth for a two-year period (Fall 2013 to Fall 2015). Seasonal pollen sedimentation was compared with meteorological variables from a nearby weather station. Bulk pollen sedimentation, dominated by *Pinus* (pine) and *Quercus* (oak), followed a clear seasonal pattern that peaked during the spring/summer (SS), coinciding with maximum temperature and precipitation, minimum relative humidity and moderate winds from the SSE. Pollen sedimentation lags (PSL) were observed for most pollen types, as substantial amounts of pollen were found in the traps outside of their respective flowering seasons. Two pollen assemblages were clearly differentiated by their taxonomic composition, corresponding to spring/summer and fall-winter (FW). This pattern is consistent with existing interpretation of the sediment varves, specifically, that varves are formed by two-layer couplets that represent the same seasonality as pollen. We concluded that pollen sedimentation in Lake Montcortès exhibits a strong seasonal signal in the quantity of pollen, the taxonomic composition of the pollen, and relationships between the pollen and meteorological variables. Thus, varved sediments provide a potentially powerful tool for paleoecological reconstruction at seasonal resolution. This method could be used not only to identify paleoenvironmental trends, but also to identify annual layers and therefore date sediments, even in the absence of evident sediment laminations. A satisfactory explanation of PSL will require further studies that examine internal lake dynamics and pollen production/dispersal patterns.

Keywords: Laminated sediments, Varves, Palynology, Pollen influx, High-resolution paleoecology, Sediment traps, Seasonality

3.1- Introduction

Varved lake sediments are useful not only for high-resolution paleoecological studies, but also for bridging the temporal gap between ecology and paleoecology. Indeed, the annual/seasonal domain is the ideal time frame to produce truly continuous long-term, high-resolution ecological time series, which successfully merge ecological and paleoecological data (Rull 2014). The combination of ecological data from varved lake sediments and modern observations may yield long, high-resolution ecological time series comparable to the continuous, long-term climate series obtained by linking paleoclimate data derived from tree rings and similar proxies, with instrumental climate measures at annual/seasonal resolution (Mann et al. 1999). Formation of annually laminated lake sediments requires a seasonal climate, and variable flux to the sediment of components from multiple autochthonous and allochthonous sources. The preservation of varves is favored in small, deep lakes with a permanent (meromictic) or seasonal (monomictic/dimictic), hypoxic or anoxic hypolimnion. Varves are especially well preserved in meromictic lakes with a clear chemical contrast between the epilimnion and hypolimnion, which differ in water density, thereby maintaining stratification and preventing water column circulation (O'Sullivan 1983; Ojala et al. 2012; Zolitschka et al. 2015). Lakes with varved sediments are more frequent in northern, temperate regions. In Europe, more than 60 lakes with annually laminated sediments have been studied, of which more than half have continuous varve chronologies for at least the last 100 years. Middle and late Holocene records are frequent, and in some cases varved sediments extend back to the late glacial and early Holocene (Ojala et al. 2012). These particular types of sediments have been used for a variety of paleoecological studies, most notably to calibrate radiometric (^{14}C , ^{210}Pb , ^{137}Cs , ^{32}Si) chronologies and to obtain high-resolution paleorecords of Earth's magnetic field, solar forcing, volcanic and seismic activity, climatic change, ecological shifts and human activities (Zolitschka et al. 2015).

In the Iberian Peninsula, four lakes with annually laminated sediments have been studied to date, a Pliocene paleolake (Muñoz et al. 2002) and three extant lakes with Holocene varves: La Cruz, with laminations that date from AD 1579 to the present (Romero-Viana et al. 2008, 2011), Zóñar, with intermittent varved sections over the last 2500 years (Martín-Puertas et al. 2009) and Montcortès, the longest, continuous varved record retrieved thus far, representing the last ~1550 years (Corella et al. 2011, 2012) (Fig. 3.1). Preliminary paleoclimate, paleoecological and paleolimnological studies of the Montcortès record were conducted at multi-decadal to centennial resolution (Corella et al. 2011, 2012; Rull et al. 2011; Scussolini et al. 2011; Rull and Vegas-Vilarrúbia 2014, 2015). The only high-

resolution study to date is an annual reconstruction of the extreme rainfall events that occurred since the mid-14th century (Corella et al. 2014). The varve chronology of this lake (ca. AD 400 to the present) is ideal for studying the transition from the Medieval Warm Period (MWP) to the Little Ice Age (LIA) and ongoing Global Warming (GW), at the annual resolution. A recent initiative was launched to increase the resolution of climatic and ecological reconstructions, which includes the study of present-day varve formation and preservation, so that they can be used as modern analogs of the past. Modern sedimentation was monitored using sediment traps in the lake to record seasonal variations in sediment flux and composition, a widely used method in studies of lakes with laminated sediments (Bloesch and Burns 1980; Mieszczankin 1991; Mieszczankin and Noryskiewicz 2000; Punning et al. 2003; Giesecke and Fontana 2008; St. Jacques et al. 2008; Huguet et al. 2012; Zolitschka et al. 2015).

In this paper, we present results of two years (Fall 2013 to Fall 2015) of pollen trapping, and investigate potential relationships between seasonal patterns of pollen sedimentation and relevant climate variables. The primary aim of this study was to identify seasonal pollen sedimentation patterns that would be useful for interpreting past records from the same lake. In this study, we concentrated our efforts on pollen sedimentation features; analysis of pollen-vegetation relationships, which is required to interpret past pollen records in terms of vegetation changes, is beyond the scope of this study, but will be addressed in the future. Pollen seasonality could be useful to resolve annual sediment layering, and therefore achieve high-resolution dating, even if physical sediment features are difficult to discern, e.g. when sublayers are absent, turbidites are present, or there are no laminations at all (Tippett 1964; Lotter 1989; St. Jacques et al. 2008). Sediment trap studies have been used to monitor pollen sedimentation in many parts of Europe, across a wide array of environments and vegetation types, and have provided insights into the importance of intra-annual weather conditions and pollination patterns (Mieszczankin 1997; Mieszczankin and Noryskiewicz 2000; Punning et al. 2003; Giesecke and Fontana 2008; Giesecke et al. 2010; van der Kaap et al. 2010; Pidek et al. 2015). To our knowledge, however, this is the first study to use submerged pollen traps in varved lakes on the Iberian Peninsula. Previous studies of this type were carried out in non-varved, high-mountain Pyrenean lakes to examine plankton sedimentation and its relationship to climate seasonality (Pla-Rabes and Catalan 2011). Our aim is to maintain the traps in Lake Montortès to study pollen sedimentation over the medium to long term.

3.1.1- Study site

Lake Montcortès is situated on the southern flank of the Central Pyrenees, in the Pallars Sobirà region of Catalonia (Spain), at 42° 19' N, 0° 59' E and 1027 m altitude, with a surface of 12.36 ha. The lake lies in karst terrain that is primarily characterized by Triassic limestones, marls and evaporites, and Oligocene carbonate conglomerates. Triassic ophyte outcrops primarily occur in the southern Quaternary lacustrine sediments that surround present-day water bodies (Corella et al. 2011). The catchment is small, and the lake is fed primarily by groundwater, with intermittent small creeks and scattered springs. Most water is lost to evaporation and a small seasonal outlet at the north end of the lake. The lake is roughly kidney-shaped, with a diameter between 400 and 500 m and a maximum water depth of 30 m near the center (Corella et al. 2014) (Fig. 3.2). Climate data from a nearby weather station (La Pobla de Segur), which is situated ~9 km to the South (Fig. 3.2) at 513 m elevation, show the annual average air temperature of the area is 12.8°C, which ranges from 2.9°C in January to 23.2°C in July. Total annual precipitation is 669 mm. February is the driest month (33.4 mm) and May is the wettest month (88.4 mm). Maximum and minimum temperatures recorded at this location were 41°C and -20°C, respectively. The maximum daily precipitation recorded was 138 mm.

The lake lies near the altitudinal boundary of the Sub-Montane belt, which is located in the Pyrenees at 800–1000 m elevation, depending on local features (Vigo and Ninot 1987). Four major forest formations occur in the lake region (Fig. 3.3): (1) Mediterranean sclerophyllous forests represented by *Quercus rotundifolia* woods; (2) Sub-Montane deciduous oak forests, which experience higher levels of precipitation and are dominated by *Quercus pubescens* and *Q. subpyrenaica*; (3) conifer forests of *Pinus nigra* subsp. *salzmannii*, which are usually secondary and replace the deciduous oak forests in the lower and southern regions (Folch 1981), but are probably natural here (Bolòs et al. 2004); and (4) higher-elevation forests of *Pinus sylvestris*, which mark the transition between Sub-Montane and Montane belts. Portions of these conifer woods were likely planted. *Pinus* forests are monospecific with a poorly developed understory, whereas oak woodlands are more taxonomically diverse.

The evergreen *Q. rotundifolia* communities have a well-developed understory with several shrubs (*Q. coccifera*, *Rhamnus* spp., *Prunus spinosa*, *Buxus sempervirens* and *Lonicera japonica*) and an herbaceous stratum with nemoral (shade-adapted) species such as *Rubia peregrina*, *Teucrium chamaedris*, *Asparagus acutifolius* and *Brachypodium retusum*. The *Q. pubescens*-*Q. subpyrenaica* deciduous forests include a variety of other trees in the arboreal

stratum, notably *Pinus sylvestris*, *Fagus sylvatica*, *Tilia cordata* and some *Acer* species. The understory is dominated by *Buxus sempervirens*, *Coronilla emerus*, *Amelanchier ovalis*, *Colutea arborescens*, *Cytisophilum sessilifolium* and *Vuburnum lantana*. In the herbaceous stratum, the most common species are *Primula veris*, *Hepatica nobilis*, *Brachypodium phoenicoides* and *Campanula persicifolia*. There are two main types of regional shrubland: one dominated by *Amelanchier ovalis*, *Buxus sempervirens* and *Rhamnus saxatilis*, and another dominated by *Arctostaphilos uva-ursi* with *Buxus sempervirens*. Herbaceous communities primarily consist of meadows and pastures of *Aphyllanthes monspelliensis* and *Arrhenatherum elatius*, herbaceous cereal crops (*Hordeum* sp., *Avena sativa*, *Triticum* sp., *Secale cereale*) and some forage plants (*Medicago sativa*), with several weeds (*Lolium rigidum*, *Papaver rhoeas*, *Polygonum aiculare*, *Bromus* sp.). Abandoned croplands, colonized by shrubs and ruderal species, and badlands devoid of vegetation or with scattered shrubs and herbs from other communities, also occur in some areas (Carreras et al. 2005-2006) (Fig. 3.2).

The local vegetation around the lake is closely tied to microclimate conditions and is fairly diverse in comparison to surrounding regional patterns. A recent detailed study by Mercadé et al. (2013) recognized 534 species of vascular plants distributed across 52 vegetation units of the European CORINE biotope classification (Vigo et al. 2005-2008). Aquatic and semi-aquatic habitats are represented by submerged vegetation, sometimes with floating leaves (*Potamogeton*, *Myriophyllum*, *Chara*, *Utricularia*, *Ranunculus*), and a macrophyte fringe surrounding the lake that is dominated by the grass *Phragmites*, with *Cladium* and *Typha*. Hygrophilous vegetation grows on wet or inundated soils. The most common vegetation consists of sedges, such as *Carex*, *Eleocharis* and *Scirpus*. *Juncus* species, together with *Cyperus* and *Plantago*, dominate the plant communities on temporarily flooded soils. Several types of meadows and pastures around the lake are characterized by *Arrhenatherum* (hay meadows), *Trifolium* (calcicolous grasslands), *Filipendula* (mesophilous grasslands), *Festuca* and *Aphyllanthes* (xerophilous grasslands) and *Poa* (dwarf grasslands). Shrubby vegetation is primarily represented by communities dominated by *Buxus*, *Genista* and *Thymus*. Forest formations around the lake are dominated by various *Quercus* species and small stands of *Fraxinus*, *Populus* and *Salix*. Anthropogenic habitats consist of intensive pastures, crop fields (*Hordeum*, *Medicago*, *Chenopodium*), fruit-tree orchards (*Malus*, *Prunus*, *Pyrus*) and ruderal communities (*Arctium*, *Artemisia*, *Galium*, *Pastinaca*, *Urtica*, *Melilotus*, *Polygonum*). A very detailed description of the vegetation around the lake can be found in Mercadé et al. (2013).

The region is rather densely populated and the lake has historically been an important water source for the numerous surrounding villages and farmhouses. An artificial pond was recently built to use the water from the lake for fire-fighting purposes. Cultivation (wheat, oat, barely, olives, rye, hemp and legumes) and livestock husbandry (cattle and sheep) have also increased in the area during the last millennium and possibly longer (Rull and Vegas-Vilarrúbia 2015). Currently, cereal and alfalfa fields, intermingled with pastures for cattle and horses, and hay meadows, are common and heavily exploited. The lake and its catchment area are part of the European Natura 2000 network for the protection of species and habitats (http://ec.europa.eu/environment/nature/natura2000/index_en.htm).

In Lake Montcortès, varved sediments extend down to a depth of 543 cm, which encompass the last 1548 years. Varves are thin (1.16 mm average thickness) and are composed of two (three) layers, intermingled with occasional turbidites (6.8 mm average thickness). The basic varve unit is a biogenic couplet of two layers, a white calcite layer and a brownish organic layer (Fig. 3.1). A third, grayish detrital layer may be present between the calcite and organic layers. According to Corella et al. (2012), the white layer corresponds to spring/summer and is characterized by rhombohedral calcite crystals, whereas sediments of the brownish layer correspond to fall/winter and are primarily composed of amorphous organic matter, diatoms, detrital carbonate and quartz grains within a clayey matrix. The grayish detrital layer is deposited during phases of increased runoff and consists of irregularly shaped detrital calcite, quartz and feldspar grains, terrestrial plant remains and clay minerals (Corella et al. 2012). Detailed limnological study of the lake dynamics in relation to varve formation and preservation is in progress.

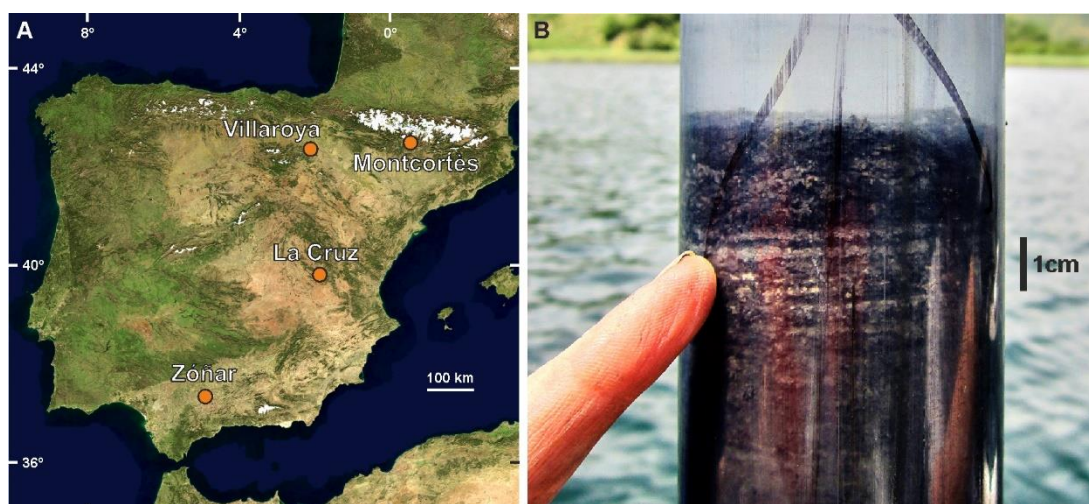


Figure.3.1 - A) Map of the Iberian Peninsula showing lakes with varved sediments that have been studied to date. B) Close-up of the top of a gravity sediment core retrieved from Lake Montcortès in 2013 that shows the varves of the previous years, with a clear alternation of white (spring/summer) and brownish (fall/winter) layers (see text)

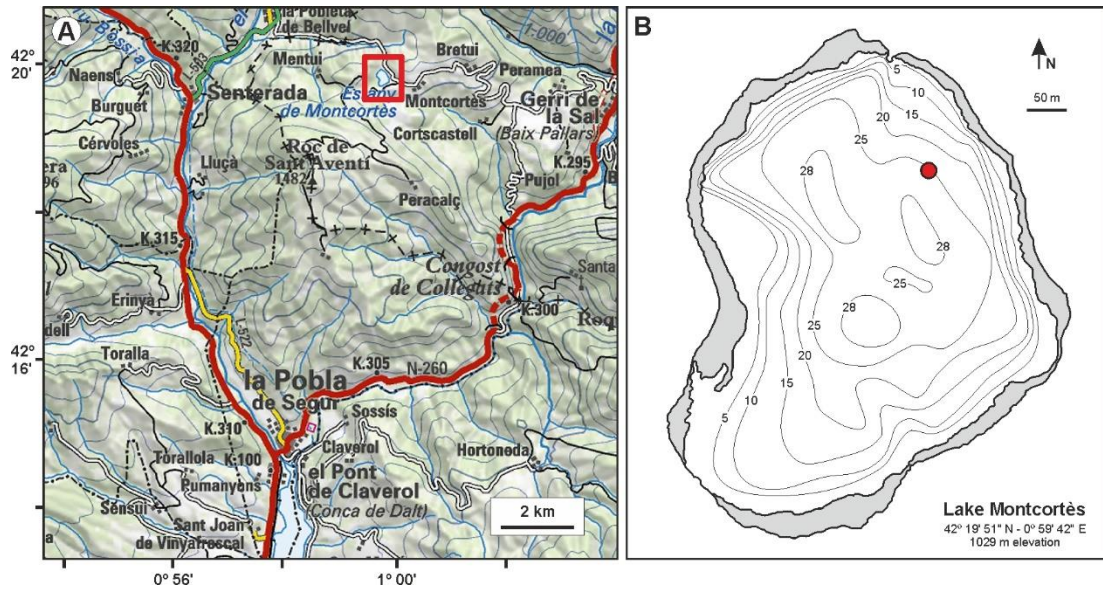


Figure.3.2 - A) Map of the region around Lake Montcortès, indicating the location of the lake (red box) and the meteorological station used in this study (La Poble de Segur). Base map: Institut Cartogràfic I Geològic de Catalunya (www.icc.cat). B) Bathymetry of Lake Montcortès with the location of sediment traps (red dot)

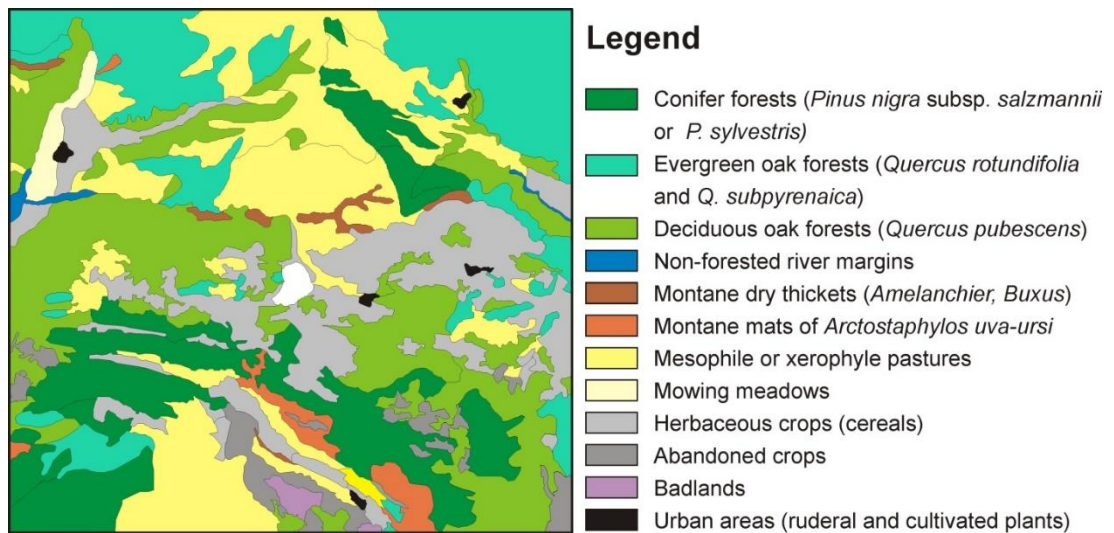


Figure.3.3 - Regional vegetation map of Montcortès using the CORINE system (CEC 1991). The lake is represented as a white area in the center. Modified from Ninot (2006) and Ferré and Carrillo (2007)

3.2- Materials and methods

A set of sediment traps was suspended from a floating platform located above the deepest part of the lake, where the sediment cores were collected for paleoecological study. Each trap consisted of an opaque cylindrical plastic tube (8.5 cm diameter and 80 cm long), with the top open and the base sealed, and was suspended by a plastic line at a water depth of 20 m, i.e. ~5 m above the bottom. The content of the pollen traps was collected quarterly at the end of each season: March (winter), June (spring), September (summer) and December (fall). Sediments were allowed to settle in the laboratory for a minimum of 48 hours. The supernatant was decanted and filtered through a glass-fiber filter, which was added to the sediment to minimize losses. A tablet of exotic *Lycopodium* spores (batch no. 1031, 20,848 spores/tablet, on average) was added to the sediment prior to chemical processing, which included acid digestion (HCl and HF), acetolysis and storage in glycerine (Bennett and Willis 2002). Microscope slides were prepared with glycerine, without sealing. Pollen identification was made following previous, lower-resolution studies (Rull and Vegas-Vilarrúbia 2014, 2015; Rull et al. 2011). Pollen was counted until a minimum of 300 pollen grains per sample had been enumerated—excluding *Pinus* and *Quercus*, which were super-abundant (~40-90% of the total counts)—and the diversity of the sample was saturated (Rull 1987). Total pollen counts averaged 729 (range 305-1118) and exotic *Lycopodium* counts averaged 70 (range 23-171). Taxonomic classification of plants and the grouping of pollen into vegetation types followed Mercadé et al. (2013). Diagram plotting and statistical analyses were performed with Psimpoll 2.7 and MVSP 3.22, respectively.

The meteorological variables used in this study were obtained from the nearest weather station, La Pobla de Segur, which is located ~9 km to the south (Fig. 3.2) at 513 m elevation. The meteorological variables considered were average, maximum and minimum temperature (T_m , T_x , T_n , respectively, in °C); average, maximum and minimum relative humidity (H_m , H_x , H_n , respectively, in %); average, maximum and minimum pressure (P_m , P_x , P_n , respectively, in hPa); total precipitation (PPT; in mm); wind velocity (W ; in $m\ s^{-1}$) and wind direction (W_d ; in °) at 10 m above the ground. Seasonal values for these variables were obtained by averaging daily values for each season, using raw meteorological measures from the reference station.

Cluster analysis was used to identify seasonal pollen assemblages. In this case, we used the Gower similarity coefficient and the centroid clustering method, which have proven

to be suitable for similar purposes using pollen data (Rull 2001, 2003). Spearman rank correlation coefficient was used to study relationships between pollen and meteorological variables. This non-parametric correlation method is recommended when the requirements for using the parametric Pearson product-moment correlation coefficient are not met (Siegel and Castellan 1988). In our case, we used the Spearman index because of the low sample size ($n=9$). Canonical correspondence analysis (CCA) was used to define new multi-dimensional variables that account for maximum variance in the dataset, and to graphically display pollen and meteorological data simultaneously in the space of these new variables (Jongman et al. 1995). All statistical analyses were carried out on percentage data using MVSP version 3.22.

3.3- Results

Total pollen sedimentation displays a clear seasonal pattern, with maxima during the spring/summer and minima during the fall and winter (Fig. 3.4). The two years studied had similar patterns except for the dramatic maximum recorded in the spring of 2015, with values more than three times higher than in the spring of 2014. The major components of the pollen assemblages were *Pinus* and *Quercus*, with their percentages oscillating between 15% and 35%, respectively, throughout the year, except during the spring, which is the flowering season of both taxa, when *Pinus* increased to 50-65%. In the case of *Quercus*, the seasonal pattern is less apparent, and the spring percentages were below 30% (Fig. 4.5). In both cases, the supply of pollen to the sediment traps was continuous throughout the year, although parent plants were no longer in bloom. This phenomenon, called pollen sedimentation lag (PSL), is better assessed using influx values (Fig. 4.6). A large fraction of *Pinus* and *Quercus* pollen settled onto the sediments during the spring, but a significant portion of pollen settled later, particularly between summer and winter. This negative exponential trend occurred in both years, with lower decreasing rates in 2014 than in 2015. In both cases, however, summer to winter values were very similar, indicating that, no matter the intensity of the spring peak, the background signal for the rest of the year was almost the same.

The most important pollen taxa, in terms of abundance and seasonality (Fig. 4.7) illustrate that trees tend to bloom before (winter/spring) herbs (spring/fall), a trend that is also reflected in patterns of pollen sedimentation. In general, percentage pollen peaks coincide with the flowering season of each taxon, but almost all plant species exhibited PSL, expressed by the presence of pollen outside the flowering season. Taxa with a lower PSL, that is, with pollen sedimentation patterns that are very similar to flowering patterns, such

as *Corylus*, *Fraxinus*, and *Artemisia*, are all represented by a single species in the lake (Mercadé et al. 2013). The genus *Olea* had the highest PSL. On the other hand, *Plantago* and *Chenopodium* had an intermediate PSL. Possible presence of several species from the adjacent flora with similar pollen morphology, but with different flowering seasons, might also explain these patterns. In families such as Poaceae and Cyperaceae, which include many genera and species, this is certainly the case. In general, aside from *Pinus* and *Quercus*, the most abundant pollen type belongs to *Cannabis*, especially during the fall, when it reaches values of 40% or more. Cluster analysis yielded two groups that represent two distinct pollen assemblages, the spring/summer assemblage and the fall/winter assemblage (Fig. 4.8). The only exception was the sample from Fall 2014, which was more similar to the spring/summer samples.

Regarding the relationship between pollen and meteorological variables, a preliminary visual inspection showed that the influx of total pollen and of pollen from major types (i.e. *Pinus* and *Quercus*) roughly matched seasonal trends in temperature and precipitation (Fig. 4.9). Pollen maxima occurred during the flowering season of the involved taxa (spring), one season before temperature and precipitation maxima (summer). The relationship between pollen and relative humidity was inverse. Moreover, maxima of pollen influx coincided with moderate wind velocities with a predominant SSE (~150°) direction, whereas pollen minima coincided with slower winds with a WSW (~250°) direction.

Individually, a number of pollen taxa exhibited significant correlations with meteorological variables, whereas others did not (Table 3.1). Some of the relationships are worthy of mention. The variables with greatest significant correlations were wind velocity, wind direction and relative humidity, whereas pressure did not show a significant correlation. Pollen taxa that lacked significant correlations with meteorological variables were *Alnus*, *Artemisia*, *Buxus*, *Cerealia*, *Corylus*, *Plantago* and Poaceae (others). *Olea*, *Pinus*, *Quercus* and Cyperaceae (others) were negatively associated with relative humidity and wind direction and positively correlated with wind velocity. *Chenopodium* and *Juniperus/Cupressus* were correlated with temperature (together with *Pinus*) and total precipitation. *Cannabis* was correlated only with wind velocity.

A synthetic analysis was conducted using Canonical Correspondence Analysis (CCA). Fig. 4.10 shows the scatter plot with the first two axes, which accounted for 70.74% of the total variance. The strongest gradient corresponds to axis 1 (56.80% of the total variance), which was highly correlated with relative humidity and pressure with its positive values, and with wind velocity with its negative values. Along this gradient, samples were ordered

according to a seasonal gradient from spring (left) to winter (right), with summer and fall occupying intermediate positions. Pollen taxa were also ordered according to the same gradient: *Pinus*, *Quercus* and *Fraxinus* were close to spring samples and *Juniperus/Cupressus*, *Corylus*, *Cannabis* and *Artemisia* were located in the fall-winter cluster. The remaining taxa occupied an intermediate position. The spring group was highly correlated with temperature, precipitation and wind velocity. The fall group was also highly correlated with wind direction, which in this season comes from the WSW. This wind direction contrasts with the winds during the spring/summer, which blow from the SSE. Taxa most associated with WSW winds were *Juniperus* and *Cannabis*, which are typically fall taxa.

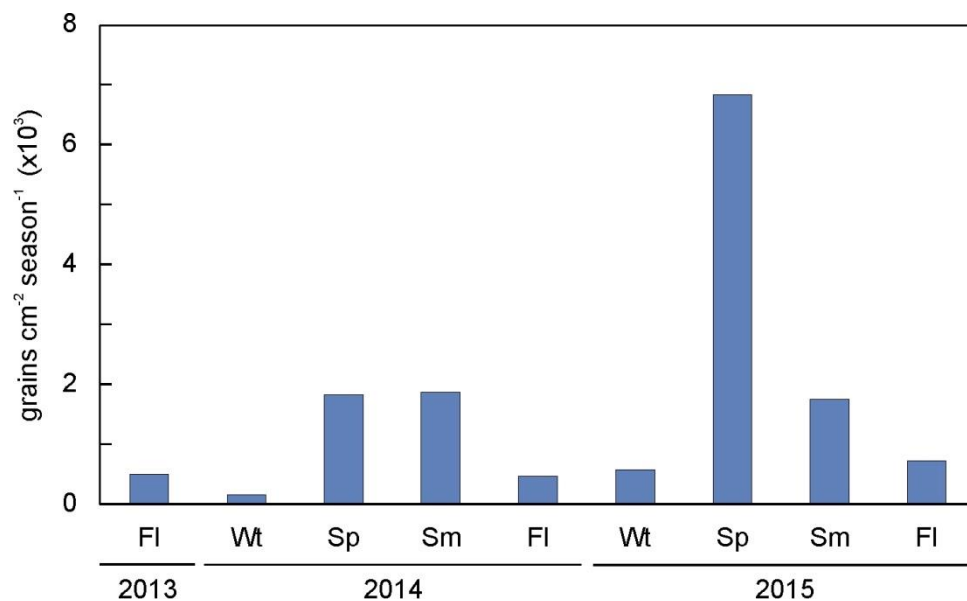


Figure.3.4 - Seasonal trends of total pollen sedimentation expressed as influx units (number of grains per cm² per season). Sp – spring, Sm – summer, FI – fall, Wt – winter

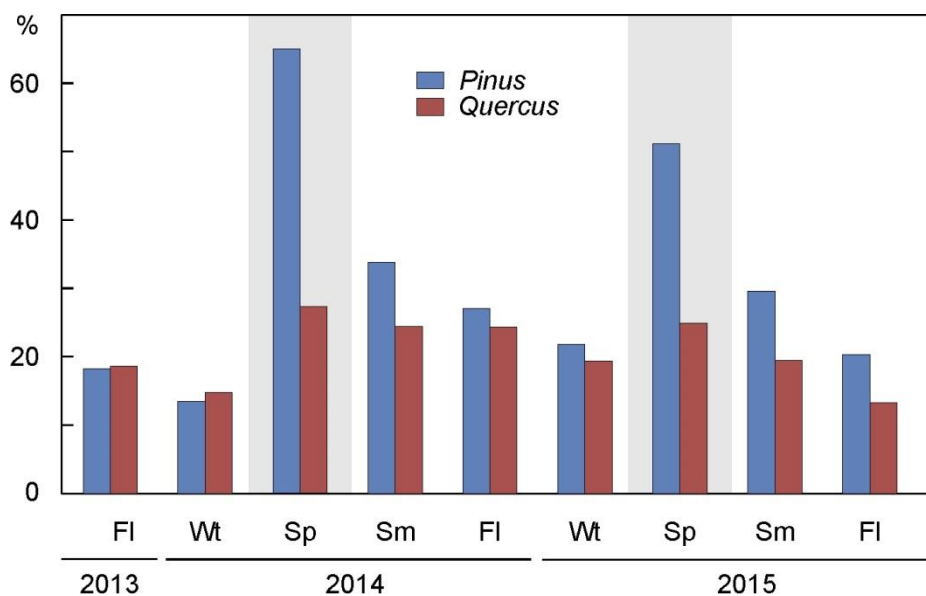


Figure.3.5 - Pollen percentages of *Pinus* and *Quercus*, the two major components of the pollen assemblages, throughout the year. Grey bands represent the flowering season of *Pinus* and *Quercus* species present in the Montocrtès region, according to Bolòs et al. (2000). Sp – spring, Sm – summer, FI – fall, Wt – winter

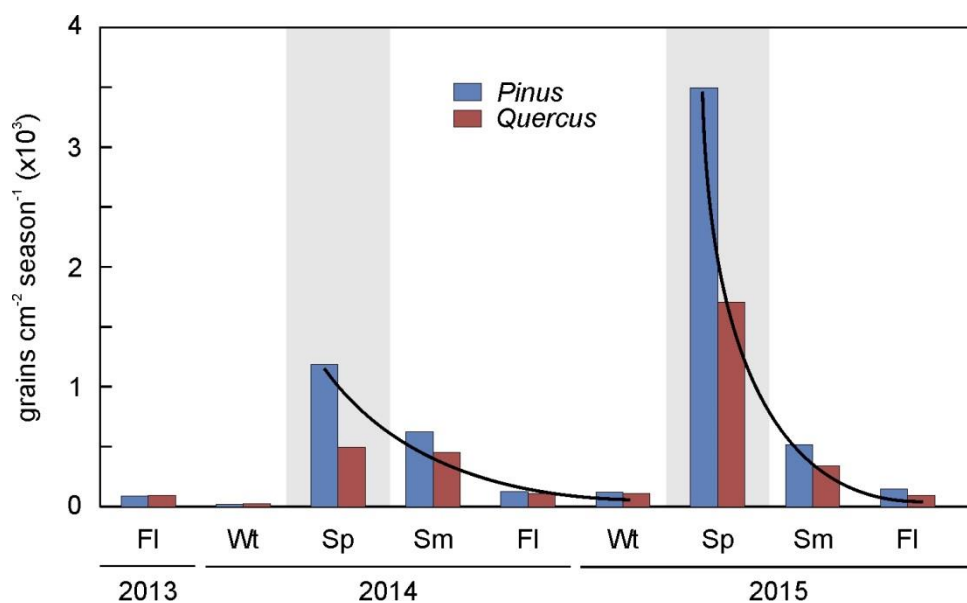


Figure.3.6 - Pollen influx values for Pinus and Quercus during the study period

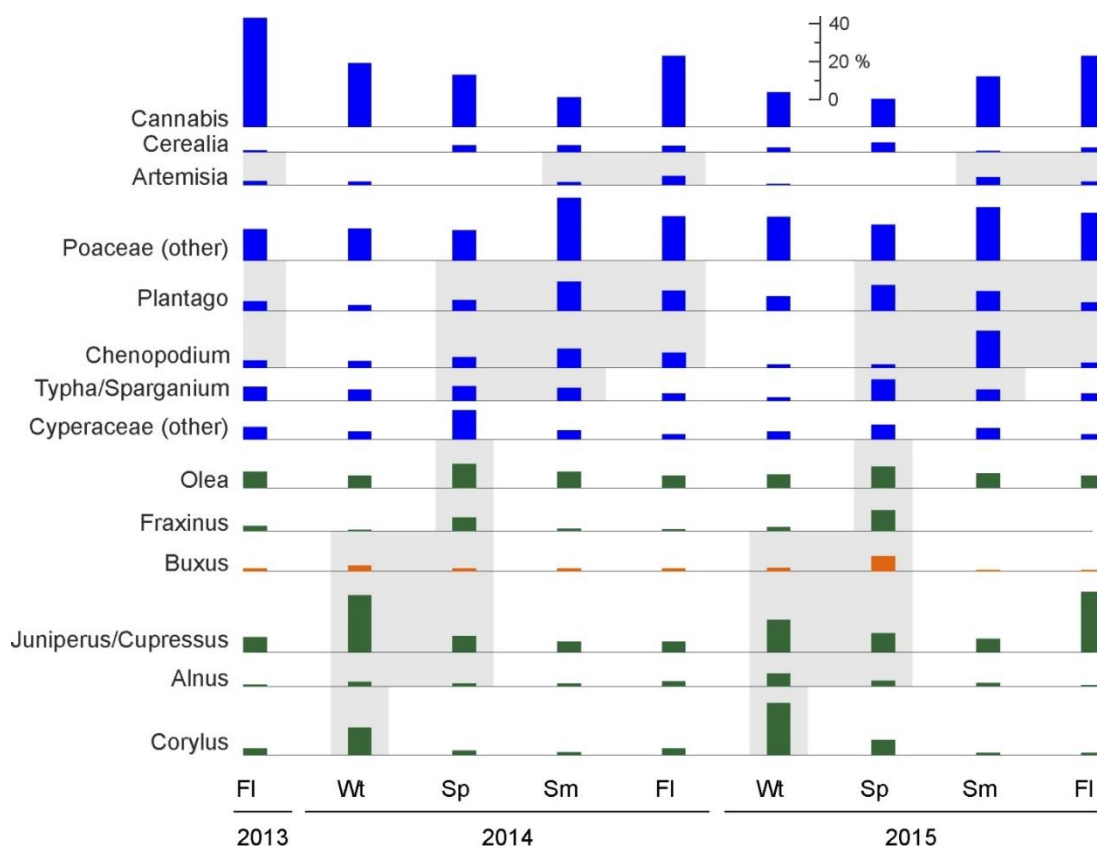


Figure.3.7 - Percentage diagram of the most relevant pollen taxa during the study period. Percentages were calculated, excluding the super-abundant Pinus and Quercus (Fig. 5). Taxa are ordered by their respective flowering seasons (grey bands) (Bolòs et al., 2000), from bottom to top and from left to right. The flowering season of all species of the different genera present in the Montcortès region (Marcadé et al., 2012) was considered. 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. Sp – spring, Sm – summer, FI – fall, Wt – winter

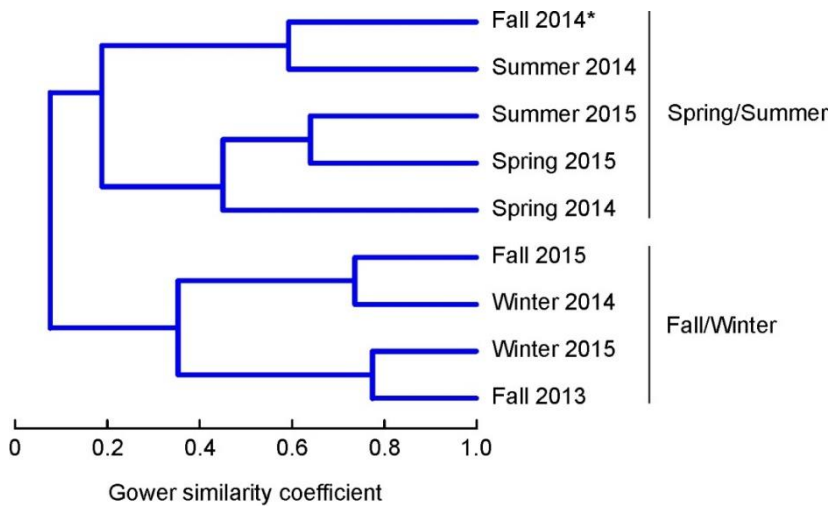


Figure.3.8 - Cluster analysis using the Gower (1971) similarity coefficient and the centroid clustering method. The asterisk indicates the only sample that does not follow the spring/summer vs. fall/winter pattern

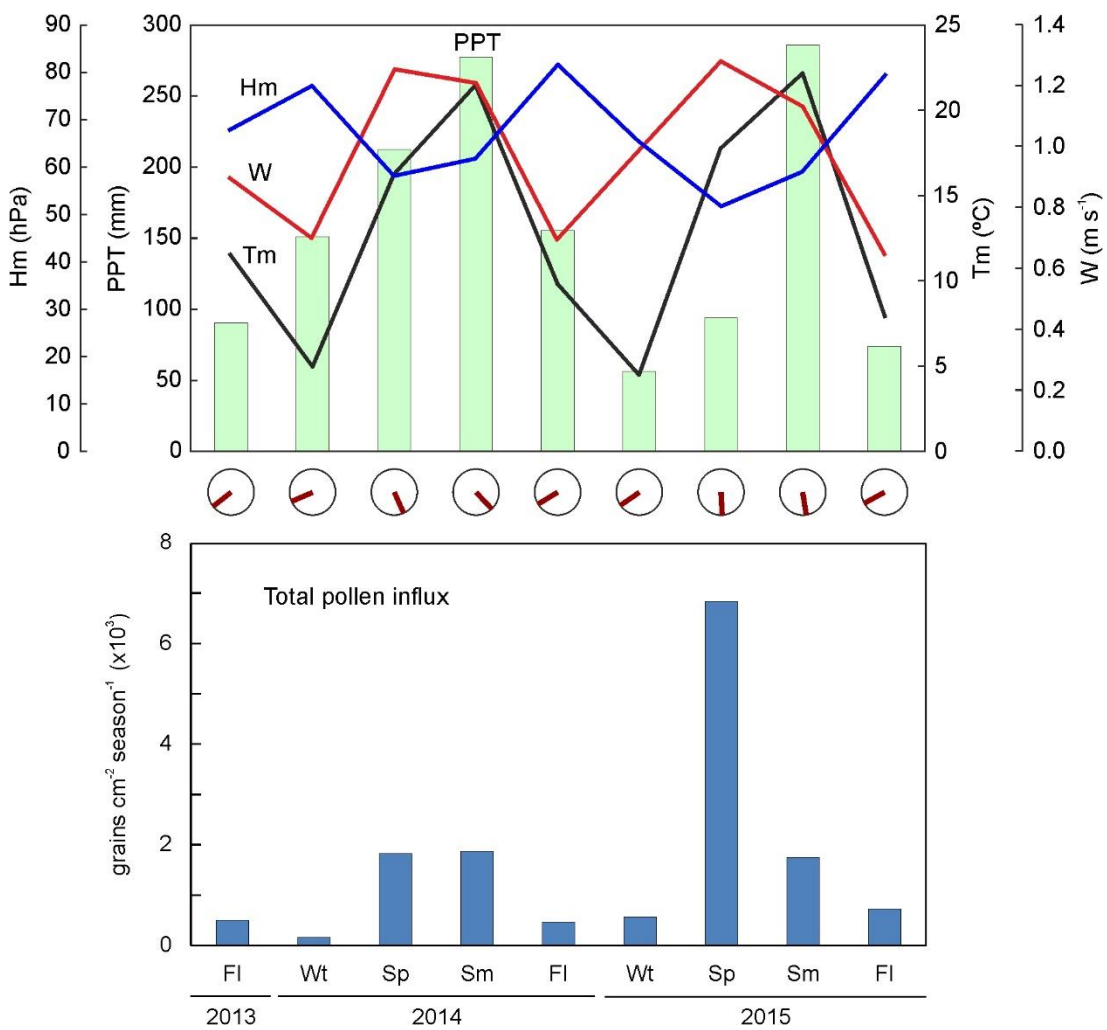


Figure.3.9 - Relationships between pollen influx and the most relevant meteorological variables. Average temperature (Tm), relative Humidity (Hm) and wind velocity (W) are represented by lines. Total precipitation (PPT) is represented by bars. The predominant direction of the wind (Wd) is shown in circles

Pollen taxa	Tm	Hm	Pm	PPT	W	Wd
<i>Alnus</i>	-0.200	-0.167	-0.217	-0.100	0.250	0.117
<i>Artemisia</i>	-0.008	0.661	0.025	0.226	-0.661	0.368
<i>Buxus</i>	-0.150	-0.383	-0.417	-0.100	0.500	-0.033
<i>Cannabis</i>	-0.383	0.750	0.150	-0.200	-0.767*	0.533
Cerealia	0.300	-0.500	0.000	0.067	0.617	-0.533
<i>Chenopodium</i>	0.683*	-0.033	-0.417	0.900**	0.083	-0.500
<i>Corylus</i>	-0.533	-0.033	-0.167	-0.467	0.117	0.317
Cyperaceae (others)	0.567	-0.883**	-0.017	0.233	0.850**	-0.717*
<i>Fraxinus</i>	0.117	-0.577	0.017	-0.176	0.678*	-0.427
<i>Juniperus/Cupressus</i>	-0.667*	0.150	0.267	-0.700*	-0.300	0.650
<i>Olea</i>	0.650	-0.850**	0.050	0.267	0.867**	-0.867**
<i>Pinus</i>	0.667*	-0.750*	-0.200	0.517	0.800**	-0.800**
<i>Plantago</i>	0.617	-0.433	-0.050	0.433	0.567	-0.650
Poaceae (others)	0.317	0.150	0.133	0.300	-0.150	-0.150
<i>Quercus</i>	0.600	-0.700*	-0.367	0.533	0.817**	-0.767*
<i>Typha/Sparganium</i>	0.567	-0.650	-0.183	0.333	0.700*	-0.533

Table.3.1- Spearman-rank correlation coefficients between the most relevant pollen taxa and meteorological variables. See methods for abbreviations. Significant correlations (* $\alpha = 0.05$, ** $\alpha = 0.01$) are in bold. Tm – Average temperature, Hm – Average relative humidity, Pm – Average pressure, PPT – Total precipitation, W – Average wind velocity, Wd – Predominant wind direction

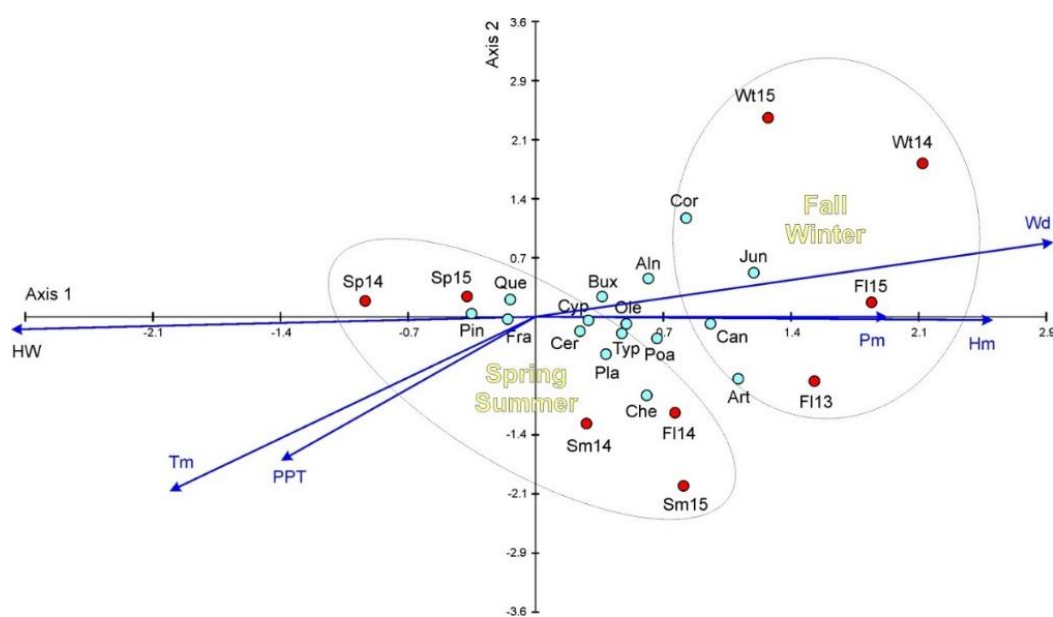


Figure.3.10 - CCA biplot using the scores of the first two axes accounting for 70.74% of the variance. Samples are represented by red dots and pollen taxa are represented by blue dots

3.4- Discussion

In general, our results show a distinct seasonal pattern in pollen sedimentation that is reflected in both total influx and taxonomic composition. In the spring/summer assemblage, *Pinus* had values of 50-65% of the total counts and *Quercus* had approximately 25%, whereas in the fall/winter, *Pinus* was below 30% and *Quercus* was lower than 20% (Fig. 4.5). Regarding the other plant species, the most significant differences were in *Plantago*, *Chenopodium*, *Typha/Sparganium*, Cyperaceae, *Fraxinus* and *Juniperus/Cupressus*, which were more abundant in the spring/summer assemblage, and *Cannabis* and *Corylus*, which were more abundant in the fall/winter assemblage (Table 3.2). This seasonal pattern appears to coincide with the varved pattern of the sediments, which are formed by two-layered couplets that correspond to the same seasons, as interpreted by Corella et al. (2012). Such a correspondence could be demonstrated by analyzing individual sediment layers, which may show that the spring/summer pollen assemblage coincides with the white layer and that the fall/winter assemblage coincides with the dark layer. Physico-chemical analyses of the bulk material collected in the other traps used in this study are in progress and can provide additional evidence for this correlation between pollen seasonality and the formation of white and dark seasonal sediment layers.

Pollen taxa	Spring/summer	Fall/winter
<i>Cannabis</i>	16.02	27.95
Cerealia	2.36	1.05
<i>Artemisia</i>	1.06	1.19
Poaceae	18.39	15.53
<i>Plantago</i>	8.66	3.92
<i>Chenopodium</i>	7.08	2.31
<i>Typha/Sparganium</i>	6.03	3.64
Cyperaceae	6.48	3.38
<i>Olea</i>	7.67	5.58
<i>Fraxinus</i>	3.57	0.95
<i>Buxus</i>	2.14	1.27
<i>Juniperus/Cupressus</i>	5.83	1.65
<i>Alnus</i>	1.44	2.00
<i>Corylus</i>	2.39	8.82

Table.3.2- . Composition of the pollen assemblages obtained in the cluster analysis (Fig. 7), using the average percentages of major pollen types (Fig. 6), excluding *Pinus* and *Quercus*

Specific aspects of the pollen sedimentation require further discussion. For example, there is a lag in pollen sedimentation (PSL), i.e. between production and deposition, throughout the year. The cause of this lag might be manifold. First, such lags may be explained by water dynamics in the lake (Punning et al. 2003). Second, PSL may arise because

of re-suspension of sediments from the uppermost layers (Mieszczankin 1997; Mieszczankin and Noryskiewicz 2000). Third, PSL may stem from the fact that pollen deposited on catchment soils during the flowering season can be washed into the lake for several months (St. Jacques et al. 2008). The first potential explanation (internal water dynamics) is currently under study. This additional study should shed some light on the potential mechanics of PSL via thermal and other density stratification. Resuspension can be identified and measured using sediment traps at different depths, in combination with aerobiological samplers at the lake surface (Bloesch 1994; Mieszczankin and Noryskiewicz 2000; Giesecke and Fontana 2008). The same combination of techniques, along with aerobiological samplers distributed across the catchment soils, might be useful for distinguishing the different processes that participate in pollen dispersal and could provide insights into the potential role of pollen washing into the lake.

The similarity in the sedimentation patterns between *Pinus* and *Quercus* pollen is also striking because the pollen grains of these two genera are different morphologically. *Pinus* pollen is inaperturate and bears two large empty sacchi, which confer unique “buoyancy” to this pollen in air. On the other hand, *Quercus* pollen is tricolporate/tricolporoidate (Erdtman 1952) and has no distinct morphological traits or ornamentation. In spite of these differences with respect to air suspension, once the pollen is in the waters of Lake Montcortès, the sedimentation of the pollen of *Pinus* and *Quercus* was quite similar, even during summer when the lake is thermal stratification is very stable. This finding could suggest that internal lake dynamics are not as important for pollen sedimentation as resuspension or catchment runoff. Aerobiological studies, however, are needed to assess this hypothesis.

Pollen of *Cannabis* (hemp) was the most abundant during the fall; however, the parent plant was not reported in an intensive floristic study of the lake catchment (Mercadé et al. 2013), or in regional surveys (Carreras et al. 2005-2006). The pollen of *Cannabis* is similar to *Humulus*; however, the criteria that distinguish them in the Montcortès sediments have already been established (Rull and Vegas-Vilarrúbia 2014). *Cannabis* is a cultivated plant whose pollen has been present and fairly abundant around Montcortès for the last 1200 years. The exact source of the pollen, however, has been impossible to locate (Rull and Vegas-Vilarrúbia 2015; Rull et al. 2011). This plant is known to have been cultivated in the adjacent lowlands (Gerri de la Sal, La Pobla de Segur and La Pobleta de Bellvé; Fig. 4.2) during the 19th century. In addition, Lake Montcortès may have been used for hemp retting, especially between the 15th and 18th centuries, but no historical documents have been found

to support this hypothesis. Currently, the source for the pollen of *Cannabis* is unknown. More studies will be required to identify the source of *Cannabis* pollen. The same is true for *Humulus*, which is very scarce in the wild and has only been observed near Gerri de la Sal (A. Mercadé, pers. commun. 28 April 2016).

The overall pollen influx patterns are consistent with the fact that, in anemophilous species, high temperature, low humidity and moderate winds favor passive flower dehydration, thereby facilitating the opening and release of pollen from the anthers (Helbig et al. 2004). These meteorological variables, however, do not provide a clear explanation for the difference in intensity of the spring pollen peaks of 2014 and 2015. Although temperature and wind velocity show almost identical patterns across 2014 and 2015, precipitation and relative humidity do not. Indeed, precipitation was significantly higher before the spring of 2014 than in 2015, whereas relative humidity was lower in the spring of 2015. These differences might have affected the release of pollen, but this hypothesis remains speculative until more local aerobiological studies are conducted. Slight differences in the location of the pollen sources cannot be dismissed, as there was a slight variation in the direction of the predominant winds between the springs of 2014 and 2015.

Individual correlations also deserve further comment, especially in the case of relative humidity, wind direction and velocity, which primarily affected *Olea*, *Pinus* and *Quercus*. Cyperaceae will not be discussed here as it may contain several species with different flowering periods and pollen dispersion/sedimentation features. *Olea* is a lowland taxon that is not common around Montcortès, which is located near the boundary of lowland and montane biomes (Rull et al. 2011; Mercadé et al. 2013). In a previous study in the central Pyrenees, Cañellas-Boltà et al. (2009) found that *Olea* pollen occurred consistently from the lowlands to the alpine zone above 2500 m elevation. The authors attributed this distribution of pollen to the effect of upward winds. This explanation is supported by our results from Montcortès, which show that dry and windy conditions favor the sedimentation of this pollen type in lake sediments. In addition, the significant negative correlation with wind direction, expressed in degrees, indicates that the source for this pollen should be from the SW (~225°), that is, in the southern lowlands, where the species grows. The same is true for the pollen of *Pinus* and *Quercus*, forests of which are better represented in the southern part of the area under study (Fig. 4.2).

The CCA plot (Fig. 4.10) yielded the same groups as the cluster analysis, which strengthened the seasonal character of the pollen succession throughout the year and showed the clear separation of the spring/summer and the fall/winter assemblages. This

analysis also provided the more relevant meteorological variables linked to seasonal pollen sedimentation as a whole. The main environmental gradient resulted from the windy, rainy and warmer character of the spring/summer seasons, with winds from the SSE, and the high pressure and high relative humidity of the fall/winter seasons, with winds from the WSW. This gradient was strongly associated with the abundance of the main pollen taxa that are characteristic of each seasonal assemblage.

3.5- Conclusions

General patterns of pollen sedimentation in Lake Montcortès during the two study years were consistent with there being a strong seasonal signal. This signal permitted the spring/summer and fall/winter assemblages to be distinguished. These seasonal differences were expressed in terms of the amount of pollen sedimentation and also in the taxonomic composition of the pollen assemblages. In addition, the main meteorological variables that influence these seasonal features of pollen were identified. Pollen seasonality coincided with the same seasonal patterns previously identified in sedimentological (varve) studies. Therefore, seasonal pollen patterns described in this study appear to adhere to a pollen-varve model that is constrained by meteorology, and which can be extrapolated down-core to be used in high-resolution paleoecological investigations. This finding needs to be corroborated with a detailed palynological analysis of the assumed seasonal sediment layers and with physico-chemical analyses of the bulk content of sediment traps. By analogy, differences in the pollen content of past varves could be explained in terms of meteorological variability, which makes pollen a potentially powerful paleoenvironmental proxy in this particular lake. In addition, pollen analysis of down-core sediments can be used to identify intra-annual seasonal patterns and to date the sediments, even in the absence of varves or at depths where the varve record has been partially disturbed. The seasonal pollen model obtained here can be applied, at least, to the last 1200 years, as all pollen types have been present with reasonably similar abundances (Rull et al. 2011). The seasonal patterns described here are sufficiently well established for use in Lake Montcortès paleoenvironmental studies. Pollen analysis of trap sediments should continue in efforts to account for potential inter-annual variability. As a general observation, the trends and relationships established in this paper should be considered empirical, with some causal relationships yet to be demonstrated. The present study can account for processes that occur after pollen has reached the lake surface. Therefore, our study can account only for factors such as the flowering season of each pollen type and internal lake processes involved

in pollen sedimentation. Other factors such as pollen production, dispersal, diagenesis or other post-depositional phenomena should be addressed with further aerobiological and sedimentological studies. In summary, our data suggest that phenological traits (i.e. flowering season) of the plant taxa involved exert a dominant control on the seasonal patterns of pollen sedimentation and inter-annual meteorological variations cause minor quantitative shifts. Sedimentological processes linked to internal lake dynamics, mainly the mixing-stratification regime and sediment reworking/resuspension, may, however, modify the original expression of biological and meteorological seasonality, and should be taken into account to explain the final pollen sedimentation patterns.

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CHAPTER 4

High-resolution (sub-decadal) pollen analysis of varved sediments from Lake Montcortès (southern Pyrenean flank): A fine-tuned record of landscape dynamics and human impact during the last 500 years.

Soundtrack: “*deep into the forest*”-Michael Nyman

Original publication (*Appendix2 in the supplementary material*):

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Abstract

A high-resolution (6 years/sample) palaeoenvironmental reconstruction using pollen, charcoal and non-pollen palynomorphs was carried out on annually laminated sediments of Lake Montcortès (South-Central Pre-Pyrenean flank). The results were combined with historical data to better understand landscape evolution and human interaction during the last 500 years. Our results show that human activities (cropping, livestock breeding and hemp cultivation and retting) have been the most important factors responsible for vegetation changes with highest intensity between 1530 and 1900 CE. By means of a sub-decadal study we have been able to evaluate short-lasting events at local and regional scales related to climate (heavy rainfall events and, high-land forest fluctuations) or to historical and well-dated and documented socio-economic events (i.e., crop promotions (hemp) or land abandonment-population emigration). The temporal extent (400 years) and continuity of *Cannabis* pollen peaks have been confirmed, and new evidence of water quality changes, likely as a consequence of hemp retting practices between the mid 17th to late 19th century, are provided. This is the first high-resolution palaeoenvironmental study carried out in a varved lake on the Iberian Peninsula so far. With these data we hope to contribute to filling the gap in high-resolution palaeoenvironmental data.

Keywords: Retting; *Cannabis*; human impact; historical data; varves; multiproxy palaeoecology

4.1-Introduction

Lake and peatlands sediments are natural archives that store information on limnological, biological, geochemical and anthropogenic processes occurring in the water body and in the catchment area (Smol et al., 2002; Veski et al., 2005). Understanding the process that leads to the recent evolution of landscapes and discerning between natural and anthropogenic causes is a challenging question that can best be empirically addressed with palaeoecological data. Changes in the spatial structure of a landscape result from natural process such as climate variability and/or soil development combined with human activity driven by socio-economic and cultural factors (Veski et al., 2005). Thus, to fully understand the changes and their drivers, it is necessary to combine data from different sources and disciplines such as archaeology, documentary sources, ecology, palaeoclimatology and palaeoenvironmental data, although such a task may not be easy. For instance, the lack of enough spatial and temporal resolution of the different data sources sometimes does not permit to obtain a complete and accurate image of the past (Jones et al., 2009; Rull et al., 2014; Sadori et al., 2015; Contreas et al., 2018). Archaeology, palaeoclimatology and palaeoenvironmental science often strive to achieve regional and long-term relevance, resulting in a coarse resolution of multi-decadal to multi-millennial scales (Rull et al., 2014; Contreras et al., 2018). In contrast, ecological, and historical data provide more constrained spatial and temporal resolutions (sub-decadal/annual/seasonal) (Rull et al., 2014; Contreras et al., 2018). In palaeoecology, a solution for this issue is to work with varved sediments that allow annual to seasonal time-resolutions or with sediment records with very high sediment accumulation rates (Veski et al., 2005; Ojala et al., 2012; Rull 2014).

Within the sedimentary archive, the last millennium is especially interesting for studying landscape and human environment interactions due to the availability of good quality and well preserved historical records (Dearing 2013; Zolitschka et al., 2015) and because it has been a key period of the development of modern vegetation types and the formation of cultural landscapes (Rull et al., 2011; Wacnik et al., 2016). Furthermore, significant climatic variations occurred in relatively short periods of time (Medieval Climatic Anomaly, Little Ice Age, and the onset of Global Warming) that might drive short-lasting vegetation disturbances only be detectable by high-resolution analyses (Wacnik et al., 2016).

Several high-resolution studies from lake sediments and some from varved records are already available for Europe using both physicochemical and biological proxies. Most of them are focused on palaeoclimate and are aimed to perform quantitative reconstructions (some examples: Feurdian et al., 2008 (pollen); Trachsel et al., 2010 (biogenic silica and

chironomids); Lotter et al., 2012 (chironomid and pollen); de Jong et al., 2013 (chrysophyte stomatocyst). Palaeoenvironmental high-resolution studies are less frequent but equally important as they are the only available tool to evaluate past biodiversity losses or to identify past key periods that can help to set conservation targets and to visualize realistic future scenarios (Ekblom and Gillson, 2017).

For the Iberian Peninsula, a considerable number of pollen records are already available. They are mainly performed at a low resolution and cover several millennia, although some exceptions at moderate resolution and covering the last millennium exist (i.e., Riera et al., 2004; Morellón et al., 2009; Ejarque et al., 2009; Rull et al., 2011, Garcés-Pastor et al., 2016). The available pollen records from the Pyrenean range mostly belong to high altitude lakes and peatlands. High altitude mountain areas have traditionally been viewed as pristine environments with low human population density where more severe climatic conditions might hamper human occupation. But, for the case of the Pyrenees and Pre-Pyrenees, it has been demonstrated that substantial human pressure and considerable exploitation of natural resources have taken place since the Mesolithic: farming, mining, logging and fire impact (Gassiot and Jiménez 2006; Palet et al 2007, Sancho and Planas 2009 Ejarque et al., 2010; Bal et al., 2011; Cunill et al., 2013; Corella et al., 2013,). Therefore, lower altitudes on the Pyrenean -montane stage (ranging from 800 to 1600 m a.s.l.)- which are very favorable for human occupation and consequently sensitive to be higher human impacted, arise as an interesting area to study human occupation history and human-landscape evolution relationships.

Lake Montcortès, which is located in the pre-Pyrenean range (1026 m a.s.l.) is a very suitable place to reconstruct past human-environment relationships. The exceptional scientific value of this lake is due to its strategic location and to the varved nature of its sediments that are ideal for performing high-resolution studies with accurate time control (Corella et al., 2011, 2012). This feature, which is uncommon among Iberian lakes, supports several studies which have demonstrated the potential to reconstruct the ecological dynamics of the lake communities and vegetation dynamics related to climate and human activities at a sub-centennial scale (Scussolini et al., 2011; Rull et al., 2011; Rull and Vegas-Vilarrúbia et al., 2015; Montoya et al., 2018). Reconstructions of past climate and past shifts in oxygenation regime at annual and sub-decadal resolutions have also been performed in this lake (Corella et al., 2014; 2016 and Vegas-Vilarrúbia et al., 2018). Moreover, modern sedimentary analogue studies and a detailed floristic inventory of lake surroundings have been published recently, both, are very useful tools that helped to interpret the sediment

record (Mercadé et al., 2015; Rull et al., 2017; Trapote et al., 2018). Among the key results found, the Lake Montcortès pollen record contains large amounts of hemp (*Cannabis sp.*) (Rull et al., 2011). Historically, *Cannabis* has been a fundamental plant for the development of human societies then, the identification of *Cannabis* pollen is a very useful tool to track and identify human activities and their impacts (van Zant et al., 1979; Mercuri et al., 2015, Peglar 1993).

Here, we present for the first time in the Iberian Peninsula, a high-resolution and continuous palynological reconstruction (6 years/sample on average) carried out in a Pre-Pyrenean varved lake. We perform a multiproxy reconstruction using pollen, charcoal, non-pollen palynomorphs (NPPs) and historical documents for the last 500 years adding unique high-resolution palaeoenvironmental and palaeoecological studies covering modern period. Our main aim is to perform a detailed, and accurate palaeoenvironmental reconstruction to investigate vegetation history, land-use and human impact around Lake Montcortès at the highest detail achieved so far. This study provides new data on historical land-use and management and on the potential use of Lake for hemp retting. Data covering the last century and therefore, the climatic instrumental record, is presented for the first time. Our data, together with the varved nature of the Lake Montcortès record and the already available palaeoenvironmental information for the lake Montcortès, combined with historical and paleoecological data available for the Pyrenees have made possible to perform a thorough picture at local and regional scales of human-vegetation interactions around the lake. The data obtained with this work may contribute to develop and test computational models of interactions between climate, landscape evolution and land-use as well as to constrain and decrease the uncertainties in future environmental projections (Dearing 2006; Hegerl et al., 2006; Dearing et al., 2013).

4.1.2- Study area

Lake Montcortès (42° 19' N; 0° 59' E and 1027 m a.s.l) is a small karstic lake situated on the south-central pre-Pyrenees in the Pallars region (Fig.4.1). It was formed by karstic processes of dissolution and collapse on Triassic evaporates. The lake's catchment is small and is emplaced in Oligocene carbonate conglomerates, Triassic limestones, marls and evaporites (Rosell, 1994). The lake is fed mainly by groundwater and lake level is controlled by an outlet stream located along the northern shore and water evaporation (Corella et al., 2016). It has a maximum water depth of 30 m. According to the nearest meteorological station, total annual mean precipitation is 669 mm, with February being the driest month and May being the wettest. Annual average air temperature is 12.8°C, with maximum and

minimum mean temperatures of 23.3°C (July) and 2.9°C (January) respectively (reference period 1961–1990). Lake alternating meromictic and holomictic conditions as has been demonstrated by Trapote et al. (2018) and Vegas-Vilarrúbia et al. (2018), remaining stratified most of the year and mixing during winter. It is an oligotrophic lake with very low nutrient content particularly for phosphorous, well buffered waters and maximum phytoplankton productivity occurring during late summer and early autumn (see Trapote et al. (2018) for more detail).

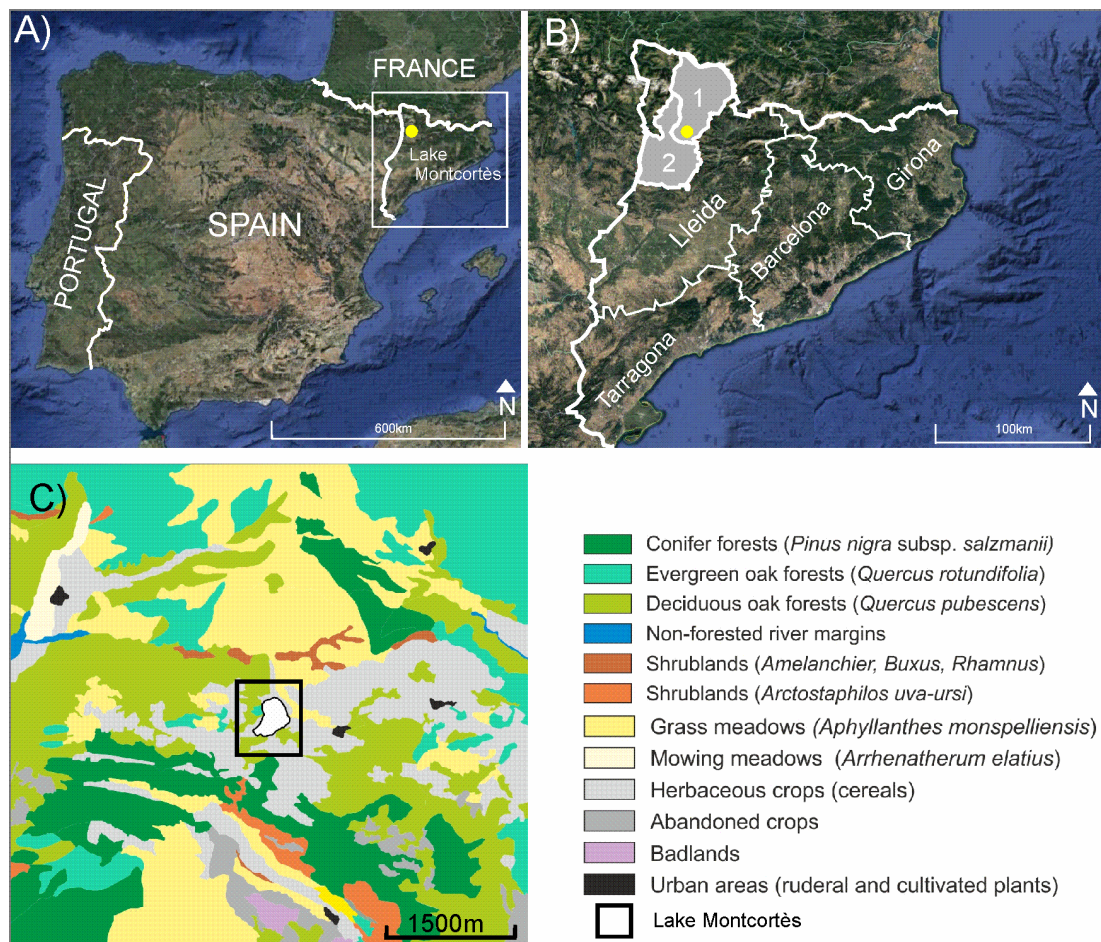


Figure.3.1- A) Location of Catalonia (squared area) and Lake Montcortès (yellow point). B) Catalonia map with its provinces and Pallars region (green shaded areas): Numbers refer to two different administrative boundaries within the Pallars region: 1- Pallars Sobirà (Montcortès location) and 2- Pallars Jussà. C) Vegetation map modified from Rull et al., 2015.

The lake lies near the altitudinal boundary corresponding to the sub-montane belt, which in the Pyrenees is situated around 800-1000 m a.s.l. elevation, depending on local conditions (Vigo and Ninot 1987). Three major forest formations occur at the lake region reflecting this boundary condition: 1) Evergreen oak forest dominated by *Quercus*

rotundifolia L. (representative of the Mediterranean lowlands); 2) Deciduous oak forest dominated by *Q. pubescens* L. and *Q. pyrenaica* L. (representative of the middle montane belt with higher precipitation); and 3) Conifer forest of *Pinus nigra* L. at lower and southern regions (probably secondary replacing the deciduous oak forest) and *Pinus sylvestris* L. at higher elevations (making the transition between Sub-montane and Montane belt) (Folch, 1981; Rull et al., 2011; Mercadé et al., 2014) (Fig. 4.1A). The lake is surrounded by a dense littoral vegetation belt dominated by *Phragmites*, *Cladium mariscus* L. and *Typha* and, to a lesser extent, represented by *Juncus* and *Scirpus* (Mercadé et al., 2013). Hay meadows, pastures (mostly for cattle and horses) and cereal crops are the most important rural anthropic habitats around the lake. Besides farming, since 1970's the most important human activity around the lake and in the area is rural tourism.

4.2- Methods

4.2.1-- Coring, sampling and Chronology

In July 2013, a 114 cm long sediment core named MONT-0713-G05 was retrieved from the deepest distal lake basin (~30 m water depth) using a UWITEC 60 mm diameter gravity corer. It was kept at the lake shore during 3 days to allow consolidation and then transported to the core repository at the Institute of Earth Sciences Jaume Almera (Spanish Research Council). In previous studies, Corella et al., (2011, 2014) built an age-depth model for the last six centuries, which is based on independent varve counting and ^{210}Pb and ^{14}C radiometric dating. Varve counting was performed on a composite sequence obtained from cores MON12-3A-1G and MON12-2A-1G and by double counting in 14 overlapping thin sections. Less than 1% of varves were interpolated using annual sedimentation rates from well-preserved adjacent varve sections. Further details of this age-depth model are provided in Corella et al., (2014). Stratigraphic correlation between core MON12-3A-1G and MONT-0713-G05 was obtained based on a detailed inspection of sedimentary structures, varve thickness patterns and characteristic features seen in specific varves that allowed the identification of 96 marker horizons (i.e., flood layers and/or distinct sub-layering in calcite layers).

4.2.2- Core sampling, pollen, charcoal and non-pollen palynomorphs (NPPs)

The varved part of MONT-0713-G05 was sampled continuously every 0.5 mm using a syringe to obtain volumetric samples, which was the highest resolution possible that allowed us to obtain enough sedimentary material for pollen analysis. Turbidites were avoided following the sampling procedure described in Corella et al. (2017) since these

sediment-laden layers represent allochthonous material eroded from the lake catchment and deposited within hours/days (Corella et al., 2015). A set of 96 samples were processed using standard palynological methods (Moore et al., 1991; Bennet and Willis, 2001), including KOH, HCl, HF digestions and acetolysis. Two *Lycopodium* tablets (batch n° 483216; 18,583 spores/tablet) were added to each sample before chemical processing. Residues were suspended in liquid glycerine and microscopic slides were mounted in the same medium. Pollen was identified according to Moore et al. (1991) and Reille (1992, 1995, 1998) and following previous Montcortès studies (Rull et al., 2011; Rull and Vegas-Vilarrúbia 2014, 2015). All samples were counted until diversity saturation (Rull, 1987) with a minimum of 300 pollen grains excluding *Cannabis-type pollen*, which was superabundant in some samples (40 - 85% respect to the total terrestrial pollen sum). Algal remains were also identified and counted to genus level. Charcoal particles were counted and classified into two groups based on size: charcoal I (< 100 µm) as indicator of regional fires and charcoal II (between 100-500 µm) as indicator of more local fires (Whitlock and Larson 2001). Fungal spores were identified following van van Geel and Aptroot (2006), van Geel et al. (2011), and López-Vila et al. (2014). The pollen sum included all pollen types except those from aquatic and semi-aquatic taxa: *Cyperaceae*, *Myriophyllum*, *Scirpus*, *Potamogeton* and *Typha-Sparganium*, hereafter referred as *Typha* according to local vegetation surveys (Mercadé et al., 2013). Pollen and spores below 3% of the pollen sum were not shown in the pollen diagram. Pollen accumulation rates (PAR) and charcoal influx in $\text{cm}^{-2} \text{yr}^{-1}$ were calculated. Diagrams were plotted and zoned using the software Psimpoll 4.27 (Bennet 2002) and the method of optimal splitting by information content (OSIC) (Bennett, 1996) considering only pollen types. Percentages for NPPs (algal remains and fungi spores) were referred to the pollen sum. Pollen groups were defined according to the present day vegetation types as previously presented for Montcortès in Rull et al. (2011) and Mercadé et al. (2013). Table 1 presents each group and the corresponding taxa included in it for the sediment record presented in this work.

Vegetation type	Pollen taxa
Conifer forest	<i>Pinus, Abies</i>
Evergreen oak forest	<i>Quercus</i> - evergreen-type
Deciduous oak forest	<i>Cornus, Carpinus, Fagus, Fraxinus, Tilia, Betula, Quercus</i> deciduous-type
Riverine forest	<i>Alnus, Populus, Salix, Ulmus</i>
Shrubs	<i>Buxus, Erica</i> -type, <i>Ilex aquifolium, Juniperus/Cupressus, Phillyrea, Pistacia</i>
Low shrubs	<i>Ephedra, Hedysarum, Helianthemum</i>
Meadows/ pastures	<i>Plantago, Poaceae</i> (others)
Cultivated trees	<i>Corylus, Juglans, Olea, Prunus</i>
Herbaceous crops	Cerealia (others), <i>Secale, Cannabis</i> -type
Ruderal/weeds	<i>Artemisia, Centaurea, Chenopodium, Echium, Rumex, Urtica</i> -type
Other	Apiaceae, Asteraceae (others), Asteraceae (fenestrate), <i>Campanula, Euphorbia, Castanea, Cerastium, Galium, Morus, Potentilla, Sanguisorba minor, Thymus, Veronica</i> -type, <i>Scabiosa, Sedum</i> -type
Aquatic plants	<i>Alisma, Cladium, Thypha, Cyperaceae</i> (others), <i>Mentha</i> -type <i>Myriophyllum, Scirpus, Potamogeton, Ranunculus</i>

Table 4.1 – Pollen groups according to the present day vegetation types, based on Rull et al., 2011

4.3- Results and Interpretation

4.3.1- Age model

The three different lithostratigraphic units previously defined in Corella et al (2014) were also clearly identified in core MON-0713-G05 (Fig.4.2) (unit 1, 0-15 cm , 2013-1902 CE; unit 2, 15-59 cm, 1901-1844 CE; unit 3, 59-100 cm 1844-1423 CE). Several marker horizons have also been detected and correlated between cores (Fig. 4.2). Sedimentation rates (SR) in cores MON12-3A-1G and MON-0713-G05 display similar values except for unit 2, where SR were 26% higher in core MONT-0713-G05 than in core MON12-3A-1G due to the thicker detrital layers deposited during the 19th century.

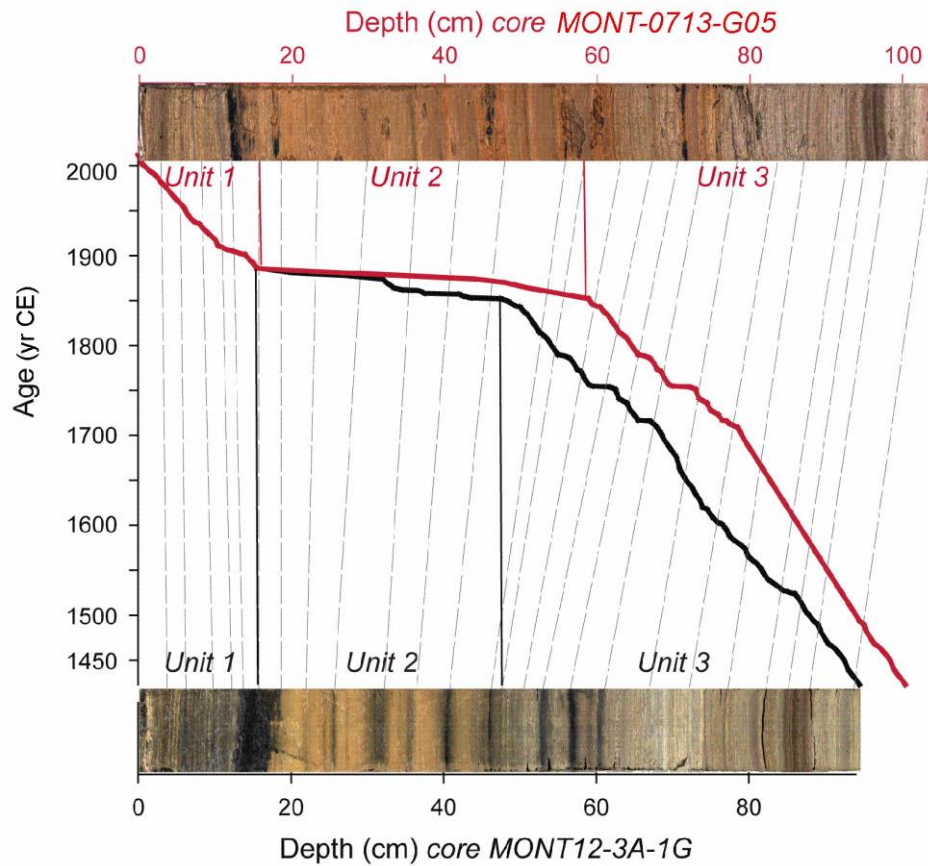


Figure.4.2- Age–depth model for the sediment cores MONT07-13-G05 (present study) and MONT12-3A-1G (Corella et al., 2014) based on varve counting for the last 500 years. Core correlation of the main sedimentary units and marker horizons are also shown.

4.3.2- Vegetation and landscape changes

Results are expressed in both percentage –for pollen and NPPs- (Fig.4.3 and 4.4) and PAR (Fig.4.5). The interpretation, in terms of vegetation shifts, is based on the percentage diagram (including *Cannabis* as a component of the regional landscape), with reference to PAR values to follow the behavior of the more significant taxa and/or vegetation groups. A summary pollen diagram excluding *Cannabis* has been added to assess vegetation changes in zones where hemp pollen attain more than 40% of relative abundance and therefore pollen signal could have been adulterated. Vegetation classification and interpretation of pollen spectra follow previous palynological studies: Cañellas-Boltà et al. (2009), Mercadé et al. (2013); Rull and Vegas-Vilarrúbia (2015) and Rull et al. (2011, 2017). This section is concerned only with vegetation dynamics and the potential processes involved that can be directly inferred from the evidence obtained in this work (pollen, charcoal, algae, and fungi spores). Other aspects needing additional independent evidence, such as the potential

influence of climatic shifts, historical events or comparisons with previous works and similar regional reconstructions, are addressed in the discussion.

Overall, the percentage diagram (Fig.4.3) is dominated by conifers (*Pinus*), evergreen and deciduous oaks (*Quercus*) and herbaceous crops, notably the *Cannabis*-type (thereafter *Cannabis*). These pollen types show significant abundance changes, especially at the base and the top of the diagram. Some autochthonous and cultivated trees (*Betula*, *Olea*), *Juniperus/Cupressus* type (likely corresponding to *Juniperus communis* L., which is abundant in the present vegetation), herbs (mainly *Poaceae*, *Plantago* and *Artemisia*) and aquatic taxa (*Typha*, Cyperaceae other than *Scirpus*) are also well represented and exhibit meaningful shifts throughout the diagram. The sequence has been subdivided into six significant pollen zones, named MC1 to MC6, which are described as follows.

Zone MC1: 100.5 – 95.5 cm, 1423-1481 CE (58 years; 11 samples; average resolution: 5.2 years/sampling interval)

This zone is dominated by trees -notably evergreen *Quercus*, the main representative taxon of the evergreen oak forests- and herbs, mainly *Artemisia* and *Poaceae* (others), belonging to the ruderal/weeds and the meadows/pastures groups, respectively (Fig. 4.3). These two herbaceous groups attain up to 50% of the pollen assemblage in this zone. Other trees (*Pinus*, deciduous *Quercus* and *Olea*), shrubs (*Juniperus*) and herbs (*Plantago*) show intermediate values. Among minor components, *Betula*, *Corylus*, *Fagus* and *Alnus* are below 10% but they attain their maximum abundances as compared to other zones. Trees such as *Quercus* (evergreen), *Pinus* and *Olea* experience an increasing trend while some of the main herbaceous pollen types, notably *Artemisia* and *Plantago*, decrease towards the top of the zone. Aquatic taxa are at their minimum values, with a slight decrease in *Typha*, which almost disappears at the end of the zone. PAR values are very low, showing an increasing trend in all vegetation types except low shrubs, herbaceous crops and aquatic plants (Fig. 4.5). Charcoal I (indicative of regional fires) reaches its lowest values spiked by a conspicuous peak near the base of the zone (99.5 cm; 1434 CE), whereas charcoal II (indicative of fires occurring in a more local scale) is present only in the form of two small peaks. Among algal remains, *Botryococcus* is the most abundant, showing an increasing trend towards the top of the zone that coincides with a small *Tetraedron* peak, which was almost absent before (Fig. 4.4). *Cosmarium* and *Pseudoschizaea* are present only as small and scattered peaks. The most abundant fungal spores are *Sporormiella* and *Glomus*, always below 10% and showing a similar trend between them.

During the time interval represented by this zone (1420 to 1490 CE), the landscape of the Montcortès catchment and its surroundings was characterized by the presence of forests, mostly evergreen oak forests, meadows, pastures and herbaceous crops, with the corresponding ruderal plants and weeds. This was not a static landscape state as oak forests were expanding at the expense of the rest of vegetation types, especially ruderal plants and weeds. Fire incidence was very low, except for a distinct burning event around 1434 CE. The presence of *Sporormiella*, a coprophilous fungi living in the dung of herbivorous animals, suggests the presence of livestock around the lake (van Geel and Aptroot, 2006). The occurrence of *Glomus* indicates that erosion of catchment soils was ongoing (Anderson et al., 1984). *Pseudoschizaea*, that also has been used as indicator of soil erosion (van Geel et al., 1989, 2003), might be indicative of cattle trampling around the lake (Ruiz-Zapata et al., 2006). The whole picture suggests a humanized landscape where anthropogenic impact was declining and wild oak forests were in expansion. *Olea* is a low-elevation tree but its pollen can be easily transported long-distance and to higher elevations than the parent plant (Cañellas-Boltà et al., 2009; Bell and Fletcher 2016); hence, its peak at the end this zone could be due to causes related with events occurring at the adjacent lowlands, which will be discussed later.

Zone MC2: 95-89cm, 1490-1536 CE (46 years; 9 samples; average resolution: 5.1 years/sampling interval)

This zone is dominated by arboreal taxa from both conifer forests (*Pinus*) and evergreen oak forests (*Quercus*), reaching overall abundances above 60% at the middle of the zone, with a decline to almost 45% at the top (Fig.4.3). Shrubs do not change but herbs of the meadows/pastures and ruderal/weeds groups (mainly *Plantago* and *Artemisia*) significantly decrease, as compared to zone MC1. The peak of arboreal pollen is due to the increase of *Pinus*, as other trees from other evergreen and deciduous forests either decrease (evergreen *Quercus*, *Betula*, *Alnus*, *Corylus*) or remain at values similar to zone MC1 (deciduous *Quercus*, *Fagus*). Concerning cultivated plants, *Cannabis* (hemp) is insignificant at the base of the zone but progressively increase to ~20% at the top. *Secale* (rye) also experiences a slight increase, whereas *Olea* (olive tree) decrease with respect to the former zone. No remarkable shifts are observed in aquatics. PAR values (Fig.4.5) experience a general increase but declined to minimal values at the top of the zone. Charcoal I undergoes an abrupt increase at the base to progressively decrease through the top, whereas charcoal II do not change with respect to the former zone (Fig.4.3). Regarding algae, *Botryococcus* continue to increase and *Tetraedron* almost disappear, whereas other types do not

experience significant changes, except *Cosmarium*, which is much less frequent. Fungal spores (*Sporormiella*, *Glomus* and *Chaetomium*) undergo a general increase (Fig.4.4).

Between 1490 and 1540 CE, evergreen and deciduous forests around the lake retracted and herbaceous crops, mainly hemp, and to a lower extent rye, expanded. The increase of *Glomus* indicates enhanced erosion, likely due to forest cover reduction, and the higher abundance of coprophilous fungi compared to former zone suggests grazing intensification. This, together with the sudden increase of charcoal, is compatible with an intensification of human impact both on the catchment area and at regional scale probably by using slash-and-burn practices for forest clearance and the enhancement of arable lands. The decline of *Artemisia* and *Plantago*, indicators of grazing, suggests the decline of pastoral activity, in which case, coprophilous fungi would indicate the presence of domestic animals associated with agriculture and/or transport activities. Pine forests are characteristic of higher elevations; therefore, their expansion would be independent of increased human activity around the lake, and will be discussed later.

Zone MC-3: 88.5-76.5 cm 1547-1717 CE (170 years; 25 samples; average resolution: 6.8 years/sampling interval)

This zone is characterized by a sharp increase of *Cannabis*, attaining values of almost 40%, and a general decrease of trees, including *Pinus*, with the exception of *Olea*, whose percentages remain stable. PAR values show that this is not a percentage artefact, as tree pollen –as well as most pollen types- actually decrease whereas *Cannabis* increase, with respect to the former zones (Fig. 4.5). Shrubs also stay unchanged. The most significant herbs, including those cultivated, also show rather stable percentages with only minor variations. Aquatic plants (*Typha* and Cyperaceae) experience a general increase at about the middle of the zone (84 cm; 1643 CE), coinciding with the appearance of *Scirpus* and a general increase of PAR values (Figs. 4.3 and 5). Charcoal I increase about the middle of the zone (ca 1643 CE, 84 cm) and peak at on the top, coinciding with the increasing trend in aquatic plants (Fig.4.3). Among algae, *Botryococcus* stabilizes in values attained at the end of the former zone but sharply peaks towards the end of this zone, coinciding with a remarkable increase of *Pediastrum*, which is very scarce earlier. *Cosmarium* is absent in this zone and for the rest of the sequence. In this zone, fungi spores experienced a general decline (Fig.4.4).

Between 1540 and 1720 CE, a dramatic shift occurred in the Montcortès landscape due to the general forest retraction and the onset of intensive and/or extensive hemp cultivation. Other crops (olive trees and cereals) remained in a situation similar to former

times. Pastoral practices, might experience a slightly decrease as indicated by the modest reduction of *Poaceae* and coprophilous fungi although *Artemisia* remained similar to the former zone. It seems that most agricultural activity was centered on hemp. Within this general scenario, the aquatic ecosystem (aquatic plants and the algal remains) changed around the middle of the zone (ca 1643 CE) probably indicating changes in lake water quality.

Zone MC-4: 76-44 cm; 1723-1874 CE (151 years; 32 samples; average resolution: 4.7 years/sampling interval)

The most distinguishing traits of this zone are the acme of *Cannabis*, reaching values of 60% to 80%, and the reduction of all trees and shrubs with no exception even *Cannabis* pollen is excluded of the pollen sum (Fig. 4.3). PAR values show that tree pollen do not decrease and their lower percentages are due to the comparatively higher rates of *Cannabis* increase (Fig. 4.5). Cereal crops, including *Secale*, slightly increase and the ruderal/weeds group remains unchanged. Some herbs that are scarce or sporadic in former zones appear more constantly and with slightly higher values in this zone. This is the case of *Urtica*-type and *Galium*. Charcoal I slightly declines and peaks near the top. Charcoal II increases its frequency and abundance, as compared to former zones. Regarding aquatic plants *Typha* and *Scirpus* attain their maximum percentages in this zone and decrease towards the top (Fig.4.3). *Potamogeton*, almost absent in former zones, starts to be present in a continuous fashion but with low values. Among algae, *Botryococcus* declines and *Pediastrum* increases. *Tetraedron*, almost absent in the former zone, reappears in the form of two peaks, at the base and nearly the top of the zone. Concerning fungal spores, *Sporormiella* and *Glomus* show similar values to the former zone including two peaks and *Chaetomium* exhibitS a similar trend but only relate to/in regards to the upper peak (Fig.4.4). A peculiar feature of these zones is an interval located in its uppermost part (61 to 45 cm; 1838-1869 CE), where *Cannabis* pollen, as well as some algae (*Tetraedron*) and fungi spores (*Sporormiella*), show large and sharp peaks coinciding with a conspicuous charcoal acme (Fig.4. 3 and 4.4). This feature is also evident in PAR values of almost all pollen groups, except for the conifer forest (Fig.4.5).

Contrary to zone MC3, in this time interval (1720-1880 CE), the increase in *Cannabis* pollen, suggesting its cultivation around the lake, was not paralleled by forest reduction. If hemp was actually cultivated this did not occurred at the expense of forests or other vegetation types, as none of them seem to have reduced their cover (see PAR values). Therefore, the increase of *Cannabis* suggests an extra source for this pollen (likely hemp retting), which might have increased pollen release to the sediments. It cannot be ignored

that a proportion of this significant *Cannabis* pollen increment can also indicate increases on cultivation at local and also at regional scale attending the great distances that *Cannabis* pollen can travel away from parental plant (Cabezudo et al., 1997; Giner et al., 2002). Increases in aquatic plants and algal remains suggest that the limnological shifts initiated in the former zone (1640 CE) were maintained, possibly exacerbated. Lake-level changes cannot be dismissed as increases in aquatic plants could be related with increases of the flooded area. Independent data (i.e. geochemical data) able to record lake-level changes, would be necessary support this interpretation. Relative high values of charcoal I and increases in charcoal II (indicatives of more regional and local fires respectively) together with increases in cereal crops and nitrophilous plants (such as *Urtica*-type and *Galium*) support human impact intensification on the area. This seems to be the phase of maximum anthropogenic influence, not only on the catchment and regional landscape but also on the aquatic ecosystem.

Zone MC-5: 15-3cm 1886-1971 CE (85 years; 13 samples; 6.5 years/sampling interval)

This zone is characterized by the rapid decrease of *Cannabis* and a general increase of trees (up to 60%, as in zone MC2) and shrubs, notably *Juniperus*. *Fagus* is an exception as its pollen is almost absent (Fig.4.3). PAR values confirm that tree and shrub pollen is generally increasing and *Cannabis* pollen almost disappears (Fig.4.5). Notably, *Quercus* (deciduous) and *Olea* attain their maximum values in this zone. *Secale* and the ruderals/weeds group (especially *Urtica* and *Galium*) also decline while other cereals remain stable but decreased at the end of the zone. Charcoal I decreases notably and charcoal II almost vanishes in this zone (Fig.4.3). Aquatic plants remain with values similar to former zones, except *Typha*, which is significantly reduced from this zone onwards. Regarding algae, *Botryococcus* sharply increases, peaking at the middle of the zone, whereas *Pediastrum* significantly declines and *Tetraedron* almost disappears (Fig.4.4). Fungal spores, with *Sporormiella* as the most abundant type, initiate a decreasing trend, almost disappearing at the top of the zone (Fig.4.4).

The results indicate that, between 1880 and 1970 CE, the *Cannabis* industry (i.e., cultivation and retting) was virtually non-existent around Lake Montcortès and forests recovered the same importance of former times, as for example, between 1490 and 1540 CE. In general, it could be stated that the landscape and the aquatic ecosystem returned to conditions similar to those observed on MC-1 before the *Cannabis* peak, with enhanced forest cover, less fire incidence and a similar trophic state of the lake. The conspicuous increase of *Olea* suggests that its cultivation in the adjacent lowlands increased.

Zone MC6: 2.5-0 cm; 1978-2013 CE (35 years; 6 samples; average resolution 5.8 years/sampling interval)

This zone is defined by a new increase of *Cannabis* to values of 20-30% and some trees, chiefly *Pinus* (attaining its maximum values throughout the diagram) and *Quercus* (deciduous), reaching values similar to zones MC1 and MC2. Other trees and shrubs decline, as is the case of *Quercus* (deciduous), *Betula*, *Corylus*, *Olea* and *Juniperus*. Herbaceous crops and ruderals/weeds attain their minimum values and aquatic plants remain stable with respect to the former zone, except for *Scirpus* and *Potamogeton* that disappear towards the top. PAR values show a general increase of all vegetation types except low shrubs (Fig.4.5). Charcoal also shows values similar to zone MC5 (Fig.4.3). *Botryococcus* attains its maximum in this zone, whereas *Pediastrum* and *Tetraedron* remain at lower values. Fungal spores are very scarce, almost absent (Fig.4.4).

During the time interval represented in this zone, conifer forests and evergreen oak forests expanded and deciduous forests receded. The significant increase in *Cannabis* contrasts with the general decline of farming and grazing activity, which requires explanation. Data from other disciplines are needed for a sound interpretation of this zone. Fortunately, historical documentation is abundant for this time period, but this will be addressed in the discussion.

Lake Montcortès pollen diagram (core MONT-0713-G05)
Analyst: M.C. Trapote

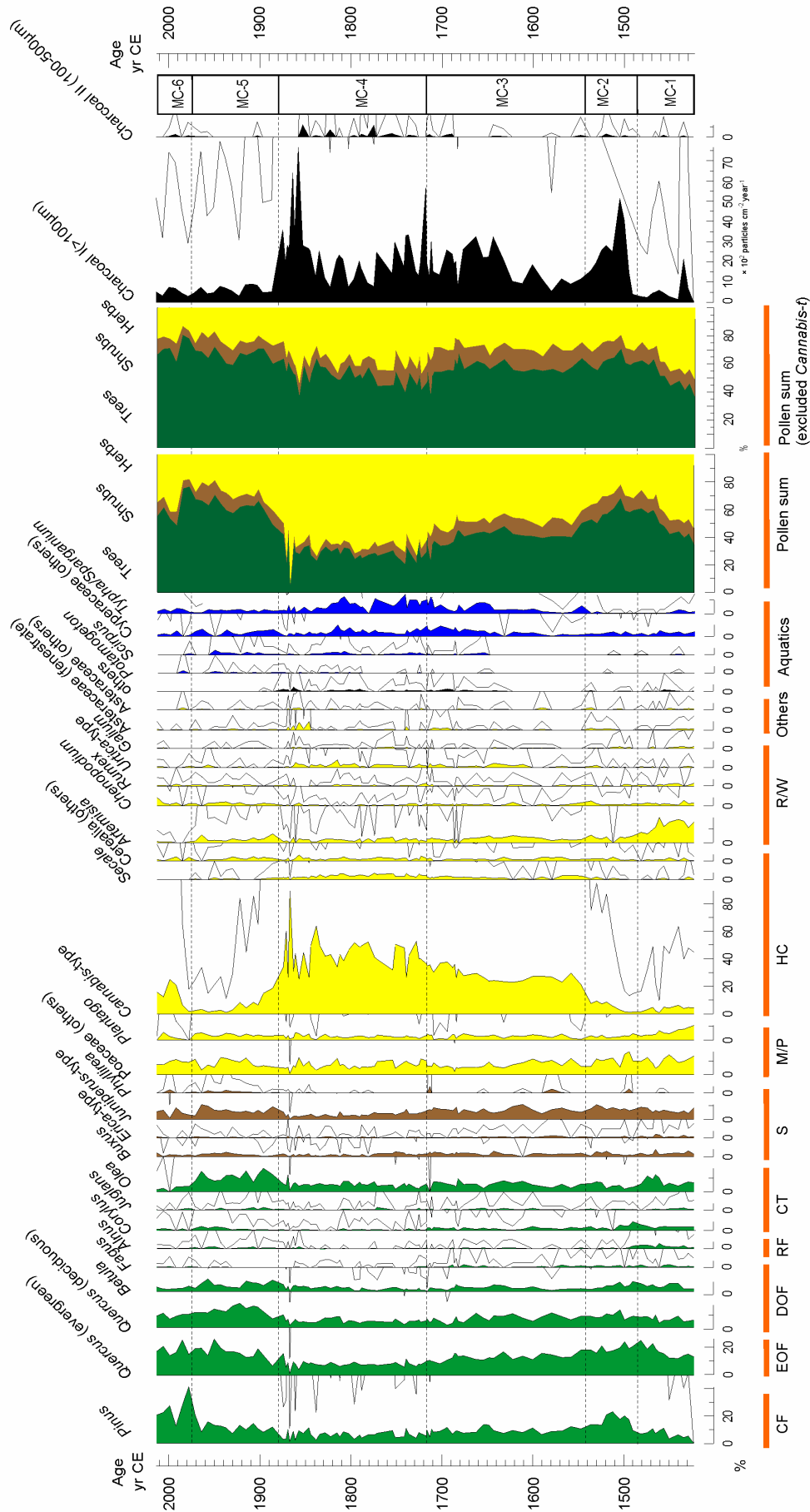


Figure.4.3- Percentage sporomorph diagram including the total pollen sum (%). Elements included in the pollen sum: CF, conifer forests; EOF, evergreen oak forests; DOF, deciduous oak forests; RF, riverine forests; CT, cultivated trees; S, shrublands; M/P, meadows/pastures; HC, herbaceous crops; R/W, ruderal/weeds. Elements outside the pollen sum: aquatic plants. An additional pollen sum diagram excluding Cannabis pollen is also shown. Charcoal pollen is also shown. Charcoal curves are expressed in fluxes for two categories of particle size. The horizontal lines correspond to statistically significant pollen zones (Bennett, 1996). Solid lines indicate (x10) exaegeration.

Lake Montcortès NPP diagram (core MONT-0713-G05)

Analyst: M.C. Trapote

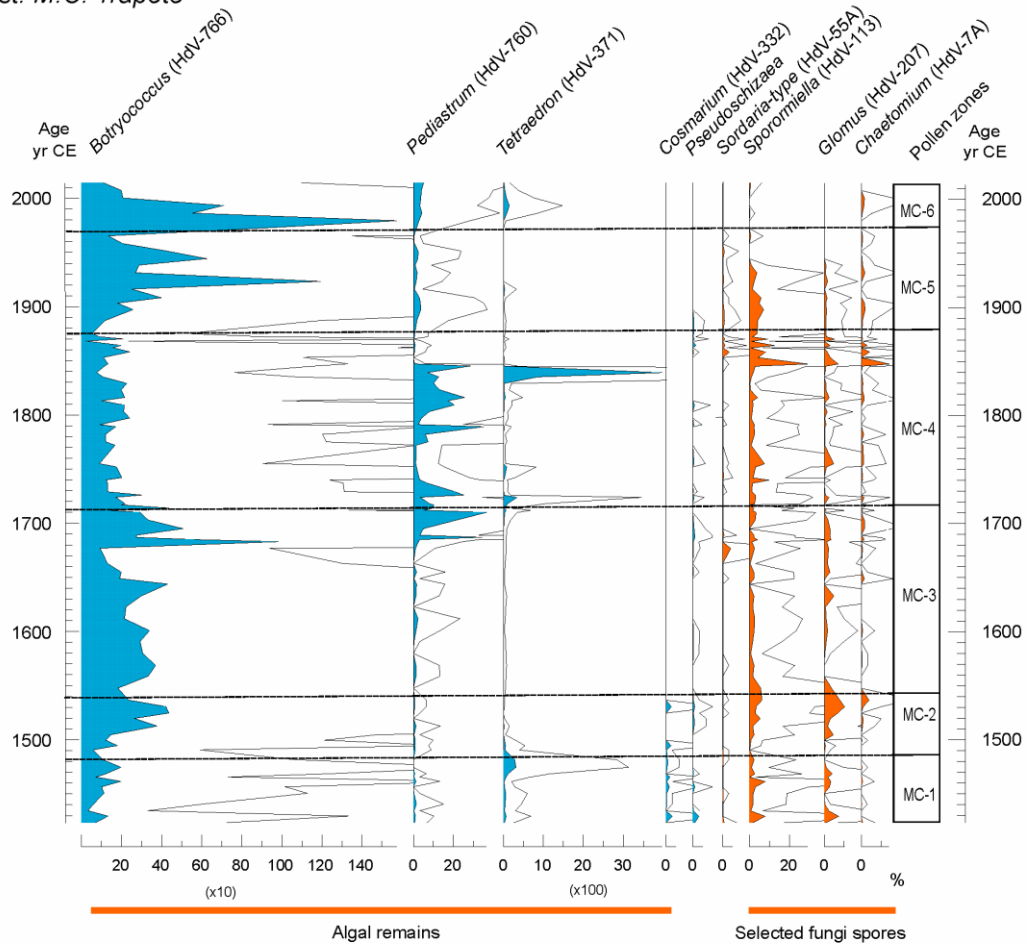


Figure.4.4- Percentage diagram for non-pollen palynomorphs (NPP) with respect to the pollen sum. The scales of Tetraedron and Botryococcus have been reduced for more clarity. NPP nomenclature based on the original publications (van Geel 1978; van Geel et al., 1981; 1989; 2003; Montoya et al., 2010; Bakker and van Smeerdijk 1982) Solid lines indicate (x10) exaggeration. Zonation as in Fig.4.3.

Lake Montcortès PAR diagram (core MONT-0713-G05)

Analyst: M.C. Trapote

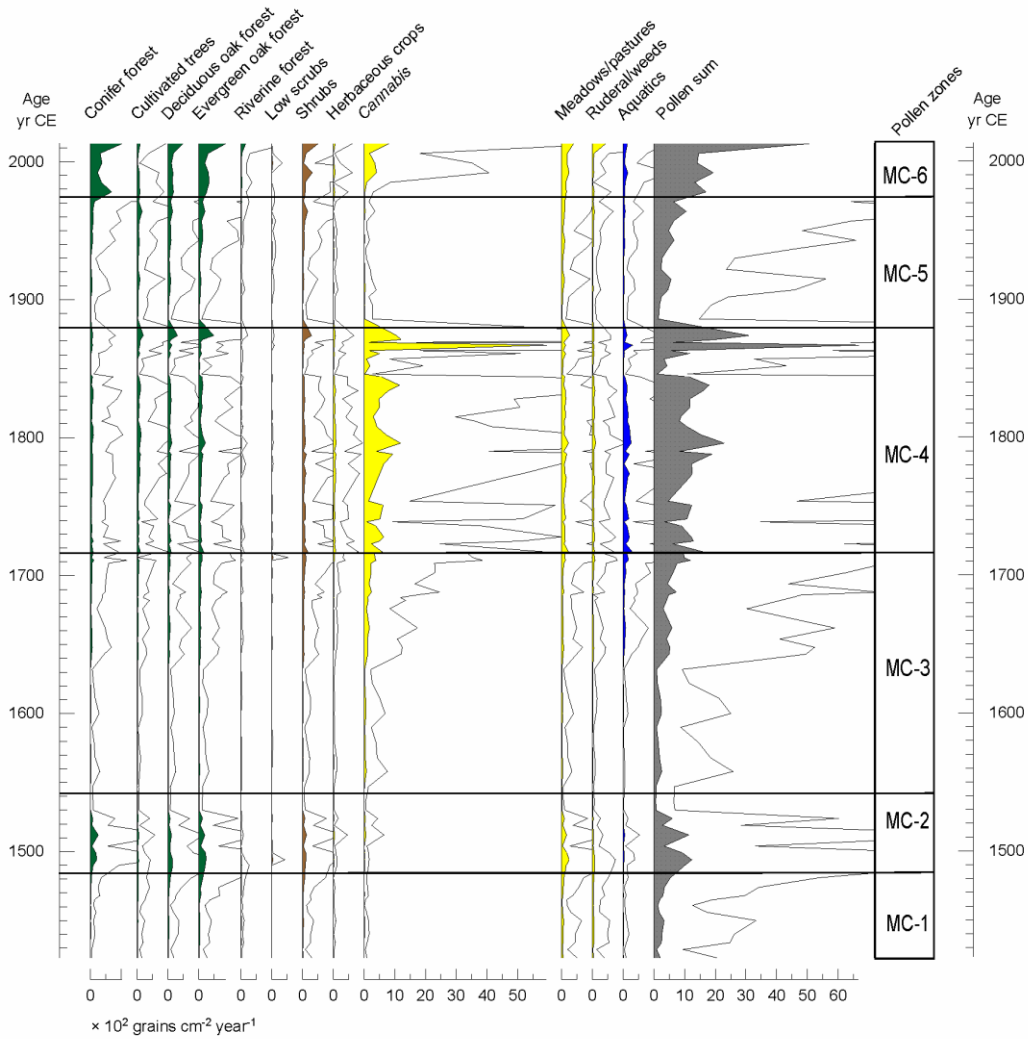


Figure.4.5 Pollen Accumulation Rates (PAR) diagram grouped by the vegetation groups detailed in Table 1. Solid lines indicate (x10) exaggeration. Zonation as in Fig.4.3.

4.4- Discussion

Figure 4.6 shows a comparison between a previous pollen study carried out in Lake Montcortès (Rull et al., 2011) and the present study. With this contribution, we have notably enhanced the average time resolution from 52 to 6 years/sampling interval which means an improvement from 9 to 79 samples for the overlapping time period. Additionally, we have analysed the recent vegetation history corresponding to 20th and 21st centuries for the first time in Lake Montcortès. The multiproxy study of the sediment record (pollen, charcoal and NPPs), the continuity between samples and the high temporal resolution covering the last 500 years have provided new insights about vegetation and the environmental history of the lake Montcortès catchment that were previously unnoticed. These new data allowed us to perform more precise comparisons with the available historical records and to disentangle local from regional events. Correlations with other sequences at local and regional scales are not easy to perform because of the lack of high resolution reconstructions covering the same period. Nevertheless, some similarities are found. Figure 4.7 shows a summary of the main findings of the present work and comparison with other studies developed in Lake Montcortès. Due to the variable human pressure on the landscape, discussion has been organised according to different degrees of anthropogenic impact.

4.4.1- Moderate human pressure (Pollen zones MC-1 and MC-2; from ~ 1423 to ~ 1536 CE)

Our record begins in the 15th century when pollen percentages suggest a humanized landscape where anthropogenic activity was progressively declining. This decline can be appreciated by forest increases at expense of pastures, meadows and ruderal taxa (Fig. 4.3). The end of the 14th century was a turbulent socio-economic moment for all of the Western Europe devastated by the “black death” epidemic. In Catalonia (Spain) and the Pallars region (where Montcortès is located) (Fig. 4.1), this moment was especially severe due to the Catalan Civil War, the contemporary framer rebellion (1462-1472 CE) and the Pallars War (1481-1487 CE). As a consequence, the population decreased and emigrated to the lowlands (see Rull and Vegas-Vilarrúbia 2015) and literature therein for more detail). This is consistent with the Lake Montcortès pollen record that showed evergreen oak forest increases and pine forest expansions in the higher-mountain areas, likely as a result of field abandonment. *Olea* expansion during this period was also in concordance (Fig. 4.3). Olive is a lowland crop, probably promoted due to lowland emigration. Its expansion, together with cereal cultivation, was recorded during the same period in other lowland lakes at that time (Estanya lake; 670 m a.s.l (Riera et al., 2004)) and in high-mountain records (Garcés-Pastor et al., 2016;

Ejarque et al., 2009 2010; Pérez-Sanz et al., 2011) owing to its high pollen dispersion capacity (Cañellas-Boltà et al., 2009; Bell and Fletcher 2016). However, human activity was still ongoing around Lake Montcortès, which is indicated by the continuous presence of coprophilous fungi and soil erosion indicators (Fig. 4.4). As recorded in historical records, the movement of large amounts of livestock through the Pyrenees was a regular practice. Farmers tried to keep livestock moving to avoid them being stolen due to poverty, famine and social instability (Bringué, 2005). Lake Montcortès, which is located on the way to the North, was likely used as a water source and rest area for livestock on the journey to the high-mountain areas, where livestock was hidden during social instability periods. Later on, from 16th century onwards, Lake Montcortès was likely used as a rest area for transhumance livestock. Lake level increases might also took place giving rise to marshy environments, as *Pseudoschizaea* is often related with humid environments (Scott, 1992), and *Cosmarium* has been related to lake level changes and increased turbidity (Reynolds, 2006; Casco et al., 2009). These conditions were probably promoted by cattle trampling near the lake shore. This is also in agreement with the lake level rises recorded for the same period in the nearby karstic Lake Estanya (Riera et al., 2004; Morellón et al., 2011). However, no changes in aquatic taxa such Cyperaceae, *Typha* or submerged vegetation were recorded in the Lake Montcortès sequence at that time.

4.4.2-. Intensification of human related activities: Cannabis pollen peak and water quality changes (Pollen zones MC-3 and MC-4; from ~1547 to ~1874 CE)

At the end of the 16th century, oak and riverine forests started to decrease locally, charcoal notably increased and herbaceous crops (hemp and cereals) expanded, meanwhile pine forest increased between 1490 to 1524 CE (Fig. 4.3). The recovery of the human population after the crisis was fast due to immigration from France (Bringué, 2005; Rull et al., 2011, 2015). The conifer forest expanded, which contrasts with the progressive decrease of oak and riverine forest around Lake Montcortès. The conifer forest expansion that took place in lake Montcortès record has been observed in other Pyrenean records (Ejarque et al 2009, Ejarque et al 2012) and it is in agreement with a period of farming decrease recorded in the Pyrenean high-mountain areas between 1430 to 1530 CE (Ejarque 2009). This was one of the colder phases of the Little Ice Age recorded nearby (Mateo and Gómez, 2004), which could have determined unfavourable for human life in the area (Ejarque et al., 2009; Mazier et al., 2009). The farming declining episode recorded in the Pyrenean high-mountain areas seem to have affected Lake Montcortès catchment where the riverine and oak forest decreased and anthropogenic activities persisted probably because its lower mountain

elevation (González-Sampéris et al., 2017). Actually, during the Little Ice Age (from ~14th to 19th centuries), and even during its colder (Fig.4.7) phases and when droughts and floods occurred (Corella et al., 2014; 2011, Morellón et al., 2012; Oliva et al.2017), intense human impact is recorded from 1550 to 1900 CE in the Montcortès record (Fig. 4.3 and 4.4). Increases in hemp, cereals, ruderal and nitrophilous taxa are the main vegetation changes recorded in the Montcortès pollen record from approximately 1500 to 1900 CE (Fig. 4.3). Charcoal also increased while forest retreated. This period was marked by a diversification and intensification in the exploitation of natural resources in the Pyrenees (crops, cattle and forest exploitation) (Reventós 2004; Bringué 2005). Forest, mainly pines, oak and beech, were used to obtain coal to feed a rising and increasing demand for iron Industry (iron forges) and for domestic use (Madoz, 1845-1850; Pèlach et al 2009; Ferrer Alòs, 2017). The Lake Montcortès pollen record showed the diversification of land uses in concordance with historical sources that documented slash-and-burn agriculture practices to obtain fields for cropping and cattle from 1500 to 1700 CE (Bringué, 2005; Ferrer Alòs, 2017). The consistent presence of coprophilous fungi and soil erosion indicators (*Glomus*) confirm the presence of livestock around Lake Montcortès (Fig. 4.4). Transhumance practices and iron forge activities were also very important activities between 1550 to 1700 CE and caused the intensification of forest exploitation to obtain fields for grazing and charcoal to fuel iron industry activities (Pèlach et al., 2009; Ejarque et al 2009). A rising intensity of charcoal production was recorded near Montcortès, in the Vallferrera Valley (Pèlach et al., 2009), coinciding with increases in the charcoal influx in the Lake Montcortès record.

The high hemp pollen percentages and the dramatic increase from 1720 to 1880 CE in the Montcortès record is coincident with one of the most important socio-economic and political moments throughout the Iberian Peninsula. Since the European discovery of America in 1492, the Spanish Royal Navy intensified its activity and hemp became a highly demanded product, mostly for supplying rigging and sails, and became strategic for commercial purposes (Díaz-Ordoñez, 2016). In this context, hemp cultivation was mandatory in Spain (for more detail, see Riera et al., 2004; Rull and Vegas-Vilarrúbia, 2014 and the literature therein). Catalonia was the second most important region for hemp production in Spain, just behind Valencia, and the one that produced the highest quality hemp fibres of the Iberian Peninsula (Sanz, 1995; Raventós, 2004). Lleida (Lerida) province (the present administrative limit where the Pallars region and Montcortès are located) (Fig. 4.1), was an important area for hemp production and fibre manufacturing (Ferrer and Alòs, 2017). The detailed proxy -data obtained and the historical data reviewed in the present work attest

that the Pallars was a renowned region for hemp production. Inhabitants of the area were well-known by their specialized skills in hemp manufacturing and by the diversity of peculiar tools for hemp manipulation characteristic of the region (Violant I Simorra 1934). This is the case of la Pobleta de Bellví at less than 6 km from Lake Montcortès, where inhabitants were highly appreciated by their outstanding skills for hemp combing (Violant I Simorra 1934). This can explain the implication of Lake Montcortès in hemp related activities.

Two main shifts can be observed in the hemp pollen curve coinciding with pollen zones MC-3 and MC-4 where percentages of 30% and more than 40% are the trend, respectively, and hemp peaked twice in 1838 and 1867 CE. The question of whether these percentages are due to hemp cultivation around Lake Montcortès or the lake was used for hemp retting has been raised in former low resolution studies (30-50 years/sampling interval, in average) (Rull et al., 2011; Rull and Vegas-Vilarrúbia, 2014). The present study presents more independent data (proxies and historical data sources), and higher resolution data required to address fully the question. Water changes are recorded in Lake Montcortès from the mid 17th century onwards. Cyperaceae, and more notably *Typha*, increased coinciding with the increases of hemp pollen that overcome the threshold of more or less constant frequencies of 30% that lasted ~200 years. At the same time, *Pediastrum* increased notably and *Tetraedron* peaked in 1838 CE, coinciding with one of the maximum hemp values (~63%). Increases in Cyperaceae and *Typha* have been related to fluctuations in water levels but also with water nutrient enrichment. *Typha* species thrives in areas of high nutrient input (i.e., nitrogen and phosphorous) mainly because of their fast growth rates and ability to take up nutrients rapidly (Newman et al., 1996; Miao and Sklar, 1998). The notable increase in *Pediastrum* 1680 to 1850 CE and *Tetraedon* peak that coincided with hemp maxima might also be related with eutrophication processes as it has been observed in other similar lakes with hemp retting (Riera et al., 2006). Furthermore, increases in both types of algal remains might be an indicator of the differential growth response to different nutrient species supply. *Tetraedron* spp. grow better in the presence of phosphorous compounds, whereas *Pediastrum* spp. responds more effectively in exploiting nitrogen sources (Berman et al., 1991; Berman and Chava, 1999). The process of hemp water retting pollutes water bodies and induces significant changes in water quality, eutrophication and oxygen depletion (Anderson, 1995; Paridah et al., 2011; Clerke and Merlin, 2013). Therefore, the changes recorded in the aquatic communities in Lake Montcortès might be a response to disturbances produced by the hemp retting process. Other proposed proxies that can provide additional arguments to corroborate retting in a water body are lithological changes

(increases on sedimentation rates, detrital material or shore reworked sediments) (Cox et al., 2001), plant fibres and seeds (that indicate the physical presence of the plant in the water body) (Clarke and Merlin, 2013), diatoms and cyanobacteria (responding to water quality changes) (Lotter, 2001; Bradshaw et al., 2006; Miras et al. 2015), biomarkers (unique to *Cannabis* plants, i.e., Cannabinol) (Lavrieux et al., 2013) and the presence of *Potamogeton* (Bradshaw et al., 1981; Riera et al., 2004, 2006). This latter case is fulfilled for Lake Montcortès as *Potamogeton* is absent in the former zones until the *Cannabis* acme, when appeared (Fig. 4.3). *Potamogeton* species grow well in eutrophic and mesotrophic waters and seem to be a good competitor regarding other submerged taxa in turbid waters (Sidorkewicz et al., 1996; Ven den Berg et al., 1999). This is in concordance with Vegas-Vilarrúbia et al., (2018), who, by analysing sedimentary pigments and physicochemical parameters of the sediment record, inferred anoxic water conditions, increased nutrient supply and turbidity during this period in Lake Montcortès. Despite the notable increase of hemp and cereals crops in MC-4, forest and/or other vegetation types did not decrease as might be expected (Fig. 4.5). This fact reinforces the idea of hemp retting as an extra source of *Cannabis* pollen into the lake. Another explanation for this surprising hemp pollen increase lies in the episode of cultivation intensification that took place during this period (from 18th century to the first half 19th century) due to technical advances and enhancement on irrigation techniques that made it possible to increase land productivity by obtaining more than one harvest per year (commonly two) (Sanz, 1995; Reventós, 2004). Cultivation intensification has also been recorded in other Pyrenean lakes and peatlands (Riera et al. 2004; Ejarque et al., 2009, 2010; González-Sampérez et al., 2017), but their lower resolution prevents detailed comparisons with Lake Montcortès and precise assessments of eventual time offsets and/or regional trends. Decreases in smaller charcoal influx (charcoal I) are also consistent with forest recovery observed in PAR values during this period (1700-1880 CE) although the frequency of bigger charcoal particles (type II) increased. These increases could be related to some agrarian techniques applied on fields surrounding Lake Montcortès as a consequence of production intensification. To maintain the intense productivity, farmers needed to fertilize the field to assure soil properties suitable for cropping. The most well-known and used method consisted of spreading manure in the fields, but it was too expensive, and depending on the crop type, the cost of manuring was higher than the profit of the corresponding harvest. Alternatively, farmers burned vegetal biomass mixed with soil to later spread and fertilize the field. Curiously, hemp was one of the most commonly used biomass sources for burning mixed with other vegetation types (Reventós 2004). With

technical advances and cropping intensification, it was possible to keep harvested products in extra stock and feed animals at home. Consequently, transhumance practices were notably reduced (Reventós 2004). The reduction of transhumance and the increase of field productivity also helped to make forest recovery possible nearby Lake Montcortès.

From 1850 to 1870 CE (pollen zone MC-4), a saw-tooth trend is mostly recorded by the hemp pollen curve (exceeding 80%) but also by other taxa and charcoal and fungal spores. Such trends are much more evident looking at PAR values (Fig. 4.5). The saw-tooth trend is recorded at the same time that extreme precipitation events were observed in the Lake Montcortès record (Corella et al., 2014, 2016). The clastic microfacies resulted from the increased runoff during flood events that took place at the sub-decadal scale and were cross-correlated with documented extreme floods occurred in most rivers in the NE Iberian Peninsula (see Corella et al., 2014, 2016 for more detail) (Fig. 4.7). The increased input of external detrital material from the watershed is clearly recorded in pollen sedimentation rates and other terrestrial proxies (charcoal and fungi). The only vegetation type that did not record the saw-tooth trend was the conifer forest, which mostly comes from high-mountain areas, giving a weak signal resulting from the watershed weathering. Therefore, the high-resolution obtained for the pollen record, in this case, gives us information about sub-decadal scale periods of runoff increases from the immediate surroundings of Lake Montcortès.

4.4.3- Low human pressure: field abandonment (Pollen zones MC-5 and MC-6; from ~ 1875 to ~ 2013)

The notable decrease in *Cannabis* pollen, as well as the decrease of other human presence indicators during 20th century in lake Montcortès record, indicates the decrease in human pressure intensity: *Secale*, *Urtica*-type decreases and the near disappearance of coprophilous fungi at the end of the century together with the considerable reduction of fire and forest recovery indicate the reduction of agro-pastoral activities and field abandonment. The dramatic and sharp hemp reduction occurred during the 20th century coincided with the dismantlement of the Spanish Royal Navy and also with important socio-political changes in the country. The need for materials for rigging, sails, clothes or trading was sharply reduced. Moreover, the appearance of new fibres (synthetic fibres and imported cotton from USA, Brazil, Mexico, Syria and Turkey) provoked almost the total disappearance of hemp fibre used for clothing in Spain (Simó, 1985). The only crop that seemed to increase during the 20th century was olive, but it could be an artefact of pollen percentages since increases in *Olea* at

that moment were not recorded in PAR values (Cultivated trees) (Fig. 4.5). A remarkable human population increase took place from 1860 onwards in the Pyrenees, which provoked a crisis at the end of the 19th century due to the lack of enough resources to maintain the increasing population. This situation forced the population to emigrate to industrialized areas (Guiardo, 2011; Farràs, 2005). Agro-pastoral activities in the Pyrenees were reduced to fewer and richer houses that were able to adapt to a high demanding market and change to a more intensive production model. The economic activities in the area changed with the advent of capitalism and the most important economic activity in the Pyrenees was the implantation and exploitation of hydroelectric power stations (Rotés 2011). Furthermore, the Spanish Civil War (1936-1939 CE) interrupted industrialization and socio-economic activities. It was not until the 1950s when the country again started with socio-economic recovery and modernization (Guiardo 2011). This can be appreciated in the Montcortès record with the declining of human indicators. Aquatic plants and algal remains also experienced changes during this period. *Typha* and *Botryococcus* (a low nitrogen and phosphorous tolerant alga (Reynolds 2006)) were reduced. This is coherent with a declining human pressure scenario. It seems that Lake Montcortès returned to similar states prior to *Cannabis pollen maxima*, likely more oligotrophic conditions that lasted until present day (Trapote et al., 2018).

From the 1970s onwards, forest continued to expand around Lake Montcortès. Coprophilous fungi disappeared, and hemp was the only cultivated plant increasing its percentages. Currently, there is no hemp cultivation known close to Lake Montcortès and, according to local people, the lake has not been used for hemp retting during the last decades. Since 1992, Montcortès is considered a site of natural heritage and it is protected by means of different administrative measures (Gentcat 2006; Xarxanatura 2000). The hemp maxima during the 21st century in Lake Montcortès is recorded from the mid to late 1990s. At the beginning of the 1970s, a renewed interest in hemp, mainly as a source for paper pulp manufacturing, took place in Spain (Gorchs and Lloveras, 2003) The European Union started to economically support hemp cultivation from the late 1980s. In the middle 1990s, EU hemp subsidy amounts reached their maxima and farmers have since been interested in growing hemp for its economic benefits (Gorchs and Lloveras, 2003; Karus, 2004). Consequently, in just a couple of decades, areas of hemp cultivation expanded again and reached their climax in Spain around 1998 (Karus 2004), coinciding with maximum hemp production in Catalonia (Gorchs and Lloveras, 2003). From the 2000s onwards, EU hemp cultivation subsidy amounts were notably reduced, and therefore, hemp production became

less economically profitable and cultivation was reduced. Currently, hemp is still used for clothing, animal feeding, oil (seeds), pulp paper, building materials and water treatment (hemp dust) among other uses (Gorchs and lloveras 2003; Clarke and Merlín 2013). The abrupt hemp peak observed during the 21st century lasted less than 20 years after almost a century of very low hemp pollen frequencies, then, decreased again until the present. The presence of this peak is related to a very specific and short-term duration historical event that can only be detected by means of high-resolution observations.

Despite a notable hemp reduction at the beginning of the 2000s, at present, Lake Montcortès still records significant amounts of hemp pollen. It was observed by Rull et al., (2017), who carried out a two year study of seasonal sediment trapping in Lake Montcortès from 2013 to 2015 CE. *Cannabis* pollen was continually recorded in the trapped material during the whole studied period at about 5% of total abundance and greatly increased during fall seasons when reached 40%. The current presence of hemp pollen in Lake Montcortès can be explained by its great air dispersion. Hemp pollen can travel far away from the parent plant (i.e., from North Africa to southern Spain, as found by Cabezudo et al., 1997; Giner et al., 2002). The mentioned trap study also found that hemp pollen was positively correlated with wind velocity supporting this idea. Another explanation could be due to an involve increasing cultivation near Lake Montcortès, as currently, there is a growing interest on hemp cultivation to recover natural and environmental friendly fibres. Moreover, the pre-Pyrenean areas have been identified as suitable for hemp cultivation due its humidity and because hemp has been reintroduced as a rotation crop together with wheat (Gorchs et al., 2006). During the trapping study period, no hemp retting was carried out in Lake Montcortès and inhabitants of the area had no notions about the effect of hemp fields on the surroundings.

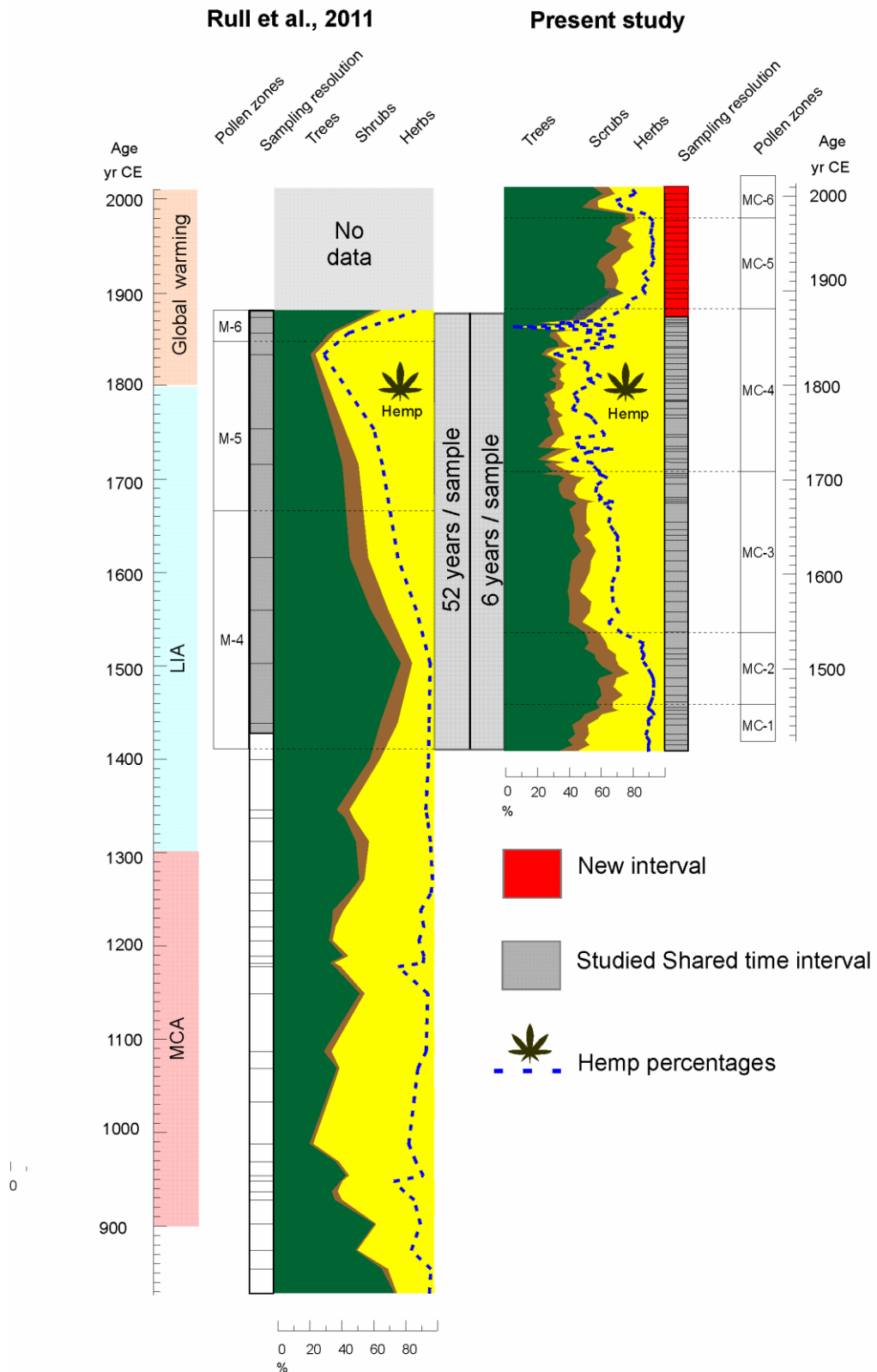


Figure.4.6- Comparison between the palynological results obtained in Rull et al. (2011) and the present work. Grey colored correspond to shared time interval. Red colored correspond to new time interval presented in this

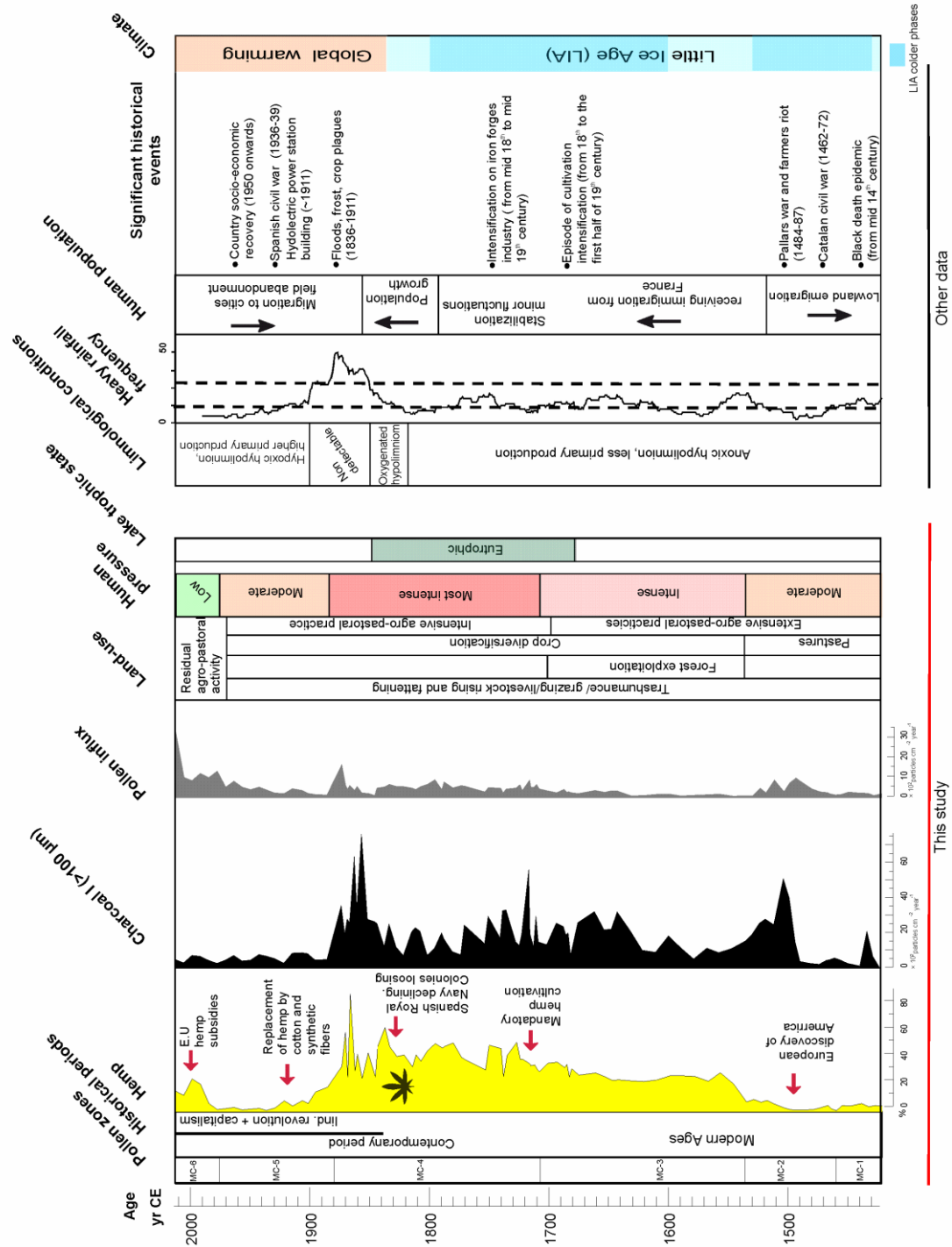


Figure 4.7 - Summary figure including the main findings of this study compared with previous studies of Lake Montcortès (Corella et al., 2014; Vegas-Vilarrúbia 2018), historical data referring to main historical events (Díaz-Ordoñez, 2016; Rotés 2011; Reventós 2004; Bringué 2005; Pérez-Sanz et al., 2011; Simó 1985; Rotés 2011; Gorchs and Lloveras, 2003; Karus, 2004) and climatic data (Oliva et al. 2017; Morellón et al 2012; Mateo and Gómez 2004).

4.5-Conclusions

We have evaluated a 500 year-long varve record from Lake Montcortès at the highest resolution achieved so far for a varved lake in the Iberian Peninsula. We have notably improved our knowledge of the recent history of Lake Montcortès and its surroundings in several terms. We have improved the temporal resolution (~6 years /sampling interval) and improved the historical and palaeoecological precision, as well as the spatial scope, with the new studied time interval (20th and 21st centuries). By means of a multiproxy analysis of biological indicators combined with independent evidence from historical sources and comparison with previous studies carried out in Lake Montcortès, we have reconstructed human-landscape dynamics in detail. The present work also helped to answer some questions that arose with former studies and to go deeply into one of the most striking features of the Montcortès pollen record: the outstanding hemp pollen percentages. We shed more light on potential consequences of human impact on the aquatic system derived from hemp retting practices.

Human activity around the lake during the last 500 years has had a greater influence on vegetation community changes than climatic factors, and only increases in the frequency of flood events could have been inferred from the studied record from the mid to the end of the 19th century. Cropping, livestock breeding, and hemp related activities have been the most important factors responsible for landscape modulation. Even during harsher climate conditions (LIA), human activities remained significant in the area. The high-resolution study provided enough data to evaluate short-lasting events at local and regional resolutions that otherwise would not be possible to identify as related to climate (sub-decadal frequency of floods and high-land forest recovery) or to historical and socio-economic events (i.e., crop promotions (hemp) or land abandonment).

The temporal extent of the *Cannabis* pollen peak (400 years) and its temporal continuity have been confirmed. The revision of new historical sources available combined with pollen and NPP indicators from Lake Montcortès provided further and detailed evidence of the local use of hemp, implying cultivation and manufacturing, as well as potential effects of retting hemp in the lake, on the aquatic communities between the mid 17th to late 19th centuries, which was not possible to confirm in previous studies at lower resolution. Further investigations using aquatic proxies at high-resolution are necessary in this sense to better assess the eutrophication degree and water community disturbance. Geochemical analyses

of sedimentary cannabinol or other hemp specific biomarker would unequivocally confirm the use of Lake Montcortès for hemp retting.

More work is needed to take advantage of the great scientific potential of the Montcortès sediment record, and also to exploit the unusual and very valuable availability of modern sedimentary pollen analogues as a tool to better interpreting the fossil signal. Studies that include and combine lake and modern analogue monitoring with high-resolution palaeoenvironmental reconstructions in varved sediments are very scarce, but it opens a range of possibilities to exploit the potential of palaeodata contained in the sediment record, i.e. it is possible to assess the yearly flux of pollen sediment rates and define rates of palaeoenvironmental changes at decadal and even at sub-decadal scales (Birks and Birks, 2006)

The present study is the first that combines all of the explained above advantages for a varved lake in the Iberian Peninsula and the Mediterranean region. The value of the data obtained in this study lies in the potential to be used to calibrate and validate future model scenarios, perform quantitative climatic or environmental reconstructions and use it as a tool to apply in the conservation and restoration of cultural landscapes. Further work should focus on improving sampling techniques to obtain higher temporal resolution for biological proxies and to obtain modern analogues for the variety of potential environmental and climatic proxies.

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CHAPTER 5

Response of aquatic photosynthetic community to 500 of anthropogenic catchment disturbance: a high-resolution (sub-decadal) pigment analysis.

*“And I find it kinda funny
I find it kinda sad
The dreams in which I'm dying
Are the best I've ever had
I find it hard to tell you
I find it hard to take....”*

Soundtrack: *“Mad world”- Tears for fears*

The content of this chapter will be submitted as:

Trapote, M. C., Vegas-Vilarrúbia, T., Buchaca, T., V., Rull. Response of aquatic photosynthetic community to 500 of anthropogenic catchment disturbance: a high-resolution (sub-decadal) pigment analysis.

Abstract

We analyzed photosynthetic pigments in one of the few existing mid-altitude Mediterranean lakes with varved sediments, Lake Montcortès, on the southern Pyrenean flank. The lake location and the varved nature of its sediments together with the long history of human occupation makes this place a key site to study and assess how anthropogenic factors and their interactions affected the aquatic community over time. Taking advantage of the high resolution and high quality paleoenvironmental data, we have examined relative effects of four human related processes (changes in land use, on lake's trophic state, soil erosion and Cannabis related activities) on the aquatic ecosystem by means of Variance Partitioning Analysis (VPA). VPA has been performed over the full record and over three selected time subsets A (1423-1525 CE); B (1530-1846) CE and C (1852-2013 CE) coinciding with main periods of change. Results shows that changes in land use was the most important factor explaining changes on aquatic community for the full record with 14.5 % of variance explained at $p < 0.001$ and for two of the three time subsets selected: subset A: 1423-1525 CE explaining 16.8% at $p < 0.05$ and subset C: 1852-2013 CE explaining 14.7 % at $p < 0.01$. Changes in trophic state was the main factor explaining variance for more than 300 years coinciding with *Cannabis maxima* on subset B: 1530-1846 with 5.7% of the variance explained at $p < 0.05$. Despite, the low human presence in the area since industrialization, last century recorded stronger eutrophication signals on pigment data, probably due to non-point nutrient sources due to historical land use and atmospheric deposition. There is still a high proportion of remaining variance probably related to other factors not accounted for in this study. Further work should focus into obtain independent environmental and climatic indicators, in order to better assess the diversity of factors affecting the aquatic community of lake Montcortès. Additionally, extending back the timeframe of research would allow accounting for ecological reference before aquatic community human affectation.

Keywords: Photosynthetic Pigments, High-resolution, Varves, Variance Partitioning , Human Pressure.

5.1- Introduction

Over time, a lake does change in response to several environmental factors. Natural and anthropogenic factors have direct and indirect influence on in-lake processes affecting the aquatic community and its ecological trajectories. On one hand, climate can affect directly a system by changing temperature and/or the hydrological balance and, indirectly by affecting vegetation development that in turn influence chemical weathering on the catchment and runoff to the lake (Simpson and Anderson 2009; Hausmann et al., 2002). On the other hand, human activities also represents an important driver of ecological change. As happens with climate, human activities can affect directly (i.e. by direct sewage discharge to the lake), or indirectly by modifying vegetation in the lake's catchment (i.e. changes on land use) (Mill et al., 2017; Bennion et al., 2011; Smol 2010). And, in some cases, the effects of human activities on the ecosystem are imposed upon natural development processes altering profoundly the course of lake development (Fritz 1989). Therefore, to understand how the aquatic community has changed and responded to perturbations at specific place, it is necessary to assess and estimate the relative importance of these factors and the manner and degree in which they interact to affect lake-catchment system over time (Lami et al 2009).

Data records obtained by historical lake monitoring are very valuable to study lake response to environmental change. However, monitoring data is often insufficient to evaluate long-term trends on aquatic community due to its short-term nature (Smol 2010; Rull 2014). Paleolimnological studies based on the analysis of the biological, chemical and physical information preserved on lake sediments offer an alternative to overcome this restriction, by allowing the reconstruction of past changes for periods predating instrumental observations (Pienitz and Vincent 2003; Rull 2014 Smol 2010).

Lakes in remote regions have been comparatively less affected by direct human activities in the past (Smol 2005; Anderson et al., 2008). Such feature make them attractive for studying influences of climate on aquatic community without signal distortion from anthropogenic influence. By contrast, lakes located in more lowland and /or near to urban and agricultural settings are prone to be under higher anthropogenic pressure that often masks the influence of natural factors on the sedimentary signal (Mills et al., 2017; Bennion et al., 2011). Although this feature can hamper assessment of the full ecosystem response to natural variability, mid-altitude lakes surge as interesting targets to carry out

paleolimnological studies. The important reason for working on sites with known human perturbation is that this brings the opportunity to study and assess the nature, magnitude and extent of human induced perturbation (Pienitz et al., 2006; Hall et al 1999;). Furthermore, these systems can provide the opportunity to identify the onset of human influence as well as to establish reference conditions prior to human perturbation, which are necessary for setting environmental management strategies if there was an environmental affectation (Bennion et al., 2011)

Among the available proxies contained in lake sediments, sedimentary pigments are one of the most accurate to provide valuable information about historical trends of lake primary production (Leavitt and Hodgson 2011). Among the varied range of ecological and paleolimnological sedimentary pigment applications, pigments have been used as indicators of a wide variety of anthropogenic impacts on aquatic ecosystems, including eutrophication, acidification, and climate (e.g. Hall et al., 1999; McGowan et al., 2008; Leavitt and Hodgson 2011; Carson et al., 2015, Buchaca et al.,201, Bunting et al., 2016). Since aquatic photosynthetic organisms respond rapidly to changes, high-time resolution with an accurate chronology of the sediment record are crucial to carry out readily the aforementioned approaches and necessary for a fruitful comparison of the sediment signal with historical documentary data and existing climate records on multi-proxy studies (Kienel et al., 2013). However, measurements of sedimentary pigments are complex and expensive and consequently, it is not easy to find long and high-resolution sedimentary pigment studies (Butz et al., 2015, 2016; Romero-Viana et al., 2010).

Lake Montcortès, which is located in the pre-Pyrenean range (1026 m a.s.l.) is a very suitable place to reconstruct past environmental changes at high-temporal resolution. The exceptional scientific value of this lake is due to its strategic location and to the varied nature of its sediments, which preserves an accurate and continuous chronology (Corella et al., 2011, 2012). There is an extensive literature evidencing that Lake Montcortès sediments have recorded both, climatic and human signal. In this case, climatic signal seems to be better reflected on those works using abiotic proxies (i.e. sediment elemental composition or by studying sedimentary microfacies) (Corella et al., 2014; 2016; Vegas-Vilarrúbia et al., 2018), while those using biotic proxies (i.e. pollen or diatoms), evidenced mainly human influence (Rull et al., 2011; Scussolini et al., 2011; Trapote et al., 2018a). Nevertheless, climatic inference was detected during the Medieval Climatic Anomaly (MCA) on pollen data (Rull et al., 2011). The extensive literature documenting human pressure through historical and paleoenvironmental data on lake's Montcortès region, suggested that it was a crucial place

for past human community development. Among others, hemp cultivation and/or hemp retting, cereal cropping and livestock rising, took place in Montcortès at different intensities over or more than one millennium (Rull et al 2011, Rull et al 2015, Montoya et al., 2018 and Trapote et al., 2018a). There is evidence that such activities considerably affected the aquatic ecosystem (Vegas-Vilarrúbia et al., 2018; Trapote et al., 2018; Scussolini et al.2011). This is more clearly evidenced in a very recent work carried out in Lake Montcortès using sedimentary redox proxies and sulphur bacterial and cyanobacterial pigments, in order to differentiate periods of distinct water column oxygenation and stability associated with climate and human factors (Vegas-Vilarrúbia et al., 2018). The authors clearly distinguish pre- and post- industrial oxygenation trends showing an unprecedented trend of change coupled to climate change (Vegas-Vilarrúbia et al., 2018). The strategic location of lake Montcortès in a historically and well-known human occupied area and the already available high quality paleoenvironmental and historical data covering several centuries, makes Lake Montcortès an excellent study site to examine the response of the phytoplankton community to environmental changes induced by human activities and occurring in lake-catchment system.

This chapter describes main changes occurring on lake's Montcortès photosynthetic community since 1423 CE to nowadays at sub-decadal resolution (6 years/sample on average) and focus in to quantify the effects of environmental drivers on pigment-inferred abundance of photosynthetic bacteria and algae including cyanobacteria. Since no suitable climatic reconstructions are already available for such specific area, and the sample resolution covering climatic instrumental period is not enough to establish numerical significant relationships between proxy and climatic data; we will focus on the available historical and paleoenvironmental data from biological proxies at the same resolution obtained from other studies carried out in lake Montcortès. As explained above, in former studies, the signal inferred from biological data was mainly related with human activities. Hence, by means of a multi-proxy approach, we will make particular reference to changing land-uses and derived processes occurring on the lake and catchment area that in turn would be related directly and indirectly with climate and human activities affecting in-lake process. The advantage of the selected study period lies in the fact that covers significant climatic variations (Little Ice Age (LIA) and the onset of global rising temperature (global warming)); and in the availability of high quality and high-resolution historical and palynological data belonging the same core and at the same time resolution that pigment analyses were done here. Such circumstance substantially improve accuracy by reducing uncertainties on data treatment and improve the reliability of the resulting inferences after data analysis.

Therefore, our main aims are (1) to describe main changes on photosynthetic community during the last 500 years at high temporal resolution (6 years per sample on average); (2) to identify the main factors driving change, specially referring to catchment disturbances, and to determine key periods of change on the aquatic community; (3) to quantify the relative contribution of each factor and their synergies in explaining photosynthetic community behavior on lake Montcortès.

5.1.2- Study site

Lake Montcortès is a karstic lake in a closed basin located in the Pre-Pyrenean Range (NE Spain) in the Pallars Sobirà region (42° 19' N; 0° 59' E) at 1029 m a.s.l. (Fig. 5.1). The lake's catchment area is small (watershed surface area ~1.39km²) and lies on Oligocene conglomerates and Triassic rocks mainly comprising carbonates, evaporites, claystones and shales. The lake is relatively small and kidney-shaped with a surface area of 0.14 km² and a maximum water depth of 30 m. It has no permanent inlet, it is mainly fed by groundwater and runoff and two ephemeral streams located on the southern area of the watershed drain the lake (Corella et al., 2011). The lake is situated in a transitional climatic area between the Mediterranean lowlands and the Middle Montane Belt within the sub-Mediterranean bioclimatic domain (Vigo and Ninot, 1987). According to the nearest meteorological station, la Pobla de Segur (at less than 20 km from Montcortès), total annual mean precipitation is 668.5 mm. February and May are the driest and wettest months, respectively. Annual average air temperature is 12.8 °C, with maximum and minimum mean temperatures of 23.3 (July) and 2.9 °C (January). The lake's nearest surroundings are dominated by herbaceous vegetation types represented by pastures (for cattle and horses) and hay meadows and crops (alfalfa and cereals) (Rull et al., 2011; Mercadé et al., 2013). Oak and Pine forests are the main forest formations of the area. The lake's shoreline presents a steep talus and a littoral vegetation belt dominated by hygrophyte communities (Mercadé et al., 2013).

The mixing regime on Lake Montcortès alternates holomictic periods, with long duration of thermal stratification and hypolimnetic anoxia and, meromictic periods (Trapote et al., 2018b, Vegas-Vilarrúbia et al., 2018). Lake water is alkaline and oligotrophic (Table.5.1). Prevailing phytoplankton communities alternate seasonally, with cryptophytes dominating during winter, diatoms during spring and chlorophytes during summer and autumn (Table.5.1). It has historically been an important water resource for numerous surrounding villages and farmhouses with a long history of human occupation (Scussolini et al., 2011; Rull

et al., 2011; Trapote et al 2018a; Vegas-Vilarrúbia et al., 2018). Nowadays, Montcortès town has a total population of 26 inhabitants (Idescat, 2015).

Limnological data from Lake Montcortès	
Variable	Range
Conductivity (μS)	~ 400-500
pH	~ 8.4-7.2
Ca (mg L^{-1})	~ 80-145
Mg(mg L^{-1})	~ 15-20
K (mg L^{-1})	~ 2.2-2.7
SRP (mg L^{-1})	~ 0.01-0.07
TP (mg L^{-1})	~ 0.05-0.8
TN (mg L^{-1})	~ 24-71
Secchi Disk (m)	~3.5-8.5

Table 5.1- Summary of limnological variables of lake Montcortès from Trapote et al., (2018b). Solubre Reactive Phosphorous (SRP); Total Phosphorous (TP); Total Nitrogen (TN).

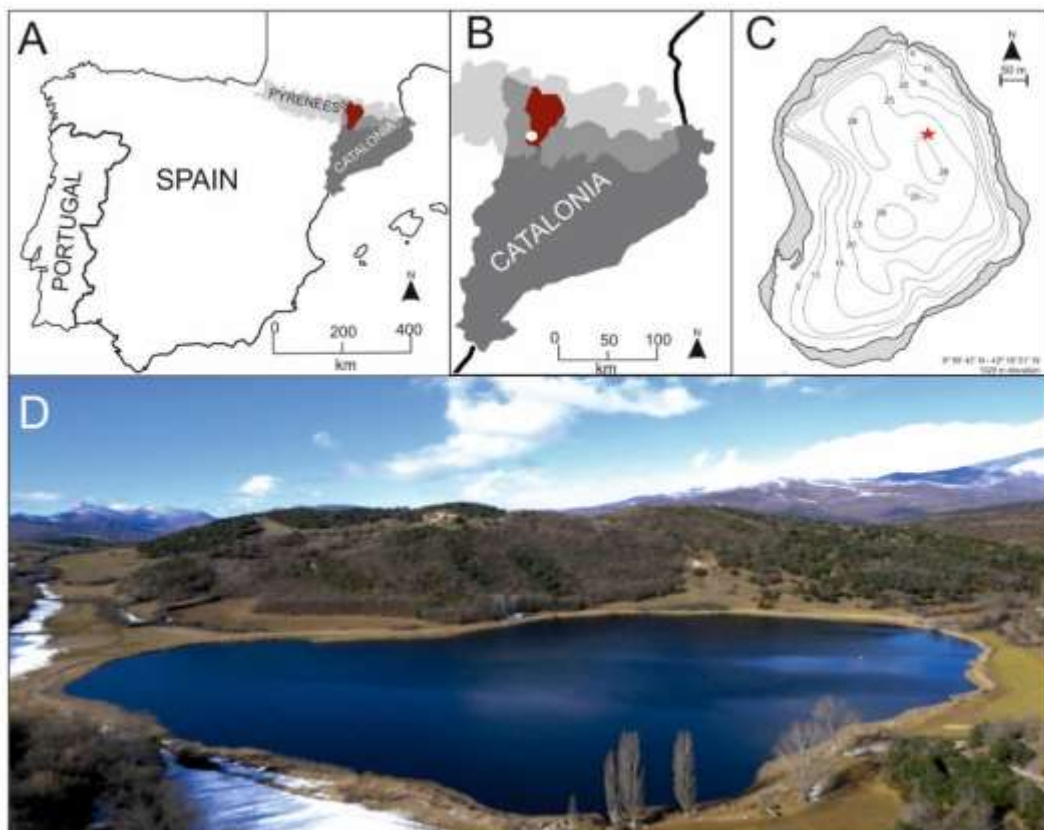


Figure 5.1- A and B) Map of Spain and Catalonia (dark green), in red Pallars region where Montcortès lake is located (white dot). C) Montcortès bathymetry and D) aerial photo of the lake.

5.2- Methods

5.2.1- Coring, sampling and Chronology

Core MONT-0713-G05 was retrieved from the deepest distal lake basin of Lake Montcortès (~ 30 m water depth, using a UWITEC 60 mm diameter gravity corer, in July 2013. Core sampling was performed continuously in the varved part of the sedimentary record avoiding turbidites. Samples were extracted every 0.5 mm using a syringe (see Trapote et al. 2018a for more detail). A set of 96 samples were obtained for pigment analysis. They were frozen and stored in dark prior to pigment extraction.

The age-depth model for MONT-0713-G05 was recently published on Trapote et al., (2018a). Chronology was based on independent varve counting and ^{210}Pb and ^{14}C radiometric dating from a composite sequence and then transferred to MONT-0713-G05. Further details of this age-depth model are provided in Corella et al., 2014 and Trapote et al., 2018a.

5.3.2- Pigment analysis

The method used for pigment analysis is the same that presented on Vegas-Vilarrúbia et al 2018. The frozen sediment samples were dried and the pigments were extracted in 5-mL of 90% acetone using probe sonication (Sonopuls GM70 Delft, The Netherlands) (50W, 2 min). The extract was centrifuged (4 min at 3000 rpm, 4 °C), filtered through Whatman ANODISC 25 (0.1 μm) and analysed with ultra-high-performance liquid chromatography (UHPLC). The UHPLC system (Acquity Waters, Milford, MA, USA) was equipped with an UPLC HSS C18 SB column (dimensions: 2.1 \times 100 mm; particle size: 1.8 μm) and with Photo Diode Array (PDA) (λ : 300–800 nm). The PDA channel was set at 440 nm for pigment detection and quantification. After a sample injection (7.5 μL), the pigments were eluted with a linear gradient from 100% solvent B (51:36:13 methanol:acetonitrile: MilliQ water, v/v/v 0.3 M ammonium acetate) to 75% B and 25% A (70:30 ethyl acetate: acetonitrile, v/v) for 3 min, followed by 0.45 min of isocratic hold at 75% B and 2 min of linear gradient to 100% of solvent A. The initial conditions (100% B) were linearly recovered in 0.65 min. The flow rate was 0.7 mL min⁻¹. Pigments were identified checking retention times and absorption spectra against a library based on photosynthetic bacterial cultures (Institut d'Ecologia Aquàtica, University of Girona, Catalonia, Spain), cyanobacterial and algae (Buchaca, 2009).

5.2.3- Biological data and environmental variables

In this chapter, we focus on the role of carotenoids, as these have proved to be better biomarkers than chlorophyll derivatives to differentiate between algal groups, due to their varied functions and resistance to degradation (Goodwin, 1980; Leavitt and Hodgson 2002). Among the pigment pool identified by UHPLC analysis, key markers (Table.5.2) were selected and used as a taxonomic tool representing the variability and diversity of Lake Montcortès phytoplankton community. The selected pigments were expressed in both concentration (nmol g^{-1}) and relative percentage of the total marker selected

Pigment name	Main taxonomic group affinity	Broader groups	Grouping referred to Fig. 5.2 and 5.3.
Canthaxanthin	Cyanobacteria	Cyanobacteria	Cyanobacteria
Zeaxanthin	Cyanobacteria		
Echinenone	Cyanobacteria		
Oscillaxanthin	Oscillatoriaceae		
Aphanizophyll	N ₂ -fixing cyanobacteria		
Isorenieratene	Green sulphur bacteria (Brown group)	Phototrophic Bacteria	Sulphur bacteria
Okenone	Purple sulphur bacteria		
Lutein	Chlorophytes	Algae	Green algae
Alloxanthin	Cryptophytes		Flagellate algae
Diadinoxanthin	Dinoflagellates		
Diatoxanthin	Diatoms		
Fucoxanthin	Diatoms and crysophytes		Siliceous algae

Table 5.2- Marker pigments and their main taxonomic group affinity. Column 4 shows the grouping represented in the stratigraphic diagram of figures 2 and 3 following Buchaca et al., 2005.

In order to identify main factors driving change in Lake's Montcortès aquatic community, pigment data was compared with previously published data sets from already existing paleoenvironmental reconstructions carried out in the lake and from the same core. We used proxy data from Trapote et al., 2018a as environmental variable or predictors (Table 5.3). Table 5.3 shows the selected proxy-data and the given indicator value according to the author's interpretation.

Type	Units	Selected indicators	
		Variables	Related process
Pollen grains	%*	MEADOWS/PASTURES	Changes on land use
Pollen grains	%*	CROPS	
Pollen grains	%*	CULTIVATED TREES	
Charcoal particles	INFLUX	CHAR I (Charcoal <100 µm)	Changes on soil erosion
Fungi spores	%	GLOM (<i>Glomus</i> -fungi)	
Charcoal particles	INFLUX	CHAR II (Charcoal 100-500 µm)	
Algal remains	%	PEDIASTRUM	Changes on lake's trophic state
Pollen grains	%	TYPHA	
Pollen grains	%	CANNABIS	Changes on <i>Cannabis</i> related activities

Table 5.3- Selected proxy data as environmental variables and its given indicator value following Trapote et al 2018a.

Stratigraphic diagrams were plotted with Psimpoll 4.27 software (Bennet, 2009) and divided into zones using Optimal Splitting by Information Content method (Bennet 1996).

Principal component analysis (PCA) was used to summarize data about pigment data set composition with linear analysis option being used because early detrended correspondence analysis (DCA) revealed short gradient lengths (< 2.5) for DCA axis 1 (Šmilauer and Lepš 2003). All analyses were performed on Hellinger transformed data (Legendre and Gallagher 2001) since in this case, and after several tests, Hellinger transformation gave data distributions closer to normality.

Rate-of-change (RoC) of community data was used to estimate the amount of compositional change per unit time in the stratigraphical data (Birks 2012). In this case, RoC was estimated by applying Euclidean distance as dissimilarity coefficient on Hellinger transformed data. It has been performed on pigment data and algae remains data, as well as on pollen data for terrestrial plants (excluding *Cannabis*) and aquatic plants (including all taxa). Proxy data used is expressed in influx units to avoid artifacts derived from changes in sediment accumulation rates. RoC analysis was used to evaluate magnitude and synchronicity/non-synchronicity of changes occurring among sets of biological indicators corresponding to three different environments: in-lake environment (pigment and algal remains), transitional area between lake and catchment (pollen from aquatic plants), and on the catchment itself (pollen from terrestrial plants); it will help to test the connection

between lake and catchment. Correlations among RoCs were performed using Pearson's product-moment correlation coefficient to quantify its trend similarity.

Variance partitioning was used to estimate the explanatory power of the selected predictors and their interactive effects as related to the response variable (pigment data). Forward selection as well as collinearity test were used to reduce redundancy between the explanatory variables. The significance of each remaining variable was tested with permutation tests for constrained analysis (with 999 unrestricted permutations) (Borcard et al., 2011).

Multivariate analysis and significance test, RoC calculations and correlation coefficients were performed with the R statistical software (R Development Core Team, 2009). We used the Vegan package (Oksanen et al., 2012) for PCA, RDA and significance tests; the paleoMAS package (Correa-Metrio et al., 2011) for estimating RoCs and; PerformanceAnalytics (Peterson et al., 2018) for correlation coefficients.

5.3- Results and interpretation

5.3.1- Pigment sedimentary record.

The stratigraphic profile (concentration units; nmol/g) of the pigment data set is shown in (Fig. 5.2). Marker pigments are grouped by main taxonomic group affinity as indicate in Table.5.2. The plot shows general algal production indicators including cyanobacteria (β -carotene), a summary diagram of main algal groups relative abundances (%) and chlorophyll *a* and *a*-phorbins ratio (chl-*a*/*a*-phorbins) which gives information about pigment preservation (Buchaca et al., 2007). Common trends are identified to describe sedimentary pigment behavior from the bottom to the top of the sedimentary sequence. Overall, cyanobacteria groups dominates in concentration units and relative abundances (more than 50%), except for the most modern samples (last 20 years, from ~1980 CE to the present) when phototrophic sulphur bacteria and at less extent, other groups as well, results in a growing prominence. Low concentrations are common from the beginning of the sequence in ~ 1423 CE until ~ 1480 CE. Thereafter, all groups peak near to ~ 1490 CE to decrease afterwards, when they stabilize showing a relatively constant pigment concentration for the next 322 years until ~ 1840 CE. From this point onwards, all pigment concentrations decrease to reach very low values around 1850 CE and remained low until ~ 1900 CE. Pigment concentrations increase again from ~1920 CE to ~ 1980 CE and, most of them; reach its maximum values during this period. B-carotene, indicator of total algae

biomass, also records these general changes. From ~ 1922 to 1992 CE a general increase and higher abundance of primary producers is recorded in comparison with the rest of the sequence sulphur bacteria records major changes compared with other groups, oscillating from very low values, almost disappearing (i.e. from ~ 1850 to 1910 CE) to reach the highest concentration values of the record (i.e. from 1970 CE to nowadays). . From ~1980 CE to the present day, cyanobacteria decrease and other groups as diatoms and cryptophytes dominate the algal assemblage for the first time in the full record. On the other hand, chl-a/a-phorbins ratio remained roughly stable indicating that there were no major changes on sedimentary pigment preservation conditions. Major change on chl-a/a-phorbins is observed from ~ 1850 to 1910 CE.

The vertical zonation of the sediment core established 8 statistically significant zones. Those zones with less than 5 samples were added to the contiguous zone and maintained as sub-zones. Therefore, six main zones were identified on the pigment sedimentary record. Moreover, three time subsets have been established based on zonation and coinciding with main phases of change (Fig.5.2).

Zone 1 (Z-1; from ~ 1423 to 1481 CE) shows low concentrations of photosynthetic compounds where cyanobacteria dominated. This scenario abruptly change in Zone 2 (Z-2; from ~1481 to 1530 CE) where all groups peak near year 1485 CE. In this zone, sulphur bacteria's peak stands out followed by the peaks of cyanobacteria and N₂-fixing cyanobacteria. The transition between Z-1 and Z-2 and the synchronous increase of all algal groups suggest a change towards more suitable conditions for growth, with no differential response among algal groups. High sulphur bacteria concentrations suggest persistent lake stratification and long lasting bottom waters anoxia.

Zone 3 (Z-3; from ~1530 to 1852 CE), which last for 322 years, records (in average) lower concentrations than in the previous zone and shows a roughly stable trends in pigment data except for sulphur bacteria, which fluctuate broadly and shows short periods of concentration values below 5 nmol/g with subsequent recovery to values above 40 nmol/g. This zone is interpreted as a period of phytoplankton dynamics stability, where the major changes on pigment signal are related with fluctuations of the water column oxygenation and/or light availability on profundal waters as indicated by fluctuations in sulphur bacteria (Van Gemerden and Mas, 1995).

Zone 4 (Z-4; from 1852 to 1915 AD) comprises 2 subzones. At sub-Zone 4.A (Z-4.A; from ~ 1852-1886 CE), all groups experience significant decrease and some of them, sulphur

bacteria and dinoflagellates, almost disappear. This is maintained in sub-Zone 4.B (Z-4.B; from ~1886-1915 CE) where sulphur bacteria and dinoflagellates remain with very low concentration values and diatoms decrease while cyanobacteria and N₂-fixing cyanobacteria significantly increased together with chlorophytes. As inferred by the almost absence of sulphur bacteria signal, increases in water oxygenation seem to take place during this period and therefore, changes in water mixing regime which probably benefit cyanobacteria, N₂-fixing cyanobacteria and chlorophytes.

In Zone 5 (Z-5; from ~1915-1978 CE) pigments concentrations continue to increase. All groups except sulphur bacteria display larger values than in previous zones. This zone seems to mark a period of transition where anoxia restarts and/or lasts for longer periods and, although cyanobacteria and N₂-fixing cyanobacteria still dominate, diatoms, chlorophytes and cryptophytes have greater representation.

Finally, Zone -6 (Z-6; from ~ 1978-2013 CE) comprises 2 subzones. At sub-Zone 6.A (Z-6A; from ~ 1978-1992 CE) the increasing trend continued on sulphur bacteria to reach values above 90 nmol/g, which suppose 9 fold increases compared with the previous zone. Something similar, at lower scale, happens with chlorophytes which reach its maxima in this sub-zone, as well as dinoflagellates at a lesser extent. Nevertheless, cyanobacteria and N₂-fixing cyanobacteria decrease progressively and diatoms almost disappear. The absence of signal for cryptophytes in this sub-zone can be attributed to detection problems as high values of bacteriochlorophylls in this case obscured cryptophytes signal and hampered quantification of HPLC peak. In sub-Zone 6.B (Z-6.B; from ~ 1999- to 2013 CE) diatoms, cryptophytes and sulphur bacteria increase and reach their maxima, while chlorophytes decrease and cyanobacteria and N₂-fixing cyanobacteria also decrease to slightly increase in the most modern samples. This is the first time in the full sequence that cyanobacteria do not dominate the assemblage and decrease from relative abundances above of 50% to ~20%.

Lake Monticortés sedimentary pigments grouped by main taxonomic group (nmol/g; core MONT-0713-G05)

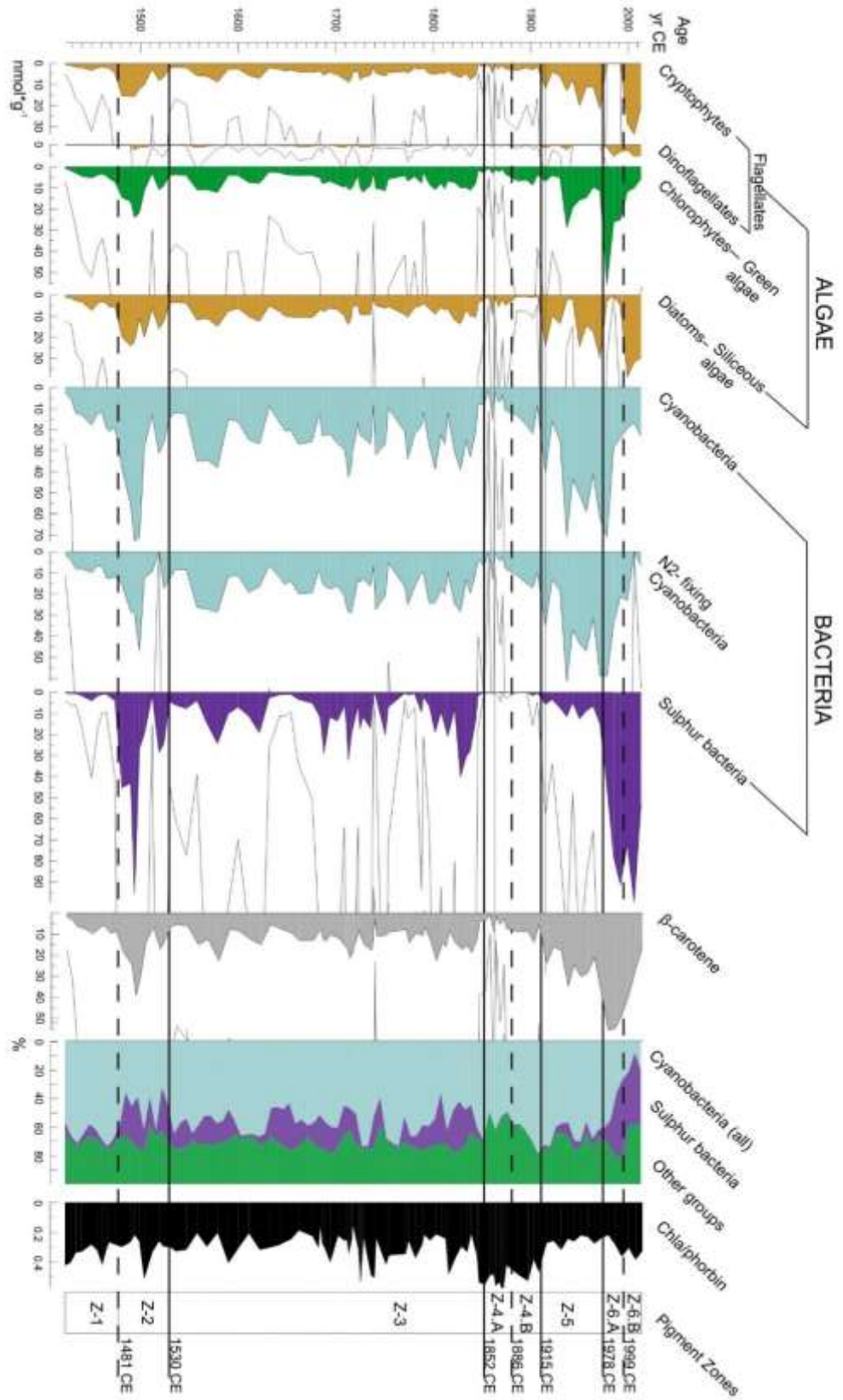


Figure 5.2. Time series of main algal groups and total biomass indicators (β-carotene). Summary diagram of relative abundances of the aquatic photosynthetic community and chl-a:phorbol ratio as an index of pigment preservation state.

5.3.2- Principal component analysis

Principal component analysis of the twelve chemically stable pigments used in this study (Fig.5.3) has been performed to provide a better background for the interpretation of the stratigraphic profile. Two principal components were discriminated, which captured 64.4% of the variance and resulted significant under the broken stick model (Fig.5.3A). PC axis 1 explains 44.1 % and PC axis 2 20.3%. To simplify the graphic presentation of the PCA biplot, those pigments with correlation loadings lower than 10.11 were eliminated from the main plots (diadinoxanthin, canthaxanthin and fucoxanthin).

PCA distinctly and orthogonally separates bacteria and cyanobacteria related pigments (axis 1) from algae related pigments (axis 2). Axis 1 is positively and highly correlated with okenone and, to at less extent, with isorenieratene; both are markers of anoxic sulphur bacteria (obligate anaerobes), and is negatively related with cyanobacteria (all) indicated by aphanizophyll (N_2 -fixing cyanobacteria), zeaxanthin and enchinone (cyanobacteria) and oscilaxanthin (cyanobacteria-oscillatories) (Table 5.2). This opposite relationship might be interpreted as a nutrient gradient from bottom to surface waters. Nutrients are released from the bottom to epilimnetic waters when stratifications breaks during mixing periods favoring algal growing, in this case mainly cyanobacteria, and hampering sulphur bacteria development under oxic conditions (Butz et al., 2016). On the other hand, PC axis 2 has a clear and positive correlation with Diatoxanthin (diatoms) and Alloxanthin (cryptophytes) while it is weakly and negatively correlated with Lutein (chlorophytes). Axis 2 would relate with turbulence conditions that occur during mixing periods. Turbulence is commonly required for planktonic diatoms to maintain their position in the photic zone (Rühland et al., 2015). Such mixing in lake Montcortès occurs during winter when cryptophytes are the dominant genera (Trapote et al., 2018a), which is in agreement with the high correlation between diatoxanthin and alloxanthin on axis 2. Among cyanobacteria, PCA biplot also shows grouping along axis 2 although with low loadings. General pigments of cyanobacteria are positioned on the positive part of the axis 2 which agree with higher nutrient availability and turbulence. While those belonging to N_2 -fixing and oscillatoria are positioned on the negative part which agree with nutrient restriction in the epilimnion and stratified waters since N_2 -fixers and oscillatoria, has competitive advantage on well-stratified lakes due to its oscillation motion ability (Reynolds 1984).

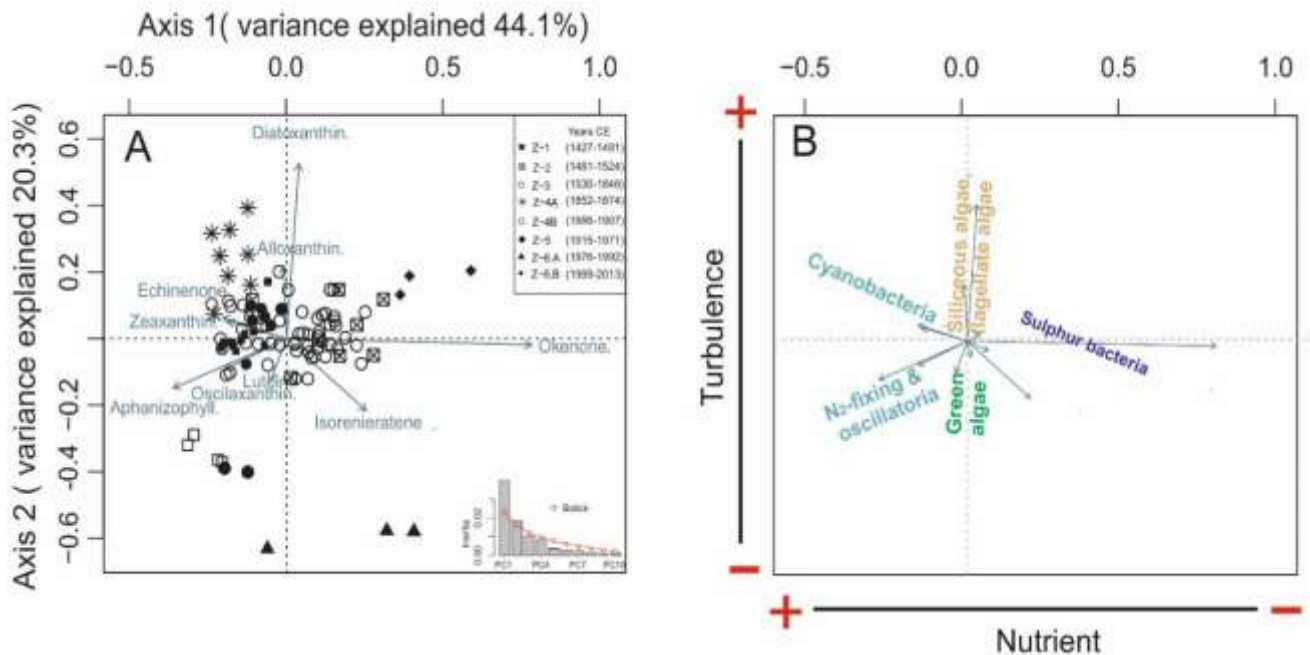


Figure 5.3- A) Principal component analysis of pigment data on the two principal axis; B) Main algal groups distributed along PCA axis and interpretation.

5.3.3- Rate of change of Lake Montcortès biological proxies

Trends in temporal patterns of change on aquatic and terrestrial community are broadly similar (Fig.5.4A). This is also evident by looking correlation coefficients among RoCs of the different data sets that show values from 0.4 up to ~ 0.7 all at $p \leq 0.001$ significance level (Fig.4B). The highest correlation coefficients among all data sets belongs to the relationships between terrestrial and aquatic plants and between pigments and terrestrial plants (0.69 and 0.62 at $p \leq 0.001$, respectively) which might be interpreted as an indicator of the close relationship between in-lake and catchment processes (Fig.5.4). RoC trends are all similar, but there are differences in the magnitude of changes, i.e terrestrial pollen data show the lowest RoC values, whereas aquatic plants and algal remains show the highest values. RoC stratigraphic diagram (Fig 5.4) has been divided into the same zones and time subsets as the pigment stratigraphic profile in order to better compare periods of change, regarding all data sets and photosynthetic community. major fluctuations occurred between 1530 and 1852 CE (on subset B).

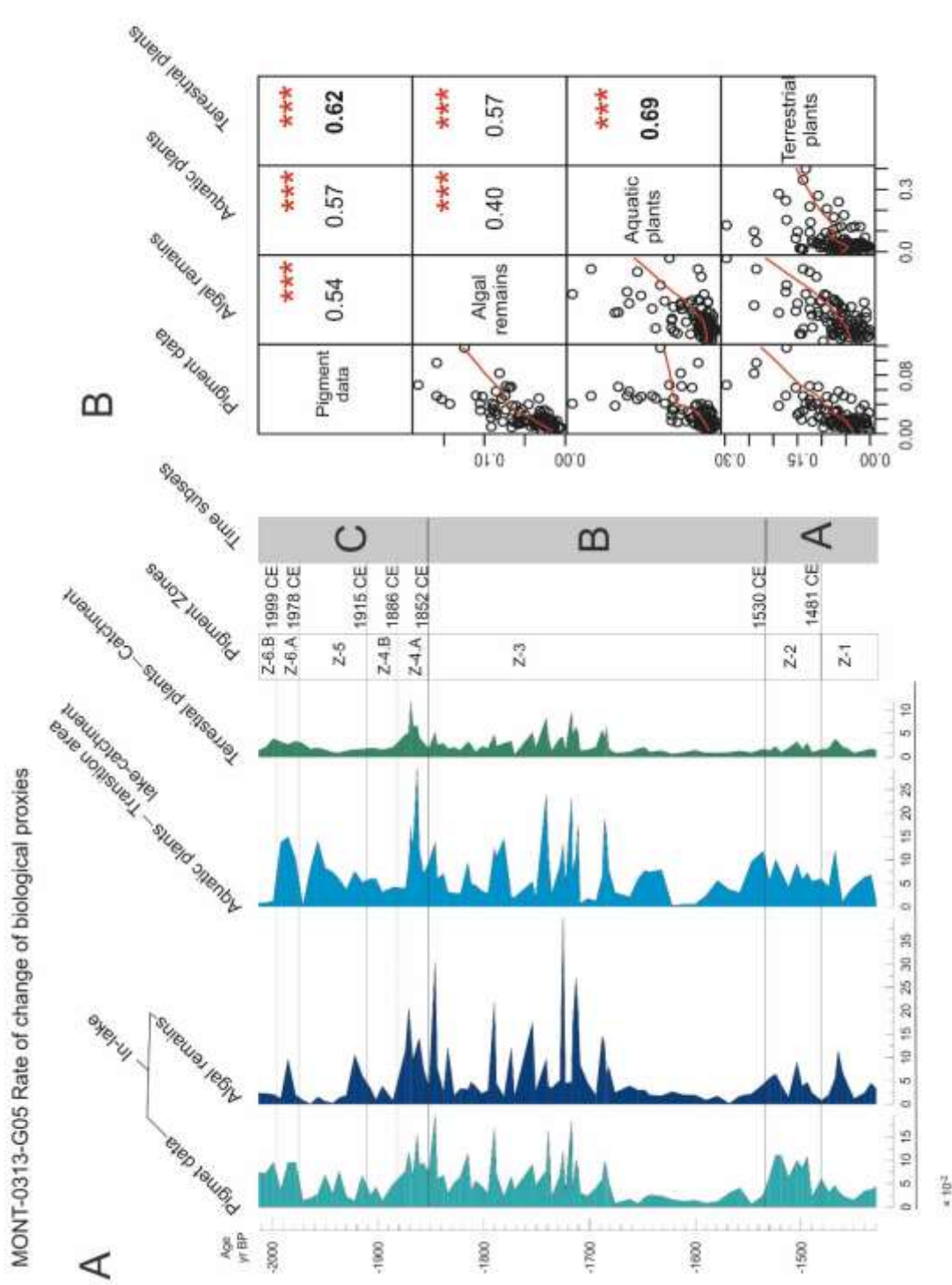


Figure 5.4. A) Diagram of the rate of change of biological proxies (pigments, algal remains, and pollen from aquatic and terrestrial plants. B) Correlation matrix among RoC of biological proxies: on the upper half correlation values and in the lower half the scatter plot corresponding to each correlation. Significance at different levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

5.4.3- Variance partitioning analysis

Variance partitioning analysis (VPA) is a statistical method used frequently in paleoecological studies to identify the relative magnitude of the effects of potential forcing factors on lake ecosystem through time (Simpson and Anderson 2009). Therefore VPA has been used here to estimate the fraction of historical variance of pigment fossil assemblages explained by categories of selected predictor variables (factors) and their combined interactions. Table 5.4 shows explanatory variables remaining as the main sources of variance after forward selection. Variance inflation factor was calculated for all predictors and resulted below 3 units in all cases, indicating low collinearity, which is a desired condition to avoid inflated proportions of explained variance and redundant relationships among factors on the application of VPA analysis (Broadcard et al., 2011).

UNITS	SELECTED VARIABLES	SOURCE OF VARIANCE (Factors)
%*	CROPS	Changes on land use
%*	CULTIVATED TREES	
%	GLOM (<i>Glomus</i> -fungi)	Changes on soil erosion
%	PEDIASTRUM (Chlorophyceae)	Changes on lake's Trophic state
%	CANNABIS	Changes on <i>Cannabis</i> related activities

Table 5.4- Remaining variables after forward selection used on VPA.

VPA results are always expressed in proportion values of adjusted R^2 ($AdjR^2$). Results obtained from VPA analysis corresponding to the full record, suggest that in the absence of climatic data, changes on land use is the factor that strongest correlates pigment data explaining 14.4 % of a total 22.8 % variance at $p \leq 0.001$ (Fig.5.6I). In this case, all factors (testable fractions) resulted significant (Fig.5.6I). Shared proportions among factors are not very common and with low values indicating factor independence and low collinearity and/or the combined response in relation to aquatic data does not follow a linear function.

To investigate which factor was the most closely correlated with aquatic community changes during main periods of change, VPA has been performed on the three time subsets defined following the obtained stratigraphic zonation (Fig.5.2). Table 5.5 shows time periods comprising each subset and number of samples.

Subset A, from ~ 1423 to 1530 CE, VPA shows that 29.3% of the pigment variance was explained by our selected factors at $p < 0.05$ level of significance (Fig.5.6II) Land use is the

most important source of variance (16.9 %). It is worth to mention that soil erosion obtained negative values, which indicates that the proportion of variance explained by soil erosion in this case is worse than a set of random deviates (Borcard et al., 2011).

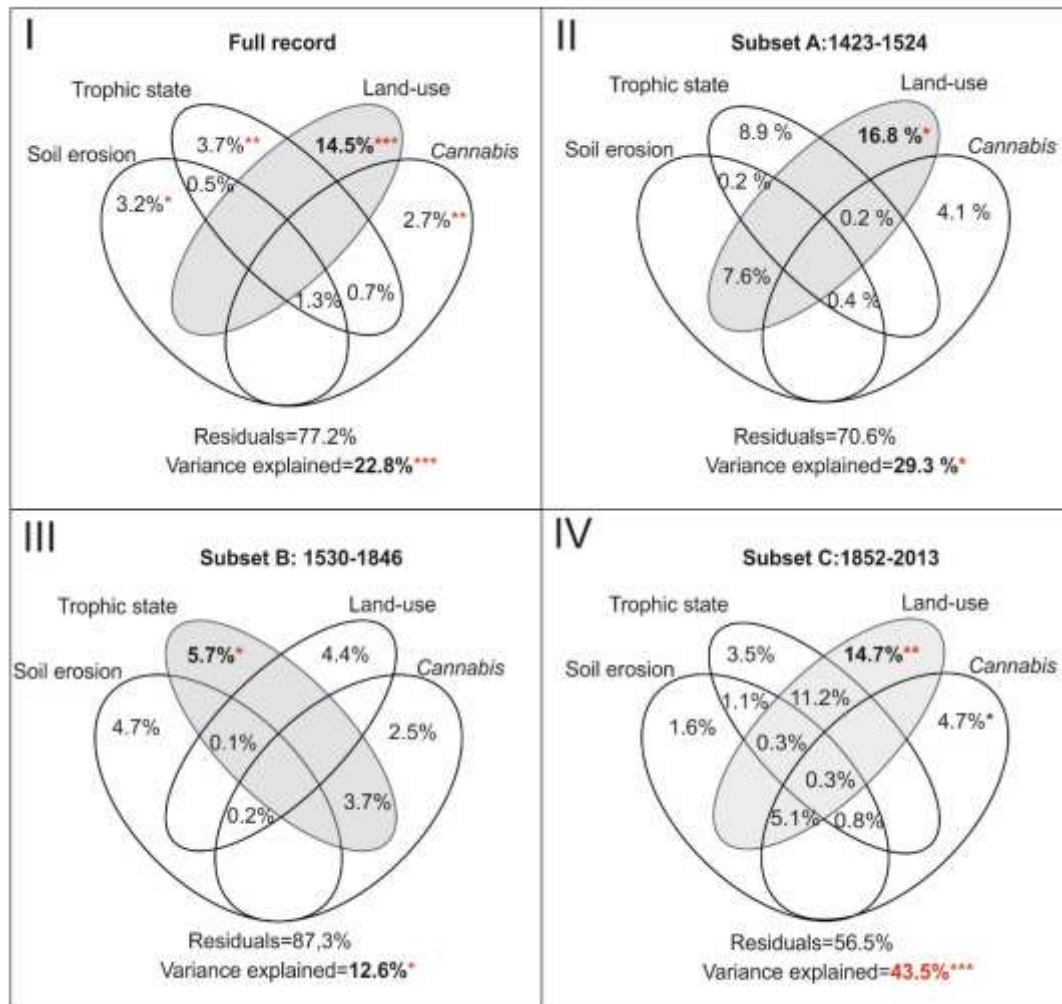


Figure 5. 6- Variance partitioning analysis (VPA) of the full record (I) and three time slides (II to IV). Significance at different levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Subset B, from ~ 1530 to 1852 CE, despite being the longest time-subset (322 years) and with the highest number of samples, contains less variance (12.6 % at $p < 0.05$), if compared with the rest of datasets (Fig.5.6 III). Changes in trophic state is the most important source of variance (5.7 %) and the only one significant at $p \leq 0.05$, while changes in *Cannabis* related activities is the factor with lower contribution with 2.5% of variance explained. Nevertheless, interactive effects with *Cannabis* and nutrient loading explained higher variance than *Cannabis* unique effect (3.7%).

Subset C, from ~ 1852 to 2013 CE, is the one that explained more variance among all subsets and the entire record at high significance level despite the low number of samples.

A total of 43.5% of total variance at $p < 0.001$ significance level is explained in this subset (Fig.5.6IV). Changes of land use appeared again as the most important source of variance accounting for 14.7 % of variance at $p \leq 0.01$ significance level. This subset presents greater number of interactive effects (five), when compared with other VPA indicating major coincidence of correlation among factors. Interactive effects that accounted for most of the variance are the combination of nutrient loading and land use explaining 11.2 %.

VPA Time Sections				
Subset	Time period	Years	Zones	Number of samples
A	1423-1525	107	Z-1, Z-2	18
B	1530-1846	322	Z-3	51
C	152-2013	161	Z-4A, Z4-B, Z-5, Z-6A, Z-6B	27

Table 5.5. Time subsets selected.

5.4- Discussion

Much of the following discussion focuses on to describe changes in aquatic photosynthetic community explained by selected variables (factors) (Table 5.4). Such variables would be mostly related with human activities. Other factors such as natural variability, climatic forcing, sedimentation process and biotic interactions will be discussed complementary when needed. To better organize discussion, it has been divided according to the three selected time subsets defined in the result section and that correspond to main changes occurred on aquatic community (Fig.5.2, Table.5. 4).

Overall, the photosynthetic community of Lake Montcortès was characterized by the high dominance and relative abundances on photosynthetic bacteria both, oxygenic (cyanobacteria) and anoxygenic (sulphur bacteria) as previously evidenced by (Vegas-Vilarrúbia et al., 2018). Now, with the complete pigment spectrum presented here we have been able to confirm this scenario and asses its relationships with other algal groups. Along time, all algal groups maintained rather constant relative proportions, except for the last 20 years, when cyanobacteria progressively lost their dominance. The decrease in cyanobacteria was in concordance with most recent monitored conditions (2013-2015), where cyanobacteria appeared to be essentially absent under the conditions of study (Trapote et al., 2018a). However, in the recent past, cyanobacteria blooms were recorded during 1970's on lake surveys (Modamio et al., 1988; Camps et al., 1976), which coincided with high concentration values on the record near the same date (Fig.5.2). Such coincidences suggest that pigment signal was recorded reliably on Lake Montcortès.

Subset A from ~ 1423 to 1524 CE.

Despite the low number of samples corresponding to this period VPA analysis resulted significant at $p < 0.05$ explaining 29.3 % of total variance. Changes in land use and lake's trophic state were the main factors explaining variance, although none of the factors were statistically significant. The peak on total productivity and in all algal groups with no exception on ~ 1500 CE stands out for cyanobacteria (all groups) and sulphur bacteria. High abundance or dominance of cyanobacteria are typical indicators of eutrophic lakes (Leavitt and Hodgson 2001) and often related with human activities (Taranu et al., 2015). The possibly that eutrophic conditions increased in relation with high human pressure in the area contrast with the documented decrease of human population registered at that time on Montcortès vicinities (Rull et al., 2011; Trapote et al. 2018a). Although existing palynological records indicate low human pressure and land abandonment (Rull et al., 2011; Trapote et al. 2018a), it is documented during this period, that human activity around lake Montcortès was still ongoing, mainly related with livestock rising near the lake (Trapote et al. 2018a). High amounts of livestock around the lake would be in agreement with nutrient increase in the water body. The response of photosynthetic community to increases in nutrient loading can lead to turbidity increases due to abundant phytoplankton development which result on restricted light penetration on deeper waters (O'Sullivan and Reynolds, 2003). Some cyanobacteria species possess traits that give them some advantages over other groups under low-light limitations conditions (O'Neil et al., 2012). Consequently, cyanobacteria would have taken advantage of that situation and bloomed. Moreover, persistent anoxic conditions are consistent with a high productive environment (Butz et al., 2016; McGowan et al., 2008). It seems that some threshold was trespassed during this subset and photosynthetic community responded consequently increasing productivity and favoring anoxia. The subsequent and rapid decrease on productivity and in all algal groups can be explained by nutrient restriction resulting of long periods of stratification hampering nutrient cycling from bottom to upper waters and limiting algal growth (Butz et al., 2016). The short-term durations of this change suggest that the perturbation occurred was brief and the algal community rapidly recovered.

Subset B from ~ 1530 to 1846 CE.

Among the four VPA analysis performed, this is the only one where changes on land-use was not the main factor explaining pigment data variability. Changes on trophic state was the most important factor (5,7%) while changes on *Cannabis* related activities is the factor that

explained less variation in this subset (2.7%). The latter contrast with the available information pointing that *Cannabis*' 'industry' was one of the most important human activities of the area at that time (Rull et al., 2011; Trapote et al., 2018a). Taking into account such scenario, we would expect higher value of variance explained by the *Cannabis* related factor. Shared proportions among factors may explain this apparent discrepancy. Changes in *Cannabis* related activities correlates with two of the only three interactive effects shown by VPA. Such relationships indicate that *Cannabis* factor linearly relates with changes in trophic state explaining a 3,7% of variance together. It might be interpreted as a synergy, while others on relation with changes in land use and soil erosion do not exists. The lack of relationships between changes on *Cannabis* related activities and the other factors except changes on trophic state, supports further ideas pointing that *Cannabis* was not cultivated around the lake. Instead, Lake Montcortès was used for *Cannabis* retting (Rull et al., 2011; Trapote et al., 2018 and literature therein) and its imprint on the aquatic community directly may be related with changes in the trophic state, instead of changes in land-use or soil erosion. However, changes in land-use and soil-erosion by themselves explained similar amounts of variance in comparison with changes in trophic state, although with no significance. However, their shared proportions with other factors were absent or very low > 0.3%. This fact may indicate no synergetic effect among them responding to different causes.

The 'stability' observed on algal community during this period, in comparison with the other two periods (A and C,) results unexpected in a rising human pressure scenario (Trapote et al., 2018a). Likewise, evidences of in-lake changes towards eutrophication from 17th to late 19th were found in former studies using independent proxy data (Rull et al., 2011, Trapote et al., 2018a, Vegas-Vilarrúbia et al., 2018a). In this context, we would expect significant changes in pigment data reflected on total productivity and/or a differential response among algal groups. On the contrary, no major changes in total productivity (β - carotene) and no variation on main algal groups relative composition is observed during more than 300 years (Fig.5.2), except for sulphur bacteria, which show the highest variability. One of the possible explanations is that a proportion of nutrients entering the lake due to hemp retting were not readily soluble and available for algal uptake (Cooke and Williams 1973), and therefore photosynthetic community did not suffer considerable changes related with nutrient balance. Another explanation could be that ecosystem structure and function at that moment had been able to buffer the consequences of prolonged and continuous nutrient loading process delaying its response to external changes (Bunting et al.2016, Scheffer et al.

2012; Dubois et al 2018). Light limitation derived from an increase of siliciclastic inputs and particulate organic matter to the lake from soil erosion and *Cannabis* retting also might explain this stability, as light limitation does inhibit algal growth (Huisman and Weissing 1994; Leavitt et al 2003). In Montcortès, light limitation hypothesis would be supported by the significant sediment shift occurred from 1754 CE onwards due to a higher frequency of turbiditic events (Rull et al.2011; Corella et al 2012) being a likely cause of algal growing inhibition. Moreover, this 'steady-state' condition took place entirely within the Little Ice Age (LIA) cooling (Morellón et al 2012). Lower temperatures during LIA may have shortened growing season and contributed to primary production stability. Single or combined effects of all factors explained above would explain the aquatic community steadiness during this period. Furthermore, meromictic conditions may have helped to maintain algal community stability by being a nutrient control factor and buffering the potential eutrophication response (Vegas-Vilarrúbia et al., 2018; Butz et al 2016). However, the situations suggested above do not explain the apparent inconsistency about no changes on productivity and relative pigment composition, even changes on algal remains composition has been observed (Trapote et al., 2018). In this case, it can be explained by the specific differential responses. Likely, some phytoplankton species would have been favored to nutrient increase, but not recorded by the overall pigment signal, as it may not imply changes on total productivity or changes on relative algal composition; just a shift in species dominance within algal groups.

Subset C from ~ 1852 to 2013 CE.

The low productivity and the general decrease on pigment concentration recorded at the beginning of this subset (~ 1840 to 1880 CE) contrast with the relative high pigment concentrations that generally dominate the record. This reduction coincided with heavy rainfall episodes occurred in lake Montcortès. During this period, higher frequency of detrital inputs and mass-flow turbidities triggered by major floods and mass movements took place in Montcortès (Corella et al., 2012 and 2014). Increased water turbidity and improved bottom oxygenation resulting from increases on runoff and mass movements would explain a general decrease on primary production and the disappearance of sulphur anoxic bacteria. It also agree with the rapid recovery of cyanobacteria attending to its competitive advantage under low light intensities (Carr and Whitton 1982; Whittton and Potts, 2007). This circumstance has also been observed in other meromictic lakes where strong pulses of terrigenous material were able to break up the stratification and, after few years, the system turned back to meromixis (Butz et al., 2016). Heavy rainfall signal has also been recorded by

pollen data evidencing the lake-catchment connection through the recorded signal during intense climatic events. In any case, poor preservation of pigments to explain lower concentrations during this period can be ruled out, because the chl-a/ a-phorbins ratio show higher values, thus indicating better preservation (Buchaca et al., 2007).

The main feature that define this period is the progressive increase of primary production after a generalized minimum (~1840 CE). Despite the fact that the predictors used in this study can be indirectly related with natural/ climatic processes, they still are mostly human related indicators. Therefore, given the relative high variance explained by VPA, we would interpret that human pressure increased around the lake during this period and aquatic community responded in consequence. As interpreted in subset A, increased productivity as well as cyanobacteria dominance are in concordance with eutrophication process (O'Neil et al. 2012; Mikomängi et al 2016). This scenario contrasts again with former studies carried out in Lake Montcortès that documented field abandonment and population moving to the capital cities with the onset of industrialization (~1850 CE). Actually, since population records exists, from 1850 CE to nowadays the region registered a constant decrease to reach the lower number population known to nowadays (Vegas-Vilarrúbia et al., 2018 on supplementary material). During last millennium, extensive agro-pastoral practices were common on cropping and animal feeding which were more intensive from 18th onwards. From 18th onwards, such practices implied higher animal stocking densities, field manuring and the use of fertilizers to enhance crop production altogether, in a framework of intensive *Cannabis* 'industry' (Rull et al., 2011; Trapote et al 2018a and literature therein). Such activities as well as fire occurrence might lead to excess of soil fertilization causing N and P soil surpluses that can leach into downstream aquatic ecosystems (Carpenter 1998; Bennet 2001; Bunting et al., 2016). Such nutrient surpluses on soils can last for millennia and therefore act as a non-point and diffuse fluxes of nutrients inputs to the aquatic ecosystem even when the activity has been interrupted (Carpenter 2005). The non-point nutrient sources (i.e. from soil leaching, groundwater infiltrations and atmospheric deposition) are difficult to quantify because they are often intermittent, depending on the weather effects. Moreover, they can reach the water body even coming from remote regions (frequently from uplands to lowlands) (Carpenter et al. 1998, Bennet et al., 2001). Many factors affect the intensity and frequency of nutrient mobilization and its delivery to water bodies. Features as soil texture and drainage capacity, the type of land exploitation (i.e. forage, grazing or crop production) and the method used on agro-pastoral practices on a specific place will affect diffuse nutrient delivery to water bodies (Ulén, et al., 2007). Nutrient loading to the lake from

soil nutrient surpluses derived of the historical and intense human activity in Lake Montcortès catchment, might explain the increased productivity during this period even the decaying human population on the area. Furthermore, the progressive increase in primary production coincided with the intensive use of fossil fuel burning derived from industrialization (late 19th century) and the invention of Haber-Bosch process for industrial ammonia (1913 CE) linked mostly to soil fertilizer production (N and P) that was commonly and massively used until restriction (Galloway and Cowling, 2002). Other diffuse nutrient sources, which can reach the lake by atmospheric deposition, are derived from high-pressure and high-temperature fossil fuel combustion that release significant quantities of reactive oxidized forms of N to the atmosphere (Carpenter 1998; Galloway et al., 1995; Galloway and Cowling, 2002). Increases in primary production and changes in isotopic N signal related to this phenomenon are observed in a broad range of lakes and water bodies on the northern hemisphere (Holtgrieve et al., 2011, Wolfe et al., 2013). Atmospheric deposition signal linked to the onset of the industrialization process in lake Montcortès was clearly recorded as evidenced by the increase of heavy metals (mostly lead and mercury) associated with fossil fuels (gas-oil and coal respectively) and mining intensification (Corella et al., 2017). Enrichment by atmospheric deposition has been observed on a broad range of European lakes and has been demonstrated that a point-of-change was trespassed after 1850 CE when the majority of the European lakes studied showed the first clear evidence of nutrient enrichment linked to industrialization (Battarbee et al., 2011). Therefore, although human pressure was declining in the Pyrenees and in Montcortès vicinities by this time, diffuse nutrient inputs derived from historical intensive use of manure, soil fertilizers and atmospheric deposition, likely promoted algal growth as observed in other aquatic systems (Lami et al., 2000, Battarbee et al., 2011, Ulén et al., 2007). However, other factors, more related with in-lake process as could be changes in mixing regime and internal nutrient cycling might affected and promote such situation. More work is needed in this sense to better determine the dominant factor driving this change.

From 1970 CE to nowadays, cyanobacteria reduced and do not dominate anymore. Besides of being favored by/in eutrophic waters, other commonalities among cyanobacteria, include being highly competitive for low nutrient concentration (mostly N₂-fixers). Hence, while some blooms are associated with eutrophication, other cyanobacteria dominate when inorganic N and P are low (O'Neil 2012). On Montcortès record cyanobacteria and N₂-fixing cyanobacteria pigments have had high relevance and a very similar behavior during the full record. Such situation may attest to the occurrence of nitrogen limitation at the end of the

growing season after enhanced phytoplankton production when N₂-fixers would bloom (Fisinger et al., 2014). The significant reduction of cyanobacteria on the most recent times might be interpreted as the stabilization or recovery of the lake during the last 20 years after a long time of low anthropogenic pressure in the area. To validate this hypothesis, limnological conditions of Lake Montcortès should be assessed on the absence of significance human influence by studying a sediment core spanning back several millennia before human intensification in the area. More work in combination with other limnological indicators, for example diatoms or sediment nutrient content analyses is required to better constrain main causes affecting aquatic community in lake Montcortès.

Synchronicity

Although the timing and magnitude of change in the pollen data (catchment changes) and aquatic data (in-lake changes) (Fig. 5.4) were not always perfectly matched there is clear and strong link between the lake system and catchment. This is evident on figure 5.4 where RoC trends are very similar among all indicators as well as the correlation values at high significance level. It is also illustrated on figure 5.7 by comparing pigment and pollen stratigraphic zonation that resulted almost the same. Strong parallelism in proxy data would indicate 'orchestration' of changes occurring on the water body and catchment area probably related with a common factor (human, climate, or in indirectly both). However, much of the variation remains unexplained. Further work is needed to completely explain factors affecting Lake Montcortès, which should include climatic data to assess both natural and human induced variability.

5.5- Conclusions

We carried out a high-resolution aquatic photosynthetic community dynamics reconstruction in Lake Montcortès. Three main periods of change have been identified and although pigment concentration varies broadly within the record, relative proportion among algal groups have been maintained, except for the last 20 years where cyanobacteria do not dominates the algal assemblage anymore and anoxic conditions hardened until nowadays.

Our methodology of multi-proxy investigations in combination to multivariate analysis allowed us to identify main human-related processes and its synergies driving aquatic community change for the full record and for the three time subsets identified. Land use appeared to be the most important factor except for subset B (1530 -1846 CE) where changes on trophic state and its synergies with changes on *Cannabis* related activities accounted

significant proportions of variance and sustained hemp retting as a main activity affecting Lake Montcortès during more than 300 years. In spite of the low human presence in the area since the beginning of industrialization, last century recorded harder eutrophication signals on pigment data probably due to non-point nutrient sources from the historical land use and atmospheric deposition derived of the industrialization process. There is still an important proportion of remaining variance to be explain that might belong to other factors not accounted for directly in this study, i.e. climate. Further work should be focus in to obtain independent environmental and climatic indicators to better asses the diversity and complexity of factors affecting aquatic community in Lake Montcortès its synergies. Efforts also should focus onto fine tune time resolution and aquatic community specific response i.e. by means of diatom analysis or sediment nutrient content to better asses their specific changes and causes and in-lake processes taking place. The extension of this study to the pre-impacted lake state, in order to define Lake Montcortès reference conditions would be interesting to assess transition between natural conditions towards human impacted and compare with nowadays lake conditions. This information could be useful to set management policies in other similar lakes.

5.6- References

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CHAPTER 6

General Discussion.

*“...aprendre a estimar les tares, les turbulències.
Descobrir-nos a poc a poc les carències.
Cuidar als amics com em cuiden ells a mi,
contar-nos les misèries amb formatge pa i vi.”*

Soundtrack: “Esbarzers”- La Gossa Sorda

In this section, a general discussion of all the results obtained in the preceding chapters will be presented together. Chapter 2 and 3 refer to modern sedimentary analogues as a tool for accurately reconstruct the sedimentary record, while chapters 4 and 5 focus on high-resolution paleoenvironmental reconstructions of changes occurring in the catchment (vegetation) and in-lake processes (phytoplankton community) and how they are related (lake-catchment system). As a result, this section has been divided into two subsections: Modern analogues and high-resolution paleoenvironmental reconstruction. Finally, possible directions of future work will be proposed.

6.1. Modern analogues: Two years, two different varves. Paleoenvironmental implications.

The analysis of the entire annual sediment flux and lake monitoring for two consecutive and climatically different years presented on chapter 2 and 3, revealed a strong seasonal trend for all studied proxies. It is in concordance with the annual rhythmic particle sedimentation needed for varved sediment formation (Ojala et al., 2012). Chapters 2 and 3 demonstrate that changes in calcite, pollen and diatoms were highly depending on seasonal succession of lacustrine and terrestrial life forms that, in turn, were modulated by environmental variables, thus confirming that calcite, pollen and diatoms can be used as a seasonal proxies representing specific processes in Lake Montcortès. Although two years of observations are not enough to demonstrate causal relationship between changes in proxy data and the environmental variables measured, this time lapse resulted enough to obtain high amounts and high quality data from the empirical observations. Now, with the combination of the information obtained in chapter 2 and 3, it is possible to construct a conceptual varve model for lake Montcortès and use it as a tool for future paleoenvironmental reconstructions accomplishing with the main propose of the modern analogues studies (Jackson and Williams 2004) and with one of the main aims of this thesis.

Between the two studied years, there appeared clear dissimilarities in terms of timing and seasonal signal recorded in the three proxies that have been potentially related with changes in temperature and precipitation (chapters 2, 3). Such differences indicates sediment record sensitivity to inter-annual variations. Figure 6.1 shows a scheme of the main varve differences corresponding to each year for each proxy. One of the first differences observed between the two years of study was the total amount of sedimented material being a 15% more for year 2014-2015. This might result in differences in varve thickness between years. Such differences were also in seasonal varve sublayers, due to considerable

differences in calcite amounts during spring/summer (chapter 2) (fig.6.1). With regard to the diatom sequence, the main difference between years lies on the breakage and dissolution of the diatom assemblage during spring of 2014-2015. The reason of this dissolution is unclear, although some limnological factors that differ between years can potentially explain this fact. Among factors known to affect diatom dissolution, the most common on natural systems are changes in water salinity, alkalinity and pH (Reed 1998; Ryves et al., 2006). Changes in pH and alkalinity were not significantly different between spring 2014 and 2015 (chapter 2). However, conductivity was slightly higher in 2015 in comparison with 2014 (chapter2), indicating higher water salinity during spring 2015 likely as a consequence of higher water evaporation and less precipitation during this season (Fig. 2.6 Chapter 2). In addition, 2015 was the year where higher amounts of precipitated carbonate coincided with higher rates of diatom dissolution. Poor preservation of diatom frustules also has been related with carbonate precipitation process due to higher lake water-pH at the time of carbonate deposition (Smol and Boucherle 1985). Partial diatom dissolution might result in a truncated or almost absent diatom spring signal during the warmer and dryer year 2015 (fig. 6.1b). In terms of pollen content, the main difference between the two years corresponded to total pollen influx, which was threefold in spring 2015 as compared with spring 2014 (fig. 6.1c). In comparison with calcite and diatoms, pollen appeared as a more confident indicator of seasonality. Pollen is the only one that maintained the same seasonal signal between years coinciding with the flowering season of most relevant pollen taxa (chapter 3), as inter annual-variability is expressed in terms of different pollen amounts between years (fig. 6.1c) (Chapter 3). However, looking at the other two proxies; periods of major calcite precipitation can fluctuate within spring, summer and fall (chapter 2) and diatoms may suffer breakage and dissolution depending of water conditions, Such situation would truncate diatom seasonal signal for a given year depending on weather and changes in limnological conditions (fig.6). The final resulting varve signal and configuration corresponding to 2013-2014 and 2014-2015 needs to be corroborated with a detailed varve analysis after consolidation of the sedimentary record, in comparison with the model proposed here.

The clearly different sediment signal recorded between the two consecutive studied years highlight how sensitive can be a system to inter-annual variability and the need to continue with lake and sediment traps monitoring to assess short-term changes (inter-annual) and to obtain enough data to identify long-term trends. Modern analogues studies are scarce and it is usually justified by the large amount of time needed to obtain results.

However, the quality and quantity of results obtained with this thesis makes it worthwhile, even though at the moment, it is not enough to demonstrate variable-process causality.

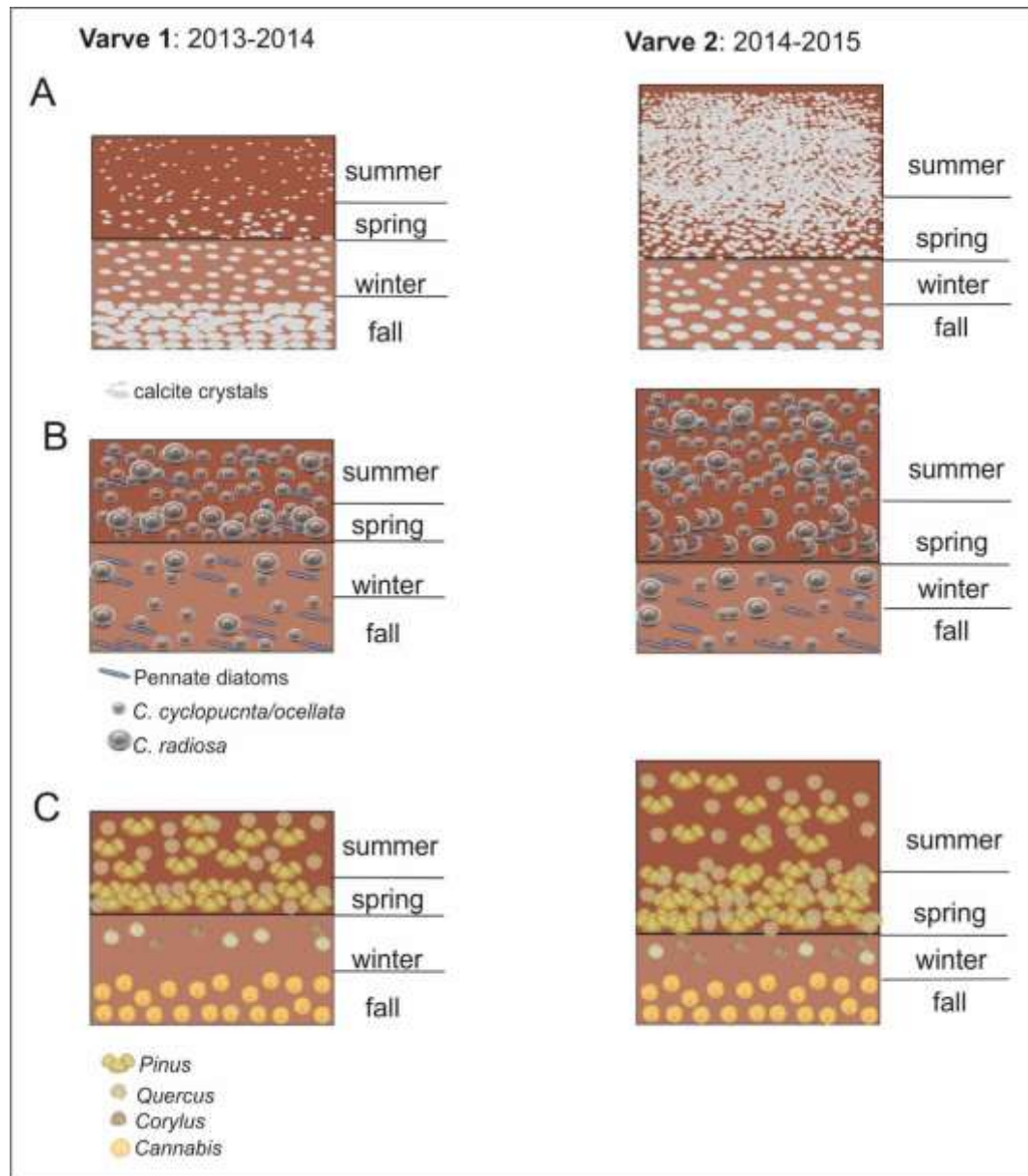


Figure 6.1. - Varve model based on Lake Montcortès modern analogue study. (A) calcite crystals, (B) diatoms, (C) pollen on a seasonal succession .

6.2- High-resolution paleoenvironmental reconstructions

To the date, former studies carried out in the lake using biological proxies have been performed on discontinuously sampling and at moderate time resolution (6-94 years; 30 years on average) (Rull 2011 and 2015, Scussolini et al., 2011, Montoya et al., 2018) . These conditions hampered the possibility to assess short-term changes, as could be those related with climate (e.g. North Atlantic Oscillation and solar variability with frequencies about 7 and

11 years respectively) (Rogers, 1977; Hurrell, 1995; Beer, 2000; Bond et al., 2001) and cultural changes affecting environment (e.g. short-term socio-economical changes). In this thesis, annual resolution was not possible to achieve, due to methodological issues. However, sub-decadal resolution allowed us to assess some short-term changes that otherwise would not have been possible to detect. One of them were heavy rainfall episodes that occurred during the last half of the 19th century (Corella et al., 2014; Vegas-Vilarrúbia et al., 2018) and which were reflected by both, pollen and pigments. Similar episodes have been observed elsewhere and used as a tool to locate specific erosion events in time and space (Clark 1986; Thompson et al., 1975). It would be interesting to test if the observed signal relates with a site-specific signal or alternatively if it reflects a regional event by studying closely located lakes. Other short-lasting events detectable with high-resolution, were fast forest recovery (20-50 years) coinciding with periods of land abandonment, or the *Cannabis* peak occurred during late 90's related with agricultural subsidies (Chapter 4). Such information helped to explain the current *Cannabis* signal on modern sedimentary analogues despite no *Cannabis* related activities were being carried out in the area (Chapter 3). Regarding aquatic community, short-lasting events detectable with high-resolution, were periods of improved water oxygenation which lasted for less than 20 years (see Fig. 5.2 Chapter 5, sulphur bacteria indicator) and the recent shift on algal community composition observed from the late 70's to nowadays.

Proxies used in chapters 4 and 5 (pollen and fossil pigments) represented two different ecological systems on a time-frame covering two different climate changes (LIA and global warming) on a well-known area of historical human occupation. In chapter 4, we investigated vegetation history, land-use and human impact by means of a multi-proxy approach and by using existing documentary historical data. On chapter 5 we have treated the existing paleoecological data with multivariate statistical methods, in order to understand changes in the aquatic community and main drivers of change, on a catchment-lake connected system. For both of them, the main signal inferred was related with anthropogenic pressure probably obscuring any potential response of the studied proxies to climatic signal, except for the heavy rainfall episodes, even during periods of known low human occupation in the area (chapters 4 and 5). Again both, pollen and pigments, showed a turning point after 1850 CE with the beginning of industrialization and the onset of global warming. However, the signal inferred had an opposed interpretation. While vegetation changes confirmed land abandonment, forest recovery and a less anthropized scenario (chapter 4), the lake community recorded a trend towards increasing eutrophication, likely

resulting from intense historical land-use legacies in combination with early industrialization effects (chapter 5). The first evidences of change towards more oligotrophic conditions appeared on late 70's and which endured to nowadays, more than a century after human occupation. Such circumstance rises the question whether lake Montcortès current conditions are representative of more 'natural' limnological conditions reached after 150 years of relative low human pressure and low human occupation in the area.

Although in some cases the inferred signal had opposite interpretations, in terms of change, both pollen and pigments responded highly synchronous (chapter 5), probably indicating a related response to the same forcing factor although with different implications.

The results obtained in this thesis provide long-term continuous data to contribute to understand current ecological changes and the past environmental history as part of a time continuum (Rull, 2014; Anderson et al. 2006). However, there are still many uncertainties and open questions to solve. Statistical analyses run in chapter 5 helped to interpret changes in aquatic community related mostly with changes in human indicators. Nonetheless, much of the pigment variability still remains to be explained and is probably related with climatic and natural forcing not accounted for directly in this work. With the possibility of significant environmental change occurring in the coming decades, such type of data become a need and a prerequisite to produce future scenarios and to test models that attempt to mimic intrinsic variability of the natural environment, ecosystem functioning and ultimately, to predict the future change (Anderson et al., 2006). This thesis represents the first step towards filling this gap in the Iberian Peninsula by exploiting one of the most valuable sedimentary archives, in terms of quality and potentiality on data resolution (varved sediments) .

6.3. Future work

The results outlined in this thesis have highlighted the high potential of data to be obtained from Lake Montcortès sediment record. However, there is still significant room of improvement

The study of modern analogues should be continued, in order to be able to assess inter-annual variability and to establish causal relationships between observed changes on proxy data and environmental variables. It should incorporate water depth sequential sediment traps to better determine sedimentological processes linked to internal lake dynamics, as could be those related with changes in primary production, calcite precipitation/dissolution

or sediment resuspension through the water column. The registration “in continuum” of physico-chemical water variables, as well as of meteorological data with a local meteorological station will help to improve robustness of paleoenvironmental interpretations. Furthermore, pollen modern analogue studies should count with airborne pollen monitoring, in order to relate pollen and vegetation cover with the pollen deposited in sediment traps and the final sedimentary signal to calibrate with fossil record and to extract a quantitative information of past community changes and/or environmental variables.

The significant amounts of sedimentary material needed for the analysis of biological indicators hampers, in this case, the possibility to assess annual resolution on lake Montcortès. However, there are some available technics currently in development that can be used to improve resolution for some specific indicators at annual resolution, i.e. for fossil pigments by means of hyperspectral imaging spectroscopy (Butz et al., 2015). Next steps should include the exploration of emerging techniques on high-resolution data obtainment from varved sediments. Improvements on sedimentary data resolution will be of special interest for the period covering instrumental climatic data as it will enable proxy-data calibration to carry out paleoenvironmental and paleoclimatic quantitative reconstructions.

It is encouraged the use of independent and complementary proxies for further paleoenvironmental reconstructions to assess the climatic signal on biological proxies. As it has been demonstrated during the course of this thesis, the human signal in Lake Montcortès masks natural or climatic signature when studying biological proxies. In this sense, efforts should be placed upon obtaining adequate climatic reconstructions at enough time-resolution for testing its importance as a driver of change on such specific area with high historical human-environment interaction.

6.4. References

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CHAPTER 7

Conclusions.

*“Seràs aquella que vas voler ser,
seràs la tres voltes rebel,
seràs un puny alçat al vent
i tu, sols tu,
faràs vibrar cinc continents.
Emancipada de qualsevol dolor,
vas eixir amb l'alegria de qui no té por,
de qui sap que el demà,
de qui sap que el demà serà millor.”*

Basat en el poema “ tres voltes rebel” de Maria Mercè Marçal

Soundtrack: “I tu, sols tu”- el Diluvi

The main conclusions of this thesis are listed below, grouped according to the general objectives corresponding to each chapter.

Chapter 2. To explore the link between varve formation and environmental variables with special regards on to understand processes related with calcite precipitation.

- Calcite showed a clear seasonal pattern closely related with the biological processes. Summer/ fall calcite precipitation was favored by high calcite saturation indices and high pH in connection with enhanced primary production and the eventual capacity of phytoplankton cells to act as condensation nuclei.
- Inter-annual differences on calcite precipitation were expressed in terms of amounts that related with temperature variability thus, thickness of calcite layers could be considered as a potential paleoclimatic proxy of temperature. Moreover, changes in calcite-crystal size would give additional information about changes in water saturation index
- Changes on diatoms amounts and assemblage composition also showed a clear seasonal pattern giving additional information of changes in water thermal stratification. However, its environmental signal will be biased due to frustule breakage and dilution.

Chapter 3. To assess relationships of pollen sedimentation with environmental and limnological variables to improve their interpretative power for paleoenvironmental reconstructions.

- General patterns of pollen sedimentation recorded a strong seasonal signal that permitted the spring/summer and fall/winter assemblages to be distinguished.
- Seasonal differences were expressed in terms of the amount and also in the taxonomic composition of the pollen assemblages.
- Our data suggest that phenological traits (i.e. flowering season) of the plant taxa involved exert a dominant control on the seasonal patterns of pollen sedimentation and inter-annual meteorological variations cause minor quantitative shifts.
- The seasonal pollen model obtained here can be applied, at least, to the last 1200 years, as all pollen types have been present with reasonably similar abundances (Rull et al. 2011).
-

Chapter 4. To carry out a high-resolution and detailed reconstruction of landscape dynamics and human interactions for the last 500 years.

- Human activity around the lake during the last 500 years had a greater influence on vegetation community changes than climatic factors, and only increases in the frequency of flood events, occurred from mid to the end of the 19th century, could have been inferred from the studied record.
- Cropping, livestock breeding, and hemp related activities have been the most important factors responsible for landscape modulation. Even during harsher climate conditions (LIA), human activities remained significant in the area
- We have achieved highest resolution so far for pollen data on a varved lake in the Iberian Peninsula (~6 years /sampling interval).
- In comparison with former studies we have improved the historical and palaeoecological precision, as well as the spatial scope, with the new studied time interval corresponding to the 20th and 21st centuries. Moreover, we shed more light on potential consequences of human impact on the aquatic system derived from hemp retting practices.

Chapter 5. To describe main changes occurred on photosynthetic aquatic community during the last 500 years and to determine the role of landscape and human-related activities in mediating lake response.

- Relative proportion among algal groups have been maintained along time, except for the last 20 years where cyanobacteria does not dominates and sulphur bacteria hardened until nowadays.
- Three main periods of change have been identified on the photosynthetic aquatic community of Lake Montcortès: Subset A (1423-1525 CE), subset B (1525-1852 CE) and subset C (1852-2013)
- Land-use appeared to be the most important factor explaining in-lake changes for the full record and time subsets except for subset B (1530 -1846 CE) where changes on trophic state and its synergies with changes on *Cannabis* related activities accounted significant proportions of variance.
- Last century recorded harder eutrophication signals on pigment data compared with the rest of the record, probably related to non-point nutrient sources from historical

legacies of intense land use, joined to atmospheric deposition derived of the industrialization process.

- There is still an important proportion of remaining variance to be explained which might belong to other factors not accounted directly in this study, i.e. climate.

Annex 1

Supplementary materials

This section contains supplementary information for chapter 2.



S1. Pink colored filter resulting after filtering hypolimnetic water. This color is due to purple sulphur bacteria living in at the hypo-metalimnetic boundary of Lake Montcortès.

Annex 2

This annex provides the original publications of:

Chapter 2:

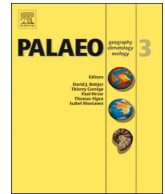
Trapote, M. C., Vegas-Vilarrúbia, T., López, P., Puche, E., Gomà, J., Buchaca, T., Cañellas-Boltà, N., Safont, E., Corella, J.P., Rull, V. (2018). Modern sedimentary analogues and integrated monitoring to understand varve formation in the Mediterranean Lake Montcortès (Central Pyrenees, Spain). *Palaeogeography, palaeoclimatology, palaeoecology*, 496, 292-304.

Chapter 3:

Rull, V., Trapote, M. C., Safont, E., Cañellas-Boltà, N., Pérez-Zanón, N., Sigrò, J., Buchaca T., Vegas-Vilarrúbia, T. (2017). Seasonal patterns of pollen sedimentation in Lake Montcortès (Central Pyrenees) and potential applications to high-resolution paleoecology: a 2-year pilot study. *Journal of paleolimnology*, 57, 95-108.

Chapter 4.

Trapote, M. C., Rull, V., Giralt, S., Corella, J. P., Montoya, E., & Vegas-Vilarrúbia, T. (2018). High-resolution (sub-decadal) pollen analysis of varved sediments from Lake Montcortès (southern Pyrenean flank): A fine-tuned record of landscape dynamics and human impact during the last 500 years. *Review of palaeobotany and palynology*, 259, 207-222.



Modern sedimentary analogues and integrated monitoring to understand varve formation in the Mediterranean Lake Montcortès (Central Pyrenees, Spain)



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ABSTRACT

Varved sediments provide unique opportunities to carry out high-resolution paleoclimatic and paleoenvironmental reconstructions with accurate time control. To better interpret the sediment record it is necessary to understand the physical, chemical and biological factors that influence varve formation and preservation. We explored the link between the annual limnological cycle and current varve deposition in the oligotrophic hard-water Lake Montcortès (Central Pyrenees). The varves of this lake consist of couplets of dark organic and light calcareous laminae. A two-year limnological monitoring (10/2013–10/2015) combined with a sediment trap study were conducted at monthly resolution. Limnological and sedimentological measurements were compared with meteorological data. Although the lake was considered meromictic in the first limnological studies, we documented total mixing of the water column both winters. In spite of this, long periods of stratification and hypolimnetic anoxia create suitable conditions for varve formation and preservation. Sediment deposition followed a clear seasonal pattern related to biological processes in the euphotic zone. During summer and fall, calcite precipitation was favored by high calcite saturation indices and enhanced primary production that promoted relatively high pH values as a result of CO₂ uptaking. There was considerable variability in the amount of calcite deposition between years, which was linked to seasonal temperature differences. In addition, calcite crystal sizes and diatom fluxes showed seasonal patterns related to calcite saturation index and changes in water stratification, which in turn were also related to temperature variability. Seasonal sedimentation patterns were strongly linked to primary producers and especially sensitive to temperature shifts. It results in a clear seasonal signal and varve formation. We compared our results with previous sedimentological interpretations of the varved record of this lake. This study improves the interpretation of Lake Montcortès sediment record extending back several millennia.

1. Introduction

Lake sediments are one of the most valuable environmental archives used for paleoenvironmental reconstructions. The sediments store past environmental changes by recording the influences of a variety of processes in the lake water body as well as in the catchment area. Among the different types, varved sediments are especially suitable for

this purpose because they allow the performance of high resolution (annual, sub-decadal) paleoenvironmental and paleoclimatic reconstructions with accurate time control (Saarnisto, 1986; Ojala et al., 2013). Formation and preservation of varves require specific conditions. Annually laminated sediment formation is favored in places with strong seasonal contrast and requires a variable flux of components from multiple autochthonous and allochthonous sources to the

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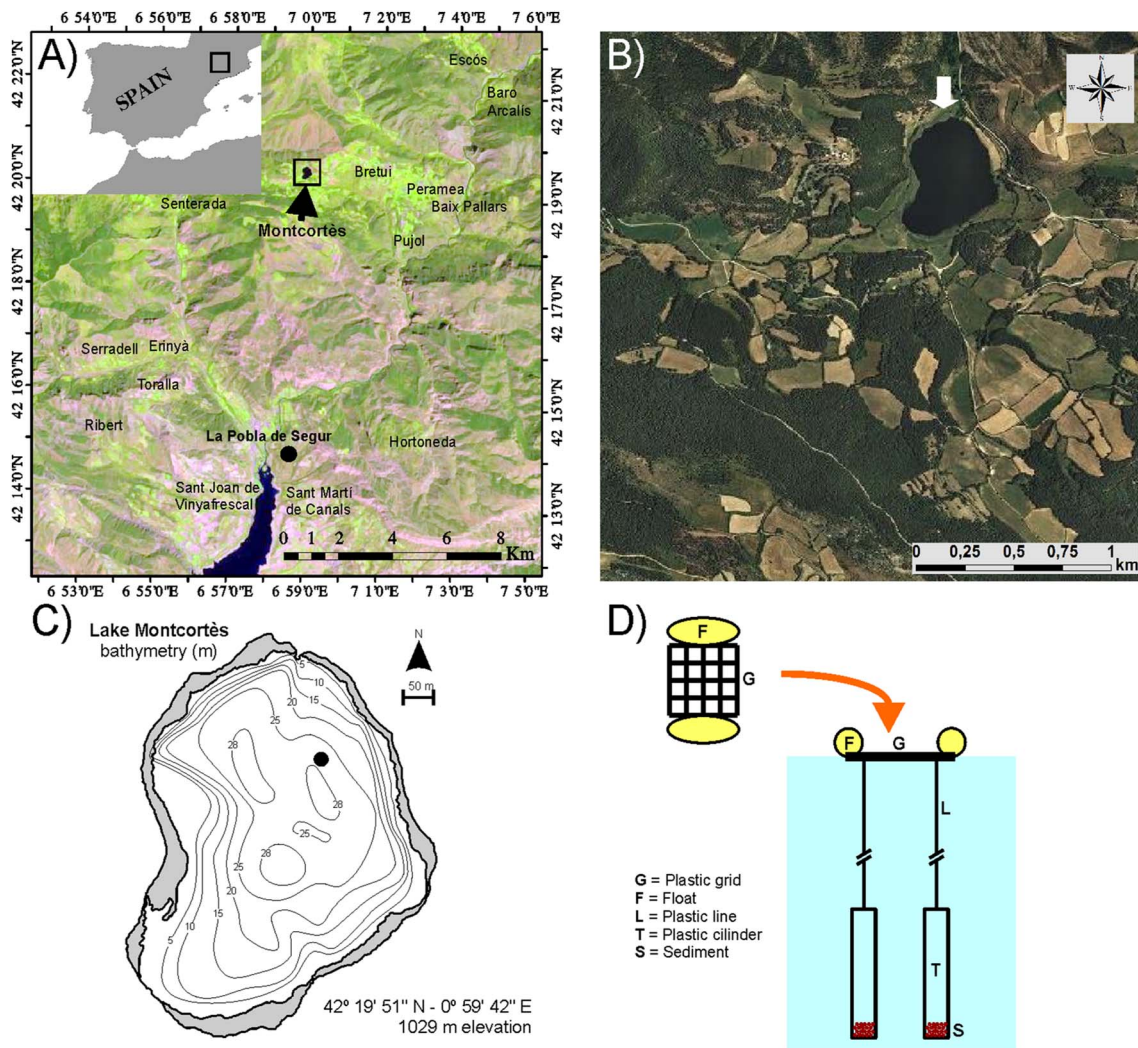


Fig. 1. Study site: A. Geographical location within the Iberian peninsula and regional map showing Lake Montcortès (square with an arrow) and the meteorological station (black point) (source: Courtesy of U.S. Geological Survey). B. Aerial photograph of Lake Montcortès (white arrow) and surrounding area. C. Bathymetric map of Lake Montcortès and location of sampling point (black point). D. Field arrangement of sedimentary traps.

sediment (Zolitschka et al., 2015). These sediments are preserved only in the absence of post-depositional reworking and sediment mixing. This condition is more common to happen in deeper lakes because they tend to be stratified and favor oxygen consumption in bottom waters. Then, prolonged (meromictic) or seasonal (monomictic/dimictic) suboxic to anoxic conditions in the hypolimnion are needed to prevent bioturbation, the most important mechanism of sediment mixing in lakes (Zolitschka et al., 2015).

Based on their composition and genesis, three main varve types (and their mixtures) can be distinguished: clastic, endogenic, and biogenic (Zolitschka et al., 2015). Deposition of clastic and endogenic varves depends mainly on seasonal runoff from the catchment and chemical precipitation of minerals from the water column (Zolitschka et al., 2015). However the mechanisms involved in biogenic varve formation are much more complex because they include bio-geochemical processes. Processes responsible of varve formation are highly variable and site-specific. Therefore, a good understanding of the local processes that promote particle flux dynamics and seasonal changes in sediment fluxes in each individual site and for a particular record is crucial to better interpret the sediment signal (Leemann and Niessen, 1994; Brauer, 2004).

The understanding of depositional conditions must be recognized as a prerequisite for a reliable varve chronology and appropriate interpretation of multi-proxy records to fully exploit the potential of varved

sediments. For this reason, modern analogue studies are highly valuable and needed. Sediment trap studies in combination with limnological and meteorological monitoring are the best way to obtain feasible modern analogues. This approach not only allows identification of the distinct pathways involved in varve formation but also determination of the period of varve deposition to assess the seasonal signal (Rodrigo et al., 1993; Miracle et al., 2000; Tylmann et al., 2012; Bonk et al., 2015).

While most published varved records are from northern and central Europe (Ojala et al., 2012), varved sequences have also been found in southern Europe and in the Mediterranean region, e.g. in the Iberian Peninsula (IP). This region is particularly attractive due to its sensitivity and vulnerability to climate change. In fact, during the last decades, temperatures have risen faster than the global average in the Mediterranean regions (Lionello et al., 2014). Also, model projections agree that future warming and drying in this area will be higher than during last century (Mariotti et al., 2015).

To date, six lakes with varved sediments have been studied in the IP, including a pliocene paleolake (Muñoz et al., 2002), and 5 extant karstic lakes: La Cruz (Romero-Viana et al., 2008), Zoñar (Martín-Puertas et al., 2009), Banyoles (Morellón et al., 2015), Arreo (Corella et al., 2011a) and Montcortès. This latter lake is the subject of our study and shows the longest continuous varved record retrieved thus far encompassing three millennia of well established varve chronology

(Corella et al., 2011b, 2016). Montcortès varves appear as couplets of light calcite and brownish organic layers that are believed to have deposited in spring/summer and fall/winter, respectively, as it occurs in biogenic varves typical of lakes located in carbonate bedrock (Corella et al., 2012). Due to the absence of modern analogue studies, these authors suggested potential mechanisms to explain varve formation based on the available literature for other similar hard-water lakes (Brauer, 2004).

Actually, among the five prevailing varved lakes in the IP mentioned above, only Lake La Cruz has been studied and monitored to obtain modern analogues to suitably interpret the sediment record (Miracle et al., 2000; Romero et al., 2006; Romero-Viana et al., 2010). In spite of the growing research interest that varved sediments have recently attracted (Ojala et al., 2012) and although some modern analogue studies are already available - mainly from north-central Europe (Tylmann et al., 2012; Ojala et al., 2013; Bonk et al., 2015) - more monitoring data is needed to properly interpret the sediment signal, especially for Mediterranean lakes.

With this study, we aim to better understand the link between varve formation and the annual limnological cycle at Lake Montcortès and provide modern analogues to better explore the potential for high resolution paleoecological and paleoclimatic reconstruction of its sedimentary record. We carried out i) in situ measurements of physico-chemical parameters in the water column and ii) measurements of sedimented material using sediment traps at monthly resolution. Special attention was paid to processes related to calcite precipitation and to understand how sediment composition varies seasonally and inter-annually related to environmental variables. It is expected that these results help to test previous hypotheses regarding varve formation in Lake Montcortès (Corella et al., 2011b, 2012).

2. Study site

Lake Montcortès is a relatively small and deep karstic lake located in the Pre-Pyrenean Range (NE Spain) in the Pallars Sobirà region (42° 19' N; 0° 59' E) at 1029 m a.s.l. (Fig. 1). The lake's catchment area is small (watershed surface area ~1.39 km²). It lies on Oligocene conglomerates and Triassic rocks mainly comprising carbonates, evaporites, claystones and shales. The lake is mainly fed by groundwater and runoff (Corella et al., 2011b). It has no permanent inlet and only two ephemeral streams located on the southern area of the watershed drain the lake. Water losses are due to evaporation and drainage from an outlet located on the northern shore that controls maximum lake levels (Corella et al., 2014). The lake is roughly circular, with a surface area of 0.14 km² and a maximum water depth of 30 m. Although Lake Montcortès has been considered meromictic (Camps et al., 1976), there is evidence of a holomictic event that occurred during the winter of 1978–79 (Modamio et al., 1988). Available data indicate that the lake water is alkaline and oligotrophic (Camps et al., 1976; Modamio et al., 1988; URS, 2010). According to the nearest meteorological station, la Pobla de Segur (Fig. 1A), total annual mean precipitation is 668.5 mm, with February being the driest month and May being the wettest. Annual average air temperature is 12.8 °C, with maximum and minimum mean temperatures of 23.3 (July) and 2.9 °C (January), respectively. The lake is situated in a transitional climatic area between the Mediterranean lowlands and the Middle Montane Belt within the sub-Mediterranean bioclimatic domain (Vigo and Ninot, 1987). Therefore, the lake is very sensitive to climate changes. The surrounding vegetation is basically forest formations of evergreen and deciduous oak trees (*Quercus rotundifolia* and *Q. pubescens*) and conifer forests of *Pinus nigra* subsp. *salzmannii* (Mercadé et al., 2013). The lake's nearest surroundings are dominated by herbaceous vegetation types represented by pastures (for cattle and horses), hay meadows and crops of cereal and alfalfa (Rull et al., 2011; Mercadé et al., 2013). The lake's shoreline presents a steep talus and a littoral vegetation belt dominated by hygrophite communities of *Phragmites australis* accompanied by *Juncus* sp., *Scirpus* sp.,

Typha sp. and *Spartanium* sp. (Mercadé et al., 2013).

Lake Montcortès is located at the *Baix Pallars* municipality which has a total population of 350 inhabitants, with only 26 belonging to the town of Montcortès itself (Idescat, 2015). Land use is limited to cereal crops and livestock pastures (Fig. 1B). The lake has historically been an important water resource for numerous surrounding villages and farmhouses with a long history of human occupation (Scussolini et al., 2011; Rull et al., 2011).

3. Methods

3.1. Limnological monitoring

Physical and chemical variables were monitored in the water column, and samples were collected monthly from October 2013 to October 2015. Profiles at 1 m depth intervals were obtained from the surface to the bottom of the water column for temperature (T), dissolved oxygen (DO), electric conductivity (EC) and pH using a multi-parameter water quality probe (Hydrolab DS5). Water transparency was determined using a Secchi disc. Light penetration was measured using a Photosynthetic Active Radiation (PAR) sensor. Water samples for chemical analyses and phytoplankton identification and counting were collected at three different depths coinciding with the epilimnion (~0.5 m), metalimnion (thermocline; from ~5 to 17 m) and hypolimnion (~20 m). Phytoplankton samples were stored in amber glass bottles and fixed with concentrated Lugol's iodine solution. Identification and counting was carried out under inverted microscope at 400× magnification following the Utermhöl method (Utermhöl, 1931). Cellular biovolume determinations were carried out according to Wetzel and Likens (1991).

Total alkalinity was analyzed using standard titration methods for freshwater samples. Total nitrogen (TN) and total phosphorus (TP) were analyzed using alkaline persulfate oxidation, while soluble reactive phosphorous (SRP) was analyzed by the molybdate ascorbic method following Grasshoff et al. (1983). Cation concentrations of calcium (Ca²⁺), magnesium (Mg²⁺), sodium (Na²⁺) and potassium (K⁺) were determined at the Scientific and Technological Center of the University of Barcelona (CCITUB) using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES) with a Perkin Elmer Optima 8300 spectrometer under standard conditions. The calcite saturation index (Ω) was calculated according to Eq. (1):

$$\Omega = [\text{Ca}^{2+}] \times [\text{CO}_3^{2-}] / K_{\text{cal}} \quad (1)$$

where [Ca²⁺] and [CO₃²⁻] are the molar concentrations of calcium and carbonate ions, and K_{cal} is the solubility product of calcite. Here, we use log₁₀(Ω) to express the calcite saturation index (SI).

Values of [CO₃²⁻] were computed from alkalinity and pH with the CO2SYS_XLS v 2.1 program (Pierrot et al., 2006).

3.2. Sampling and analyses of sediment trap material

Particulate matter settling through the water column was collected using sediment traps. Two cylindrical opaque PVC traps, 8.5 cm in diameter and with a 9:1 aspect ratio (Bloesch and Burns, 1980) were used. They were fastened to a floating platform (Fig. 1D) and placed at 20 m water depth at 5 m above the sediment surface. One trap was emptied quarterly at the end of each season for seasonal diatom and calcite crystal analysis. Trapped material for diatom analysis was preserved in formaldehyde (4%). The second trap was emptied every month for trapped material characterization and quantification. The collected material was transferred into a plastic container and stored at 4 °C prior to subsampling. To determine sediment weight and composition, sample aliquots were filtered through pre-ashed (500 °C) and pre-weighed GF/F filters until filter saturation and then oven-dried for 48 h at 60 °C. Filters for total suspended solids (TSS; g L⁻¹) analysis were weighed, and total mass fluxes (TMF) were calculated from Eq.

(2):

$$\text{TMF}(\text{g m}^{-2} \text{d}^{-1}) = \text{dry net weight}(\text{g})/\text{active area}(\text{m}^2)\text{time}(\text{d}) \quad (2)$$

To analyze carbon and nitrogen content of TSS, filters were weighed and analyzed for total particulate carbon (TPC), total particulate organic carbon (POC) and total nitrogen (TN). Samples for POC analysis were acidified to remove inorganic carbon. TPC, POC and TN were analyzed at the CCiTUB using an elemental organic analyzer, Thermo EA 1108, working under standard conditions. Total inorganic particulate carbon (PIC) was calculated as the difference between TPC and POC. Calcite contents were calculated from PIC concentrations by multiplying by 8.33, a factor referring to the molar weight of CaCO_3 . To estimate organic matter (OM) from POC content in the trapped material, we applied a widely used conversion factor for soils and sediments by multiplying POC by 1.7 (Nelson and Sommers, 1996; Bluszcz et al., 2008). We also used the theoretical relationship defined by pure organic matter in its simplest form (CH_2O) and multiplied POC by a factor of 2.5 (Leipe et al., 2011). With this approach, we obtained a range of estimated OM that covers possible variations depending upon the type of OM present in the sample.

To confirm the presence of calcite crystals and to assess seasonal variations of their size and shape trapped material from quarterly traps was analyzed by SEM. For this purpose, samples were oxidized with H_2O_2 at 200 °C, washed and then filtered using a Whatman polycarbonate filter of 0.4 μm pore size. Calcite crystals were then identified by means of spectrograms obtained in the microanalysis and characterized for size and morphology under the scanning electron microscope (SEM) with an Energy Dispersive X-ray Spectroscopy and the help of the *Inca250* software. A minimum of 170 and a maximum of 358 calcite crystals were counted per sample, and the maximum (I_{max}) and minimum lengths (I_{min}) were measured.

For diatom analysis, an aliquot of trapped material from the quarterly traps was cleaned and prepared using standard methods (Abrantes et al., 2005). At least 300 valves per sample were counted, and microspheres were added to calculate valve influx (Battarbee et al., 2001). Samples were mounted in Naphrax® and analyzed using a Polyvar light microscope at 1000 \times magnification. Diatom species were identified using Krammer and Lange-Bertalot (1986–2004).

Meteorological data were obtained from the meteorological station of La Pobla de Segur (Catalonian Meteorological Service) located 19 km of Lake Montcortès (Fig. 1A). We used these data to assess inter-annual climatic variability covering the monitoring period by calculating the deviation or anomaly of monthly precipitation and temperature values from the mean long-term reference period 1961–1990. The selected variables were mean monthly air temperature (TM), mean monthly precipitation (PPT) and wind speed (W) and the calculated TM and PPT anomalies as Anom TM and Anom PPTM, respectively (reference period 1961–1990).

4. Results

4.1. Modern limnology and water column properties

Water column temperature profiles of Lake Montcortès showed thermal stratification during most of the year, with mean surface and bottom temperatures of 14.6 °C and 5.2 °C, respectively. During winter, the surface water cooled until it reached 4–5 °C, and the increase in water density promoted thermic homogenization of the entire water column and all physical properties (Fig. 2). During spring and summer warming (April to September), surface temperature rose quickly, and thermal stratification developed. The mixing zone moved to shallower depths (5–7 m); a well-defined thermocline developed between 5 and 15 m and lasted until early winter (December) (Fig. 2A). Maximum epilimnetic temperatures of 24.7 °C were recorded during summer 2015, while the maximum during 2014 was 22.2 °C.

Dissolved oxygen showed maximum concentrations (14–17 mg L^{-1})

between 7 and 10 m in the euphotic metalimnion from May to September around the thermocline (Fig. 2B) and then decreased with depth. Secchi disc ranged from ~4 to ~9 m, and the limit of the euphotic zone was always deeper than 7 m and below the oxygen peak (Fig. 2B). Winter water column mixing events took place in January 2014 and 2015. When stratification began from March onward, oxygen depletion occurred, and anoxic conditions started to develop in the hypolimnion. Hypoxia ($\leq 2 \text{ mg L}^{-1}$) began in April, and anoxia (0 mg L^{-1}) was reached in June and remained until December (Fig. 2B). The maximum thickness of the anoxic layer was observed at the end of summer (September–October) and reached up to 15 m lake depth in 2014 and close to 17 m lake depth in 2015. This represents an anoxic layer thickness close to 10 m.

Values of pH were highest coinciding with oxygen maxima in the metalimnion. During stratification, pH values ranged from 7.1 close to the bottom to 8.7 in the epilimnion (Fig. 2C), coinciding with increasing phytoplankton biovolumes, and were near 7.8 during winter mixing. Total alkalinity was roughly constant through time and depth, with mean values ranging from 3 to 3.5 meq L^{-1} , indicating well-buffered waters even in the hypolimnion, where pH was lower (Fig. 2C). Electric conductivity (EC) did not experience significant seasonal changes along the water column, except for subtle EC minima that occurred during stratification in both years coinciding with oxygen maxima. EC anomalies, calculated as the difference between depth average and the corresponding value for each depth, were plotted to document these minima (Fig. 2E).

Ca^{2+} was the most abundant dissolved cation followed by Mg^{2+} (Table 1). Values of SRP and TP were very low. TN was relatively more abundant and increased notably in the hypolimnion during the stratification period, while it was depleted in epi and metalimnion (Table 1).

The succession of main phytoplankton groups followed a seasonal pattern during the entire sampling period (Table 1). Central diatoms belonging mainly to the genus *Cyclotella* peaked in early spring, taking advantage of turbulence after overturn. During summer and early fall Chlorophytes were the most abundant group, except in summer 2015 when diatoms remained dominant in the metalimnion, while in the epilimnion Cryptophytes dominated. Cryptophytes were dominant mainly during winters when all other groups decreased in abundance. Interestingly, a significant part of the planktonic diatoms and Chlorophytes was extremely small, exhibiting sizes of only ~5 μm .

4.2. Fluxes of trapped material

Total mass fluxes (TMF), calcite and POC fluxes are displayed in Fig. 3. The collected material from sediment traps reveals important changes during the two years of sampling. TMF followed a clear seasonal pattern in which low values occurred mainly during winter and early spring and the highest values during summer and fall (Fig. 3A). Progressive flux increases started in early spring (April–May), coinciding with oxygen increase together with increasing fluxes of POC (Fig. 3B). After that, both TMF and POC started to decrease during fall with oxygen depletion. Increases in POC fluxes were roughly followed by increases in calcite deposition (Fig. 3B and C). Calcite is mainly deposited during summer and fall, with higher values recorded in fall 2013 and summer 2015 (Fig. 3C). Periods with more intense calcite deposition also coincided with higher values of calcite saturation in the epilimnion and metalimnion. The Mg-to-Ca molar ratio did not exceed 0.37 (Fig. 3C), and epi- and metalimnion remained permanently calcite-saturated throughout the study period (Fig. 3E). The highest values of calcite saturation were recorded during spring and summer (Fig. 3E), coinciding with rising temperature and primary production. The hypolimnion also endured saturation during most of the year but presented lower saturation values than epi- and metalimnetic waters. Overall, calcite saturation values declined in late summer and fall. During the late fall of 2014, the hypolimnion was near undersaturation for calcite, and it was only during the fall of 2015 when values fell

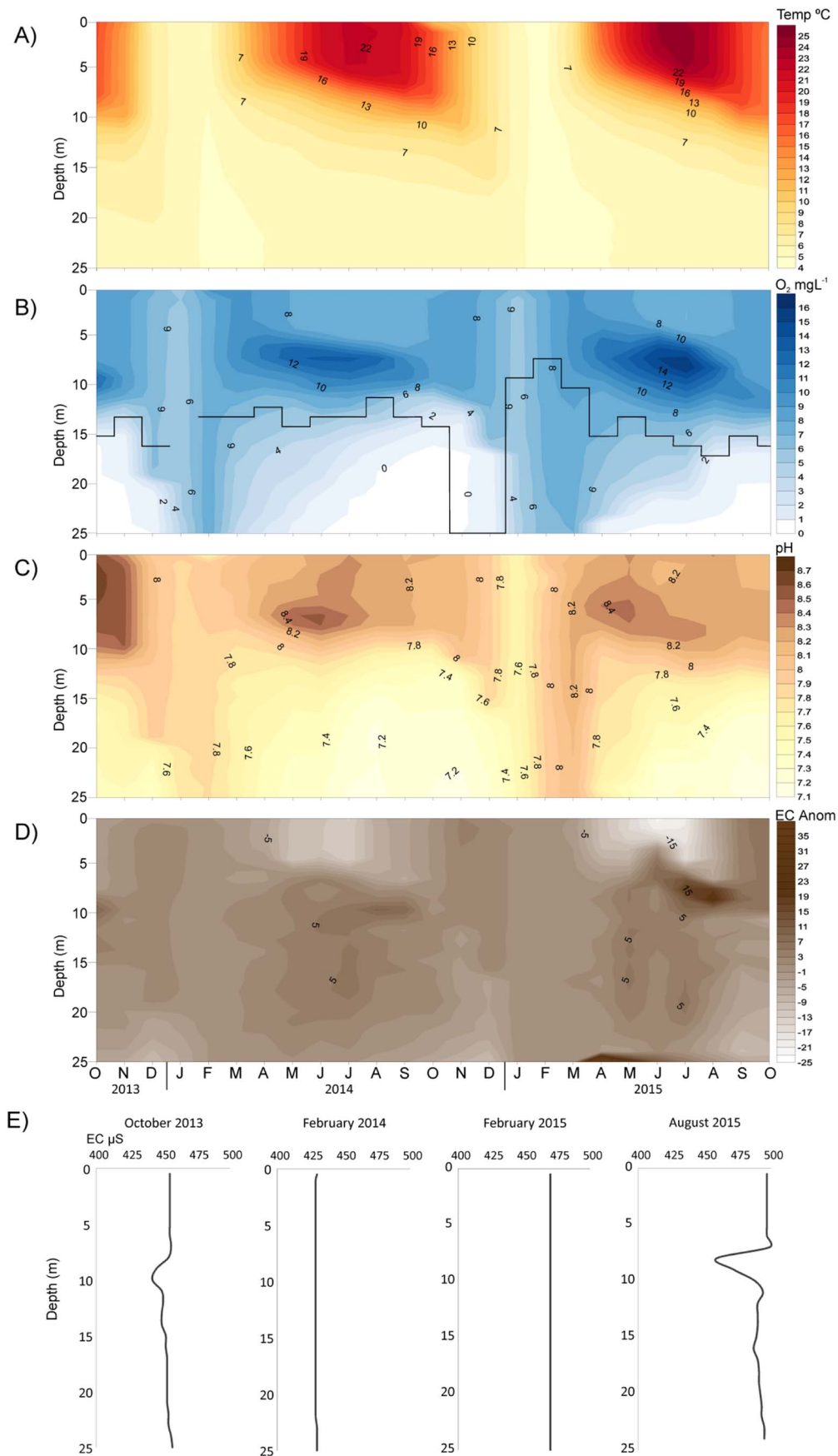


Fig. 2. Depth profiles of : A. Water temperature; B. Oxygen concentration with euphotic zone (solid line); C. pH; D. Electric conductivity (EC) anomalies and E. Four discrete electric conductivity profiles corresponding to mixing periods (February 2014 and 2015) and stratification period (October 2013 and August 2015) note the EC decreases in metalimnetic waters during stratification.

Table 1
Seasonal average concentration of cations and nutrients and main phytoplankton groups.

	2013		2014			2015		
	Fall	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
Ca (mg L⁻¹)								
Epi. ^b	128.09	127.50	102.5	145.00	107	83.57	83.57	82.78
Meta. ^c	112.99	127.95	122.67	138.36	101.21	82.97	81.71	81.56
Hypo. ^d	125.03	144.21	130.18	118.25	103.44	82.96	81.76	84.38
Mg (mg L⁻¹)								
Epi.	15.65	18.26	13.92	19.21	17.93	17.89	18.12	17.32
Meta.	17.04	18.29	16.02	19.16	18.82	17.76	17.8	17.18
Hypo.	17.38	19.23	16.2	19.19	18.56	17.76	17.61	17.50
K (mg L⁻¹)								
Epi.	2.34	2.78	2.20	2.79	2.45	2.53	2.73	2.63
Meta.	2.52	2.81	2.40	2.81	2.69	2.54	2.53	2.68
Hypo.	2.60	2.88	2.60	2.78	2.69	2.54	2.53	2.63
Na (mg L⁻¹)								
Epi.	3.04	3.66	2.26	3.63	3.31	3.24	3.60	3.63
Meta.	3.50	3.71	2.91	3.29	3.17	3.08	3.31	3.58
Hypo.	3.34	3.36	2.87	3.32	3.23	3.28	3.32	3.50
SRP (μM)								
Epi.	0.050	0.042	0.037	0.051	0.049	0.040	0.010	0.034
Meta.	0.019	0.012	0.053	0.047	0.042	0.050	0.010	0.036
Hypo.	0.071	0.036	0.047	0.047	0.049	0.057	0.011	0.050
TP(μM)								
Epi.	0.190	0.200	0.170	0.350	0.650	0.045 ^a	0.180	0.050
Meta.	0.191	0.200	0.212	0.853	0.624	0.547 ^a	0.511	0.130
Hypo.	0.198	0.201	0.264	0.697	0.900	0.512 ^a	0.402	0.396
TN (μM)								
Epi.	30.28	50.63	34.37	24.19	28.06	45.35	36.84	28.65
Meta.	43.21	50.73	37.25	27.36	34.55	45.63	39.54	28.84
Hypo.	71.47	53.81	61.68	69.81	59.55	44.84	53.45	59.45
Phytoplankton succession								
Epi.	Chlorophyta	Cryptophyta	Bacillariophyta	Chlorophyta	Chlorophyta	Cryptophyta	Bacillariophyta	Cryptophyta
Meta.	Chlorophyta	Cryptophyta	Bacillariophyta	Chlorophyta	Chlorophyta	Cryptophyta	Bacillariophyta	Bacillariophyta

^a Missing sample of January.

^b Epilimnion.

^c Metalimnion.

^d Hypolimnion.

below zero recording undersaturation values (Fig. 3E).

TMF, POC and calcite fluxes showed a similar behavior and are moderately correlated (TMF vs POC $r = +0.80$, $p(a) < 0.05$, $n = 25$; TMF vs PIC $r = +0.86$, $p(a) < 0.05$, $n = 25$ and PIC vs POC $r = +0.76$, $p(a) < 0.1$, $n = 25$). Total amounts of calcite deposition varied broadly between years, a fact that is evident by comparison with the data from 2014 to 2015 (Fig. 3C). During 2014, calcite fluxes remained below OM fluxes, while during 2013 and 2015 calcite values clearly exceeded OM during fall and from early summer to fall (Fig. 3D).

4.3. Calcite crystal characterization

Presence of calcite crystals was confirmed in all trapped samples. A clear seasonal pattern in calcite sizes was observed (Fig. 4A). Larger crystals with higher I_{max} and I_{min} correspond to fall and winter in traps and tend to decrease progressively from fall to summer through the two years of the study. Most of the crystals have blocky and polyhedral habits (Fig. 4B to G). Some crystals appeared attached to central diatom frustules of the genus *Cyclotella* (Fig. 4H).

4.4. Trapped diatoms

We analyzed diatoms from quarterly trapped sediment samples to estimate their timing and contribution to the deposited material. Higher diatom fluxes were recorded during spring and summer (Fig. 5A). This

pattern roughly matches with the total diatom cells counted in the water column (Fig. 5B). Central diatoms accounted for > 50% of the diatom composition during most of the year and reached abundances above 75% during spring and summer. Seasonal changes in relative abundances of the main species of central diatoms *Cyclotella cyclopuncta*, *C. ocellata*, *C. radiosa* and *Stephanodiscus hantzschii* are shown in Fig. 5C. *C. cyclopuncta* generally dominated the assemblage, while *C. ocellata* was less abundant with constant proportions. *C. radiosa* showed small proportions, except during spring for both years, when it increased. The differences between the diatom content of the traps and the water column during the spring of 2015 can be explained by poor preservation of the material deposited in the corresponding trap, which was considerably damaged, diluted and hardly able to be counted.

4.5. Meteorological conditions

Monthly mean air temperatures decreased from August–September to December–January, when temperatures remained above 4 °C. Maximum temperatures were reached during July of both years: 22 °C for July 2014 and 26.5 °C for July 2015 (Fig. 6A). Temperature anomalies corresponding to 2015 were generally positive and higher than anomalies in 2014, indicating a warmer year (Fig. 6A). Precipitation was high in late summer and fall for both years (Fig. 6B). During 2015, negative precipitation anomalies were persistent from December 2014 to June 2015 and positive from July to October. Comparing the same periods for 2014, December 2013 to June 2014

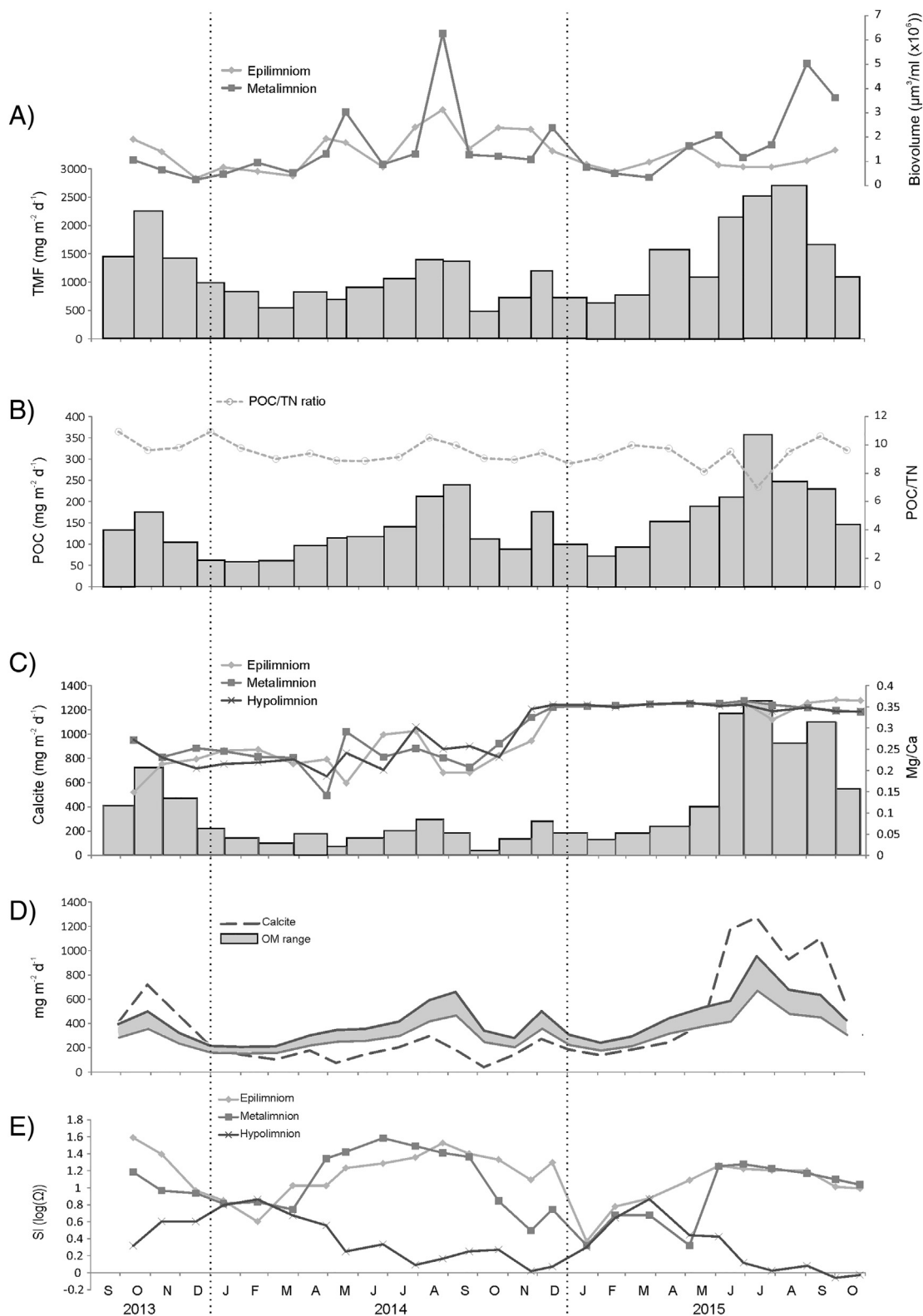


Fig. 3. Monthly fluxes of major sediment components deposited in sediment traps during monitoring period 10/2013–10/2015: A. Total mass fluxes (TMF) with changes in phytoplankton biovolume for the epilimnion and the metalimnion; B. POC fluxes and corresponding POC/TN ratios; C. Calcite fluxes with epi-, meta and hypolimnion Mg/Ca; D. Calcite fluxes versus estimated range of organic matter fluxes (OM); E. Calcite saturation index (SI) for epi-, meta- and hypolimnion.

was wetter than in 2015 with more positive anomalies, while July to October 2015 was similar to 2014. period (Fig. 6B).

Monthly mean wind speeds during the studied period reached maximum values during spring and minimum values during winter of both years. Wind speeds did not exceed 1.5 m s^{-1} during the studied

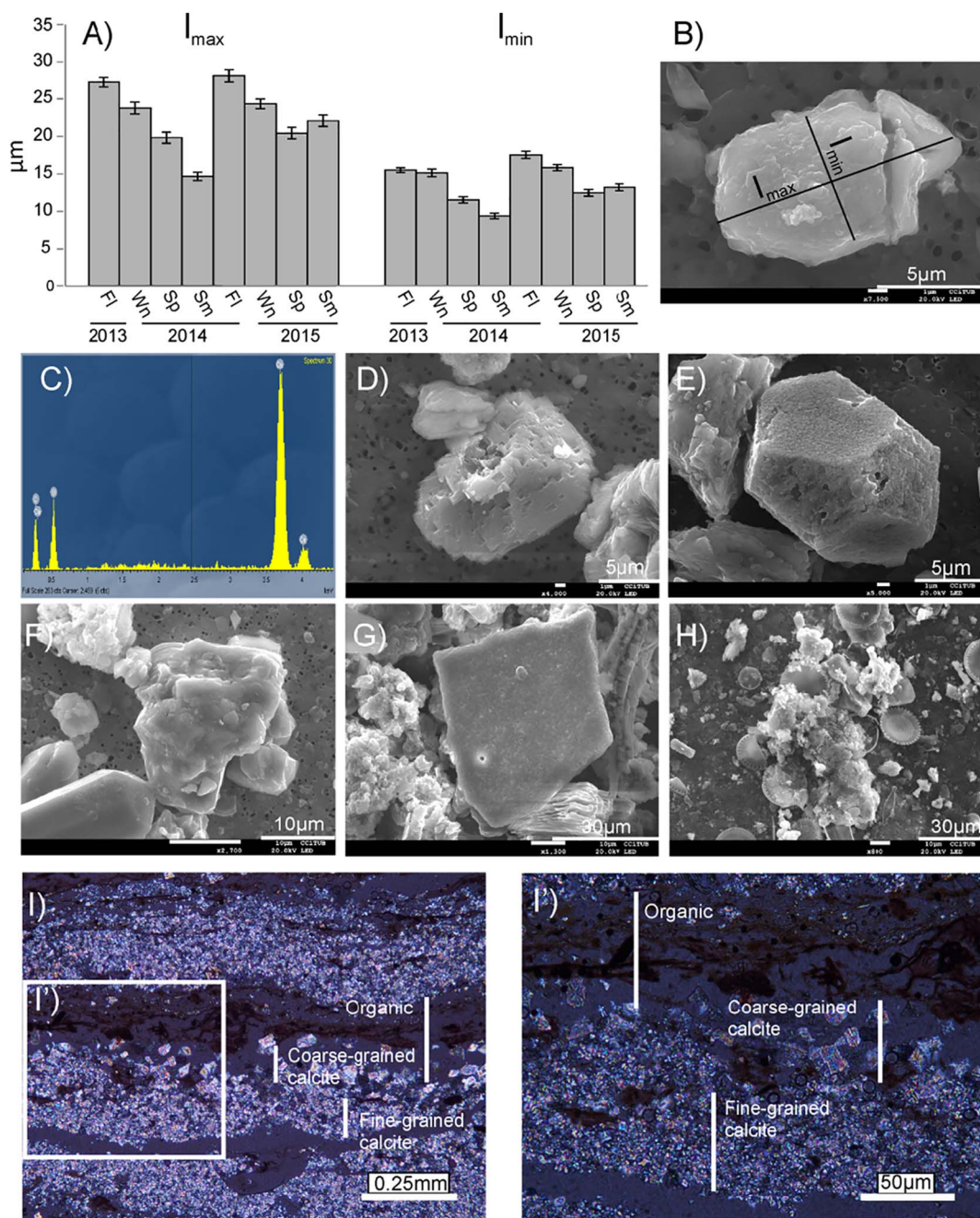


Fig. 4. Calcite crystals found in quarterly sediment traps in Lake Montcortès: A. Mean values of l_{max} and l_{min} (μm) corresponding to Fall (Fl), winter (Wn), spring (Sp) and summer (Sm) and bars indicate standard deviation; B–H. SEM images of calcite crystals: B. l_{max} and l_{min} measurements; C. Calcite spectogram; D–G. Images of calcite crystals. Thin section microscopic image showing one of the varve structures found in Lake Montcortès sediment: I. Calcite layer with fine-grained calcite crystals followed by coarse-grained calcite crystals and the organic layer; I') More magnified detail of varve structure.

5. Discussion

5.1. Varve formation: sediment fluxes and composition during the annual cycle

The annual pattern of particle flux dynamics and composition in Lake Montcortès was closely related to biogeochemical processes in the euphotic zone and changing meteorological conditions throughout the year. Fluxes of sediment reached maximum values in summer and fall that matched with maxima of phytoplanktonic growth, pH, and calcite and POC deposition in traps values. POC values were higher during summer and fall when calcite was mainly deposited, suggesting that the main origin of calcite crystals are autochthonous. Other sources of

calcite input, such as detrital carbonates from the catchment, seem to be not significant during the studied period. Well-formed blocky and rhombohedral calcite crystals and the presence of diatom frustules attached to these crystals support the autochthonous origin of calcite (Pere Anadón pers. com.). Moreover, low values of the POC-to-TN ratio indicate the prevalence of cellulose-poor autochthonous algal organic matter over lignin and cellulose-rich allochthonous organic matter derived from vascular plants (Meyers and Teranes, 2001), suggesting low input to the lake from the catchment area. Additionally, there are no discontinuities in TMF that could be attributed to external inputs, i.e., there are no TMF increases that coincide with months of higher precipitation anomalies (Figs. 3 and 6). These observations are consistent with the fact that biologically induced calcite precipitation took place

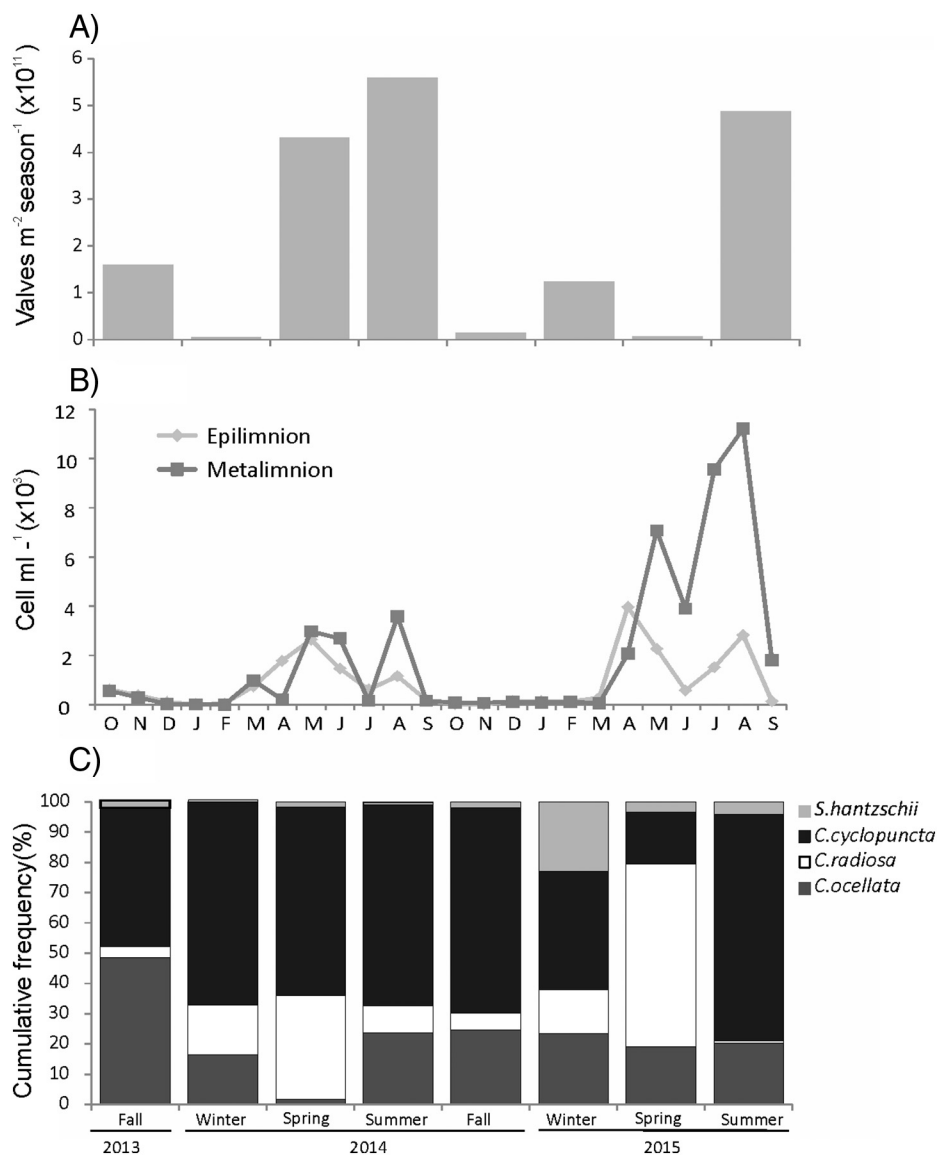


Fig. 5. Diatom dynamics: A. Fluxes of diatoms in quarterly traps; B. Diatom cell abundances in the epilimnion and metalimnion; C. Relative abundances of centric diatoms.

in Lake Montcortès.

Calcite precipitation can be triggered by CO₂ uptake during algal photosynthesis. Primary productivity affects the carbonic-carbonate system in epilimnetic and metalimnetic waters by decreasing CO₂, increasing pH, and shifting the equilibria toward the CO₃²⁻ species (Kelts and Hsü, 1978; Dittrich and Obst, 2004). This biochemical mechanism leads to calcite supersaturation in the limnetic zone (Hodell et al., 1998). In addition, when photosynthesis occurs, precipitation would be enhanced by phytoplankton providing particulate surface areas for calcite-crystal growth (Dittrich et al., 2004). In fact, the epi- and metalimnetic waters of Lake Montcortès were already supersaturated with calcite when precipitation occurred at log Ω ≥ 1 and with a rather low Mg/Ca molar ratio that indicates favorable environmental conditions for the formation of pure calcite or low Mg-calcite crystals (Müller et al., 1972). Calcite supersaturation values for calcite precipitation need to be log Ω ≥ 2 under experimental conditions (Dittrich and Obst, 2004), but it has been shown that precipitation in lakes can happen far below that threshold. Some examples are Lake Constance, where calcite precipitates at log Ω ≤ 1 (Stabel, 1986), and the oligotrophic Lake Lucerne, where precipitation occurred when log Ω was near 0.4 (Dittrich et al., 2004). A likely explanation for this discrepancy is the presence of nuclei provided by phytoplankton cells, which are

necessary for crystal growth (Dittrich and Obst, 2004). Nucleation of calcite crystals triggered by phytoplankton and small particles of picoplankton (0.2–2 μm), such as cyanobacteria or sulfur-reducing bacteria, have been shown and documented by several studies (Dittrich and Obst, 2004; Baumgartner et al., 2006). This explanation likely applies in our case, since small Chlorophyta (such as *Tetraedron minimum* and *Oocystis* spp.) and small centric diatoms (~5 μm) are dominant during periods of calcite precipitation, and persistent populations of sulfur bacteria (Chromatiaceae and Chlorobiaceae) are known to thrive at the hypolimnion-metalimnion boundary of Lake Montcortès (Cristina et al., 2000). Additionally, during stratification period, the filters we used to trap deposited material were intensely stained with the unmistakable color of purple bacteria (Supplementary Fig. 1), and their presence was confirmed by okenone and isorenieratene pigment analysis (Vegas-Vilarrúbia et al., 2018). Moreover, high calcite fluxes happened at the same time that negative EC anomalies were recorded. This slight decrease in EC could be a consequence of ion depletion caused by nutrient uptake and precipitation of calcite, but any simultaneous decrease in alkalinity and Ca concentration that could support the link between EC and calcite precipitation went unnoticed. This outcome may be a consequence of sampling resolution; in fact, variations in Ca and alkalinity might have happened between sampling data and thus remained

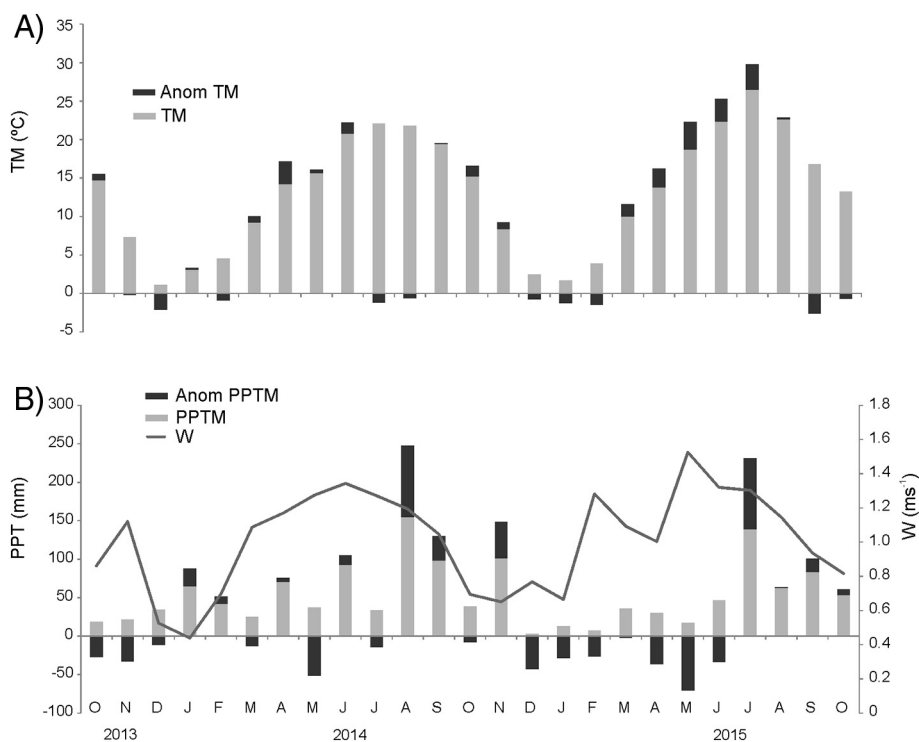


Fig. 6. Meteorological data: A. Monthly mean air temperature (TM); B. Mean precipitation (PPT) and their anomalies (black bars) along with wind speed (W) (line).

unnoticed in our records. Nonetheless, decreases in EC in epilimnetic and metalimnetic water related to calcite precipitation have been observed in other hard-water lakes (Miracle et al., 2000; Bluszcz et al., 2008).

The spring and the summer of 2015 were anomalously warm, and the calcite flux was more than twice compared to the preceding year for the same seasons (Fig. 3C). These warmer conditions were perfectly recorded in epi- and metalimnetic water temperatures that coincided with higher values of DO as a result of enhanced primary production. Thus, the influence of atmospheric temperature increase on processes involved in calcite precipitation is evidenced by the differences in the magnitudes of deposited material between both years.

Actually, 2015 was the warmest year on the record registered in Europe (NOAA, 2015) recording the second warmest summer on the record. Also, 2015, was the warmest year in Spain tied with 2011 that recorded 0.96 °C temperature anomaly (1981–2010 reference period) (AEMET, 2015). Since the late 1990ies until nowadays, increases of mean lake-water temperature between 0.5 and 0.6 °C per decade have been recorded in the majority of studied European lakes (Dokulil, 2014). Besides, the average thickness of calcite sublayers of the Montcortès record has nearly doubled since the 19th century (Corella et al., 2011b, 2012). These results suggest that variations of biogenic calcite in the sedimentary record might indicate temperature changes, at least partially. The thickness of calcareous layer in Lake Montcortès seems to be a reliable paleotemperature proxy.

5.2. Varve preservation: limnological conditions

Previous studies of Lake Montcortès found persistent anoxic conditions and sulfide production on bottom waters year-round in 1975/1976 and 1997/1998 respectively (Camps et al., 1976; Cristina et al., 2000). Based on these studies and in the laminated nature of the sediment record, Lake Montcortès was considered to be meromictic (Corella et al., 2012; Valero-Garcés et al., 2014). However, Modamio et al. (1988) reported a complete oxygenation of the water column associated with a winter mixing event (1978/1979). During the two-year period of monitoring, we observed that Lake Montcortès mixed during both

winters. Nevertheless, during 2016 the lake did not mix completely (unpublished data). The observed mixing events in Lake Montcortès were tightly related with a mean air-temperature decrease below the lake's surface water-temperature that caused denser surface water layers to sink, promoting the mixing of the water column and thus, homogenization of oxygen concentration throughout the water column. Meteorological forcing at the air-water interface is known to be the main determinant of the heat balance of many lakes (Edinger et al., 1968; Sweers, 1976). All this evidence suggests that Lake Montcortès has an interannually changing mixing regime and cannot be considered meromictic *sensu stricto* (Hakala, 2004). This is supported by a recent study documenting a 45.3% mixing recurrence during the last 500 years (Vegas-Vilarrúbia et al., 2018). With present global warming, an expected strengthening might significantly affect nutrient and oxygen regeneration within the water column.

Although meromictic conditions are especially suitable for varve preservation, short periods of mixing and seasonally suboxic to anoxic conditions during stratification seem to be enough to prevent bioturbation and allow varve preservation, even in dimictic lakes (Ojala et al., 2000; Hakala, 2004; Zolitschka et al., 2015). In Lake Montcortès this is supported by the continuous presence of varves shown by the sediment record of the last three millennia. Other factors that may affect varve preservation are resuspension of bottom sediments through wind or density-driven water circulation and bottom currents that interrupt continuous sedimentation and cause erosional hiatuses. The latter two processes can be excluded if the lake basin is deep with a small surface area and geomorphologically protected (O'Sullivan, 1983), this is the case for Lake Montcortès (Corella et al., 2012). Furthermore, lake mixing took place when minimum wind speeds were recorder both years studied (Fig. 6B).

5.3. Comparison with previous varve interpretations

The seasonal sedimentation pattern obtained from the sediment trap study is consistent with the alternating structure of light (calcite) and dark (organic) laminae observed in the sediment record of Lake Montcortès supporting the seasonal nature of the sediment laminane.

According to our study, calcium calcite layer would form mostly in summer/fall and dark organic laminae mostly during winter/spring. This is more clearly evidenced in Fig. 3D where OM and calcite fluxes are compared. Thus, our results do not totally agree with former studies that situated the formation of the calcite layer in spring/summer, and the formation of the organic layer in fall/winter (Corella et al., 2012). We did not observe conspicuous pulses of calcite precipitation, as occurs in lakes with the presence of whitening events (Miracle et al., 2000). Nonetheless, there was a clear differentiation between calcite and OM deposition in fall 2013 and summer/fall 2015, while this was not the case during 2014. In the latter case, the depositional pattern might result in a lack of sublayer differentiation and consequently of a varve or sub-layer absence for that particular year. In fact, this is coherent with Corella et al. (2011b), as they found laminated intervals with scarce light calcite layers in the Montcortès record of the last 6000 years. Knowing when and why such events happened is crucial when it comes to building varve chronologies to minimize the counting error. Varve fading or absence can lead to mistakes in the recognition of annual cycles in the sediments.

Calcite precipitation began to increase in late spring with higher deposition rates during summer lasting until fall. Consequently, the light laminae would represent the merged effect of temperature and primary production. Dark laminae (OM) would represent the productivity of the lake through the year. Calcite laminae differentiation would depend of the amount of calcite precipitated during summer/fall. Such correspondence should be demonstrated by analyzing the individual sediment layers covering the studied period but presently this cannot be accomplished as it is necessary to wait for diagenesis to consolidate the sedimentary deposits. So far, our results give further information about seasonal composition of sediment material and highlight the importance of modern analogue studies to properly interpret the sediment signal at a seasonal and sub-annual scale.

Calcite crystal-sizes from quarterly traps found in this study showed a seasonal pattern likely related to water temperature, being smaller when formed during spring and summer. Additionally, deposition of smaller crystals was concurrent with higher fluxes of diatoms and high SI. It is known that when calcite saturation is high, e.g. in spring and summer, crystals were formed rapidly and do not have time to grow. However, in fall and winter, water is not very supersaturated in calcite, and crystals can increase their sizes while travelling to the bottom (Kelts and Hsü, 1978). Thus, smaller calcite crystals in Lake Montcortès would be interpreted as occurring during periods with elevated SI related to higher temperatures and primary production. This pattern bears some relationship with the internal calcite sub-layering present in the sediment record of Lake Montcortès (fining upward with coarser calcite crystals in the lower part of the calcite layer; coarsening upward, the converse of fining upward; and a homogeneous layer of coarse crystals (Corella et al., 2012)). The seasonal calcite-crystal distribution observed in 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 in fall (Fig. 4I and I'). This texture is frequently observed in the sedimentary sequence from AD ~1350 to 1850 AD and less frequently from then until present day (Corella et al., 2012). Future micro-facies analysis of the sediment record retrieved after 2015 might show the corresponding varves and sub-layering patterns of the studied period (2013–2015).

Three *Cyclotella* species coexisted over the studied period: *C. cyclopuncta*, the dominant taxon, together with *C. ocellata*, while *C. radiosa*, which is bigger than the two other species, increased in spring, being competitive during periods of high turbulence promoted by winter mixing. The growth of highly silicified species as a response to turbulence, as well as increases in small centric diatoms during stratification (e.g., *C. cyclopuncta*), have been observed in other lakes as a consequence of changes in water stratification (Rühland et al., 2015). Changes in relative abundances of *C. cyclopuncta* and *C. radiosa* in Lake Montcortès are chiefly a response to shifts in stratification of the water

column. Therefore, increasing temperature would favor small *C. cyclopuncta* during intensified stratification periods. These findings provide new insights at seasonal time resolution to refine inferences made from the diatomological record in former studies working at coarser resolution. For instance, based on available information on diatom autoecology, the presence of *Cyclotella compta* (a synonym for *C. radiosa*) and *C. cyclopuncta* in the sediment record was attributed to warmer and cooler periods, respectively (Corella et al., 2012). Another study found frequencies of *C. cyclopuncta* and *C. radiosa* to vary inversely and only appeared together in a single sample over the last 1500 years and suggested that these shifts may be a response to nutrient availability (Scussolini et al., 2011). However, present-day data reveals an alternative scenario where both taxa coexist together and differences in relative abundances respond to seasonal changes in water stability with warmer conditions favoring *C. cyclopuncta*.

Both changes in diatom species abundances as well as in shapes and sizes of calcite crystals are related indirectly to temperature by means of changes in SI and water column stratification. Additionally, the occurrence of calcite precipitation and differences in calcite amounts between the years are related with temperature variability. Consequently, varve succession in Lake Montcortès is a direct product of seasonally changing environmental conditions and is especially sensitive to alterations in temperature.

The importance of modern analogue studies lies in the need of understanding the specific processes taking place in a specific place within specific features (hydrology, geomorphology, limnology, climate and anthropogenic influence) that shape the resulting sedimentary sequence and the resulting environmental signal. Then, general validity processes could not apply properly to successfully interpret the paleorecord. For example, Bonk et al. (2015) demonstrated that multiple calcite precipitation events took place in a single year related with changes in the mixing regime of lake Żabińskie (Poland) depending of climate conditions. These results were coherent with the sedimentary structure of the last 20 years where a maximum of four calcite layers were observed within a single year. The modern analogue study in this case provided a basis to establish a reliable varve chronology. Based on three years of monitoring data on seasonal particle pulses in Lake Van (Turkey), Stockhecke et al. (2012) found that the temporal and lateral variations of lake basin changes on particle accumulation and composition (lithology) were linked to atmospheric circulation patterns that controlled hydrological and meteorological conditions. Thus, they provide a basis for the reconstruction of past seasonal climate patterns. All of these monitoring studies successfully gave sense to modern processes to be compared with the fossil record for these specific places setting a baseline to reduce sources of error for future sediment studies. Anyhow, although monitoring studies are highly time consuming and costly, long-term water column and particle-flux monitoring should continue to better assess inter-annual variability and to improve future interpretations of the past.

6. Conclusions

Two years of sediment trap and limnological monitoring in the Lake Montcortès enabled us, for the first time for this lake, to link the limnological cycle to seasonal particle fluxes and provide modern sedimentary analogues potentially useful for improving the quality and quantity of paleoenvironmental and paleoecological information.

Varve deposition in Lake Montcortès is a direct result of seasonal limnological changes occurring in the water column and its seasonality is consistent with the annually laminated nature of the sediment record. Sedimentary patterns are closely related to biological processes occurring in the euphotic zone. There is a clear seasonal signal, useful for paleolimnological interpretation, expressed in the alternation of calcite precipitation during summer and fall and organic matter precipitation during winter and spring. Summer/fall calcite precipitation is favored by high calcite saturation indices and high pH values, in connection

with enhanced primary production and the eventual capacity of phytoplankton cells to act as condensation nuclei. Inter-annual differences were expressed in terms of the amount of precipitated calcite, which is related to temperature variability. Thus, the thickness of calcite layers in the sediment could be considered as a potential paleoclimatic temperature proxy. A hypothesis that should be tested in the future. In addition, changes in calcite-crystal size and in the composition of diatom assemblages would give additional information about changes in SI and thermal stratification.

According to our results, the varves that formed during the two studied years would likely match one of the varve types previously identified in the sedimentary record of the last millennium. Microstratigraphic investigations from sediment cores retrieved after 2015 is needed to confirm the resulting sedimentary pattern corresponding to the two studied years. Relationships between environmental and meteorological conditions and characteristics of individual varves need to be tested against longer time series of environmental and meteorological data.

Although it has been demonstrated that Lake Montcortès is not strictly meromictic but with alternating holomictic and meromictic periods, the long duration of thermal stratification and hypolimnetic anoxia create suitable conditions for varve formation and preservation. Nonetheless, the limnological monitoring and the sediment trap survey should continue to account for potential inter-annual variability, which is especially meaningful in the Mediterranean area and in the context of global warming.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.palaeo.2018.01.046>.

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Seasonal patterns of pollen sedimentation in Lake Montcortès (Central Pyrenees) and potential applications to high-resolution paleoecology: a 2-year pilot study

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Abstract Lakes with varved sediments are especially well suited for paleoecological study, from annual to even seasonal resolution. The interpretative power of such high-resolution paleoenvironmental reconstructions relies on the availability of modern analogs with the same temporal resolution. We studied seasonal pollen sedimentation in varved Lake Montcortès, Central Pyrenees (Spain), as a modern analog for high-resolution reconstruction of Late Holocene vegetation and landscape dynamics. Seasonal samples were obtained from sediment traps that were submerged near the maximum water depth for a 2-year period (fall 2013 to fall 2015). Seasonal pollen sedimentation was compared with meteorological variables from a nearby

weather station. Bulk pollen sedimentation, dominated by *Pinus* (pine) and *Quercus* (oak), followed a clear seasonal pattern that peaked during the spring/summer, coinciding with maximum temperature and precipitation, minimum relative humidity and moderate winds from the SSE. Pollen sedimentation lags (PSL) were observed for most pollen types, as substantial amounts of pollen were found in the traps outside of their respective flowering seasons. Two pollen assemblages were clearly differentiated by their taxonomic composition, corresponding to spring/summer and fall/winter. This pattern is consistent with existing interpretation of the sediment varves, specifically, that varves are formed by two-layer couplets that represent the same seasonality as pollen. We concluded that pollen sedimentation in Lake Montcortès exhibits a strong seasonal signal in the quantity of pollen, the taxonomic composition of the pollen assemblages, and relationships between the pollen and meteorological variables. Thus, varved sediments provide a potentially powerful tool for paleoecological reconstruction at seasonal resolution. This method could be used not only to identify paleoenvironmental trends, but also to identify annual layers and therefore date sediments, even in the absence of evident sediment laminations. A satisfactory explanation of PSL will require further studies that examine internal lake dynamics and pollen production/dispersal patterns.

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Introduction

Varved lake sediments are useful not only for high-resolution paleoecological studies, but also for bridging the temporal gap between ecology and paleoecology. Indeed, the annual/seasonal domain is the ideal time frame to produce truly continuous long-term, high-resolution ecological time series, which successfully merge ecological and paleoecological data (Rull 2014). The combination of ecological data from varved lake sediments and modern observations may yield long, high-resolution ecological time series comparable to the continuous, long-term climate series obtained by linking paleoclimate data derived from tree rings and similar proxies, with instrumental climate measures at annual/seasonal resolution (Mann et al. 1999). Formation of annually laminated lake sediments requires a seasonal climate, and variable flux to the sediment of components from multiple autochthonous and allochthonous sources. The preservation of varves is favored in small, deep lakes with a permanent (meromictic) or seasonal (monomictic/dimictic), hypoxic or anoxic hypolimnion. Varves are especially well preserved in meromictic lakes with a clear chemical contrast between the epilimnion and hypolimnion, which differ in water density, thereby maintaining stratification and preventing water column circulation (O'Sullivan 1983; Ojala et al. 2012; Zolitschka et al. 2015). Lakes with varved sediments are more frequent in northern, temperate regions. In Europe, more than 60 lakes with annually laminated sediments have been studied, of which more than half have continuous varve chronologies for at least the last 100 years. Middle and late Holocene records are frequent, and in some cases varved sediments extend back to the late glacial and early Holocene (Ojala et al. 2012). These particular types of sediments have been used for a variety of paleoecological studies, most notably to calibrate radiometric (^{14}C , ^{210}Pb , ^{137}Cs , ^{32}Si) chronologies and to obtain high-resolution paleorecords of Earth's magnetic field, solar forcing, volcanic and seismic activity, climatic change, ecological shifts and human activities (Zolitschka et al. 2015).

In the Iberian Peninsula, four lakes with annually laminated sediments have been studied to date, a Pliocene paleolake (Muñoz et al. 2002) and three extant lakes with Holocene varves: La Cruz, with laminations that date from AD 1579 to the present

(Romero-Viana et al. 2008, 2011), Zóñar, with intermittent varved sections over the last 2500 years (Martín-Puertas et al. 2009) and Montcortès, the longest, continuous varved record retrieved thus far, representing the last ~1550 years (Corella et al. 2011, 2012) (Fig. 1). Preliminary paleoclimate, paleoecological and paleolimnological studies of the Montcortès record were conducted at multi-decadal to centennial resolution (Corella et al. 2011, 2012; Rull et al. 2011; Scussolini et al. 2011; Rull and Vegas-Vilarrúbia 2014, 2015). The only high-resolution study to date is an annual reconstruction of the extreme rainfall events that occurred since the mid-fourteenth century (Corella et al. 2014). The varve chronology of this lake (ca. AD 400 to the present) is ideal for studying the transition from the Medieval Warm Period (MWP) to the Little Ice Age (LIA) and ongoing Global Warming (GW), at annual resolution. A recent initiative was launched to increase the resolution of climatic and ecological reconstructions, which includes the study of present-day varve formation and preservation, so that they can be used as modern analogs of the past. Modern sedimentation was monitored using sediment traps in the lake to record seasonal variations in sediment flux and composition, a widely used method in studies of lakes with laminated sediments (Bloesch and Burns 1980; Mieszcankin 1997; Mieszcankin and Noryskiewicz 2000; Punning et al. 2003; Giesecke and Fontana 2008; St. Jacques et al. 2008; Huguuet et al. 2012; Zolitschka et al. 2015).

In this paper, we present results of 2 years (fall 2013 to fall 2015) of pollen trapping, and investigate potential relationships between seasonal patterns of pollen sedimentation and relevant climate variables. The primary aim of this study was to identify seasonal pollen sedimentation patterns that would be useful for interpreting past records from the same lake. In this study, we concentrated our efforts on pollen sedimentation features; analysis of pollen-vegetation relationships, which is required to interpret past pollen records in terms of vegetation changes, is beyond the scope of this study, but will be addressed in the future. Pollen seasonality could be useful to resolve annual sediment layering, and therefore achieve high-resolution dating, even if physical sediment features are difficult to discern, e.g. when sublayers are absent, turbidites are present, or there are no laminations at all (Tippett 1964; Lotter 1986; St. Jacques et al. 2008). Sediment

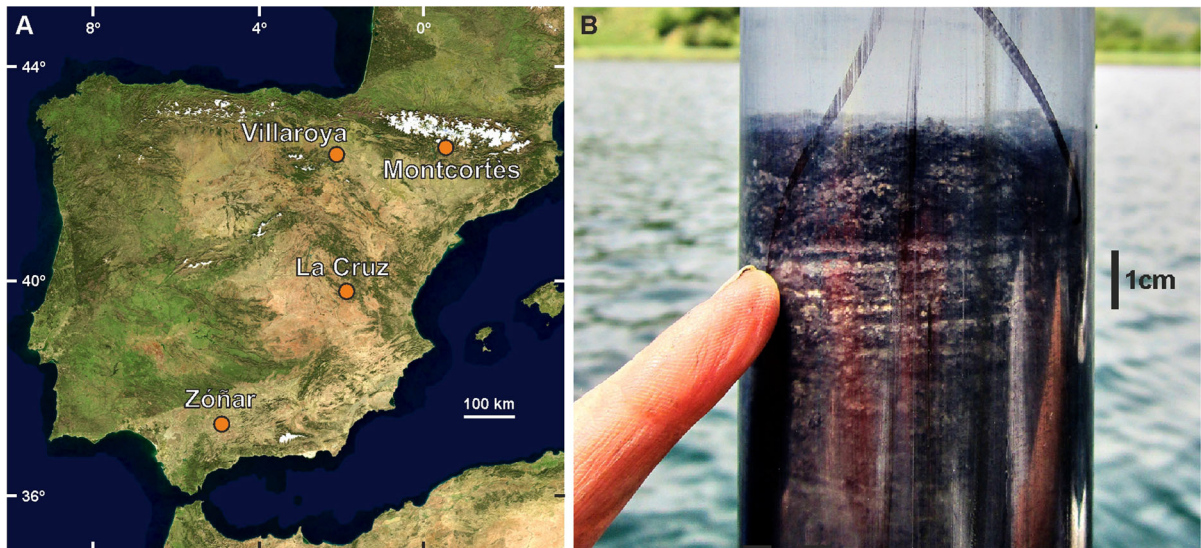


Fig. 1 **a** Map of the Iberian Peninsula showing lakes with varved sediments that have been studied to date. **b** Close-up of the top of a gravity sediment core retrieved from Lake

Montcortès in 2013 that shows the varves of the previous years, with a clear alternation of *white* and *brownish* layers (see text). (Color figure online)

trap studies have been used to monitor pollen sedimentation in many parts of Europe, across a wide array of environments and vegetation types, and have provided insights into the importance of intra-annual weather conditions and pollination patterns (Mieszcankin 1997; Mieszcankin and Noryskiewicz 2000; Punning et al. 2003; Giesecke and Fontana 2008; Giesecke et al. 2010; van der Knaap et al. 2010; Pidek et al. 2015). To our knowledge, however, this is the first study to use submerged pollen traps in varved lakes on the Iberian Peninsula. Previous studies of this type were carried out in non-varved, high-mountain Pyrenean lakes to examine plankton sedimentation and its relationship to climate seasonality (Pla-Rabes and Catalan 2011). Our aim is to maintain the traps in Lake Montortès to study pollen sedimentation over the medium to long term.

Study site

Lake Montcortès is situated on the southern flank of the Central Pyrenees, in the Pallars Sobirà region of Catalonia (Spain), at 42°19'N, 0°59'E and 1027 m altitude, with a surface of 12.36 ha. The lake lies in karst terrain that is primarily characterized by Triassic limestones, marls and evaporites, and Oligocene carbonate conglomerates. Triassic ophyte outcrops primarily occur in the southern Quaternary lacustrine

sediments that surround present-day water bodies (Corella et al. 2011). The catchment is small, and the lake is fed primarily by groundwater, with intermittent small creeks and scattered springs. Most water is lost to evaporation and a small seasonal outlet at the north end of the lake. The lake is roughly kidney-shaped, with a diameter between 400 and 500 m and a maximum water depth of 30 m near the center (Corella et al. 2014) (Fig. 2). Climate data from a nearby weather station (La Pobla de Segur), which is situated ~9 km to the south (Fig. 2) at 513 m elevation, show the annual average air temperature of the area is 12.8 °C, which ranges from 2.9 °C in January to 23.2 °C in July. Total annual precipitation is 669 mm. February is the driest month (33.4 mm) and May is the wettest month (88.4 mm). Maximum and minimum temperatures recorded at this location were 41 and −20 °C, respectively. The maximum daily precipitation recorded was 138 mm.

The lake lies near the altitudinal boundary of the Sub-Montane belt, which is located in the Pyrenees at 800–1000 m elevation, depending on local features (Vigo and Ninot 1987). Four major forest formations occur in the lake region (Fig. 3): (1) Mediterranean sclerophyllous forests represented by *Quercus rotundifolia* woods; (2) Sub-Montane deciduous oak forests, which experience higher levels of precipitation and are dominated by *Quercus pubescens* and *Q.*

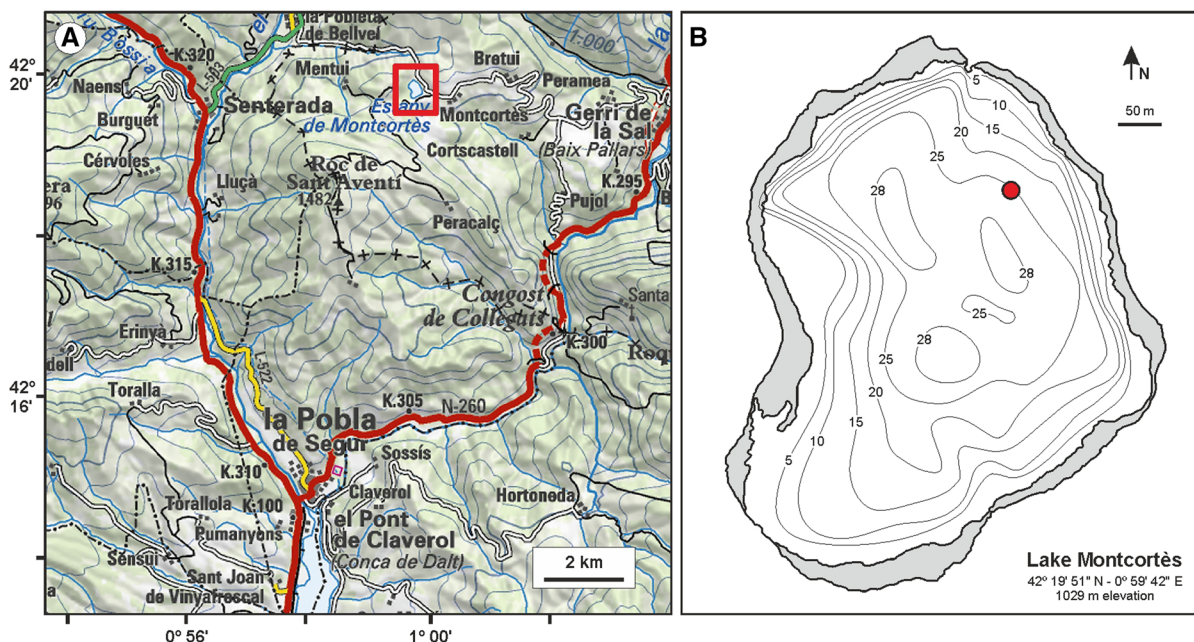


Fig. 2 **a** Map of the region around Lake Montcortès, indicating the location of the lake (red box) and the meteorological station used in this study (La Pobla de Segur). Base map:

Institut Cartogràfic I Geològic de Catalunya (www.icc.cat). **b** Bathymetry of Lake Montcortès with the location of sediment traps (red dot). (Color figure online)

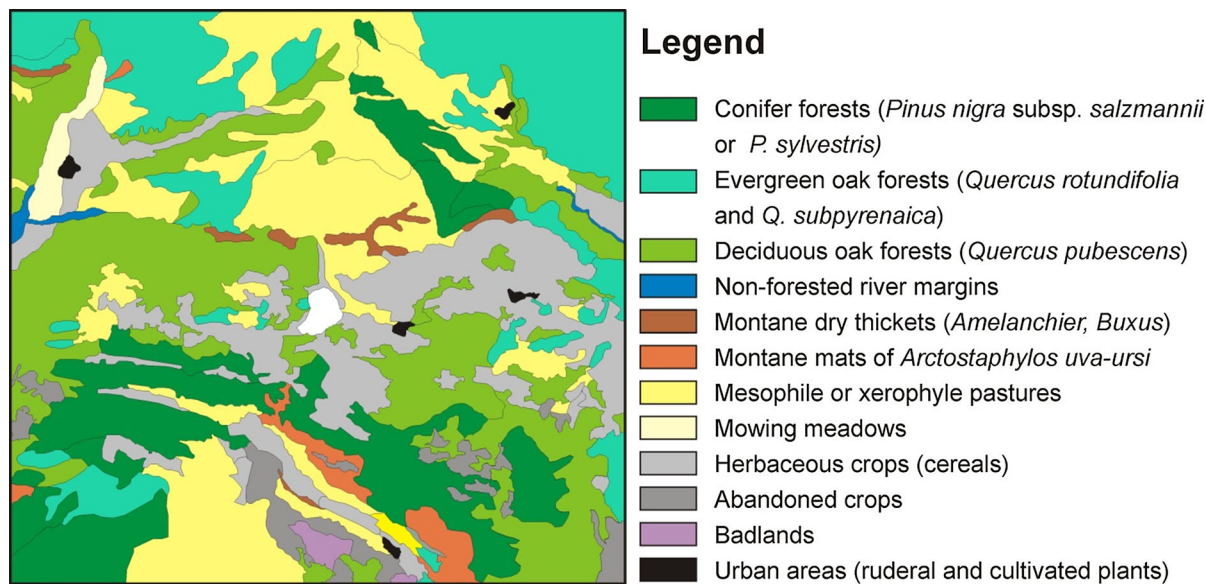


Fig. 3 Regional vegetation map of Montcortès using the CORINE system (CEC 1991). The lake is represented as a white area in the center. Modified from Ninot (2006) and Ferré and Carrillo (2007). (Color figure online)

subpyrenaica; (3) conifer forests of *Pinus nigra* subsp. *salzmannii*, which are usually secondary and replace the deciduous oak forests in the lower and southern regions, but are probably natural here (Bolòs et al.

2004); and (4) higher-elevation forests of *Pinus sylvestris*, which mark the transition between Sub-Montane and Montane belts. Portions of these conifer woods were likely planted. *Pinus* forests are

monospecific with a poorly developed understory, whereas oak woodlands are more taxonomically diverse.

The evergreen *Q. rotundifolia* communities have a well-developed understory with several shrubs (*Q. coccifera*, *Rhamnus* spp., *Prunus spinosa*, *Buxus sempervirens* and *Lonicera japonica*) and an herbaceous stratum with nemoral (shade-adapted) species such as *Rubia peregrina*, *Teucrium chamaedris*, *Asparagus acutifolius* and *Brachypodium retusum*. The *Q. pubescens*-*Q. subpyrenaica* deciduous forests include a variety of other trees in the arboreal stratum, notably *Pinus sylvestris*, *Fagus sylvatica*, *Tilia cordata* and some *Acer* species. The understory is dominated by *Buxus sempervirens*, *Coronilla emerus*, *Amelanchier ovalis*, *Colutea arborescens*, *Cytisophilum sessilifolium* and *Viburnum lantana*. In the herbaceous stratum, the most common species are *Primula veris*, *Hepatica nobilis*, *Brachypodium phoenicoides* and *Campanula persicifolia*. There are two main types of regional shrubland: one dominated by *Amelanchier ovalis*, *Buxus sempervirens* and *Rhamnus saxatilis*, and another dominated by *Arctostaphylos uva-ursi* with *Buxus sempervirens*. Herbaceous communities primarily consist of meadows and pastures of *Aphyllanthes monspelliensis* and *Arrhenatherum elatius*, herbaceous cereal crops (*Hordeum* sp., *Avena sativa*, *Triticum* sp., *Secale cereale*) and some forage plants (*Medicago sativa*), with several weeds (*Lolium rigidum*, *Papaver rhoeas*, *Polygonum aiculare*, *Bromus* sp.). Abandoned croplands, colonized by shrubs and ruderal species, and badlands devoid of vegetation or with scattered shrubs and herbs from other communities, also occur in some areas (Carreras et al. 2005–2006) (Fig. 2).

The local vegetation around the lake is closely tied to microclimate conditions and is fairly diverse in comparison to surrounding regional patterns. A recent detailed study by Mercadé et al. (2013) recognized 534 species of vascular plants distributed across 52 vegetation units of the European CORINE biotope classification (Vigo et al. 2005–2008). Aquatic and semi-aquatic habitats are represented by submerged vegetation, sometimes with floating leaves (*Potamogeton*, *Myriophyllum*, *Chara*, *Utricularia*, *Ranunculus*), and a macrophyte fringe surrounding the lake that is dominated by the grass *Phragmites*, with *Cladium* and *Typha*. Hygrophilous vegetation grows on wet or inundated soils. The most common

vegetation consists of sedges, such as *Carex*, *Eleocharis* and *Scirpus*. *Juncus* species, together with *Cyperus* and *Plantago*, dominate the plant communities on temporarily flooded soils. Several types of meadows and pastures around the lake are characterized by *Arrhenatherum* (hay meadows), *Trifolium* (calcareous grasslands), *Filipendula* (mesophilous grasslands), *Festuca* and *Aphyllanthes* (xerophilous grasslands) and *Poa* (dwarf grasslands). Shrubby vegetation is primarily represented by communities dominated by *Buxus*, *Genista* and *Thymus*. Forest formations around the lake are dominated by various *Quercus* species and small stands of *Fraxinus*, *Populus* and *Salix*. Anthropogenic habitats consist of intensive pastures, crop fields (*Hordeum*, *Medicago*, *Chenopodium*), fruit-tree orchards (*Malus*, *Prunus*, *Pyrus*) and ruderal communities (*Arctium*, *Artemisia*, *Galium*, *Pastinaca*, *Urtica*, *Melilotus*, *Polygonum*). A very detailed description of the vegetation around the lake can be found in Mercadé et al. (2013).

The region is rather densely populated and the lake has historically been an important water source for the numerous surrounding villages and farmhouses. An artificial pond was recently built to use the water from the lake for fire-fighting purposes. Cultivation (wheat, oat, barely, olives, rye, hemp and legumes) and livestock husbandry (cattle and sheep) have also increased in the area during the last millennium and possibly longer (Rull and Vegas-Vilarrúbia 2015). Currently, cereal and alfalfa fields, intermingled with pastures for cattle and horses, and hay meadows, are common and heavily exploited. The lake and its catchment area are part of the European Natura 2000 network for the protection of species and habitats (http://ec.europa.eu/environment/nature/natura2000/index_en.htm).

In Lake Montcortès, varved sediments extend down to a depth of 543 cm, which encompass the last 1548 years. Varves are thin (1.16 mm average thickness) and are composed of two (three) layers, intermingled with occasional turbidites (6.8 mm average thickness). The basic varve unit is a biogenic couplet of two layers, a white calcite layer and a brownish organic layer (Fig. 1). A third, grayish detrital layer may be present between the calcite and organic layers. According to Corella et al. (2012), the white layer corresponds to spring/summer and is characterized by rhombohedral calcite crystals, whereas sediments of the brownish layer correspond to fall/winter and are

primarily composed of amorphous organic matter, diatoms, detrital carbonate and quartz grains within a clayey matrix. The grayish detrital layer is deposited during phases of increased runoff and consists of irregularly shaped detrital calcite, quartz and feldspar grains, terrestrial plant remains and clay minerals (Corella et al. 2012). Detailed limnological study of the lake dynamics in relation to varve formation and preservation is in progress.

Materials and methods

A set of sediment traps was suspended from a floating platform located above the deepest part of the lake, where the sediment cores were collected for paleoecological study. Each trap consisted of an opaque cylindrical plastic tube (8.5 cm diameter and 80 cm long), with the top open and the base sealed, and was suspended by a plastic line at a water depth of 20 m, i.e. ~5 m above the bottom. The content of the pollen traps was collected quarterly at the end of each season: March (winter), June (spring), September (summer) and December (fall). Sediments were allowed to settle in the laboratory for a minimum of 48 h. The supernatant was decanted and filtered through a glass-fiber filter and was added to the sediment to minimize losses. A tablet of exotic *Lycopodium* spores (batch no. 1031, 20,848 spores/tablet, on average) was added to the sediment prior to chemical processing, which included acid digestion (HCl and HF), acetolysis and storage in glycerine (Bennett and Willis 2002). Microscope slides were prepared with glycerine, without sealing. Pollen identification was made following previous, lower-resolution studies (Rull and Vegas-Vilarrúbia 2014, 2015; Rull et al. 2011). Pollen was counted until a minimum of 300 pollen grains per sample had been enumerated—excluding *Pinus* and *Quercus*, which were super-abundant (~40–90% of the total counts)—and the diversity of the sample was saturated (Rull 1987). Total pollen counts averaged 729 (range 305–1118) and exotic *Lycopodium* counts averaged 70 (range 23–171). Taxonomic classification of plants and the grouping of pollen into vegetation types followed Mercadé et al. (2013). Diagram plotting and statistical analyses were performed with Psimpoll 2.7 and MVSP 3.22, respectively.

The meteorological variables used in this study were obtained from the nearest weather station, La

Pobla de Segur, which is located ~9 km to the south (Fig. 2) at 513 m elevation. The meteorological variables considered were average, maximum and minimum temperature (T_m , T_x , T_n , respectively, in °C); average, maximum and minimum relative humidity (H_m , H_x , H_n , respectively, in %); average, maximum and minimum pressure (P_m , P_x , P_n , respectively, in hPa); total precipitation (PPT; in mm); wind velocity (W ; in m s^{-1}) and wind direction (W_d ; in °) at 10 m above the ground. Seasonal values for these variables were obtained by averaging daily values for each season, using raw meteorological measures from the reference station.

Cluster analysis was used to identify seasonal pollen assemblages. In this case, we used the Gower similarity coefficient and the centroid clustering method, which have proven to be suitable for similar purposes using pollen data (Rull 2001, 2003). Spearman rank correlation coefficient was used to study relationships between pollen and meteorological variables. This non-parametric correlation method is recommended when the requirements for using the parametric Pearson product-moment correlation coefficient are not met (Siegel and Castellan 1988). In our case, we used the Spearman index because of the low sample size ($n = 9$). Canonical correspondence analysis (CCA) was used to define new multi-dimensional variables that account for maximum variance in the dataset, and to graphically display pollen and meteorological data simultaneously in the space of these new variables (Jongman et al. 1995). All statistical analyses were carried out on percentage data using MVSP version 3.22.

Results

Total pollen sedimentation displays a clear seasonal pattern, with maxima during the spring/summer and minima during the fall and winter (Fig. 4). The 2 years studied had similar patterns except for the dramatic maximum recorded in the spring of 2015, with values more than three times higher than in the spring of 2014. The major components of the pollen assemblages were *Pinus* and *Quercus*, with their percentages oscillating between 15 and 35%, respectively, throughout the year, except during the spring, which is the flowering season of both taxa, when *Pinus* increased to 50–65%. In the case of *Quercus*, the

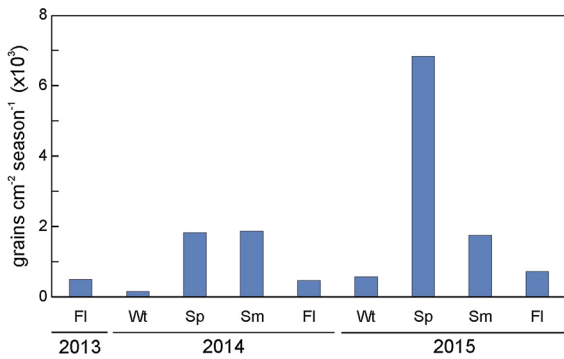


Fig. 4 Seasonal trends of total pollen sedimentation expressed as influx units (number of grains per cm² per season). *Sp* spring, *Sm* summer, *Fl* fall, *Wt* winter. (Color figure online)

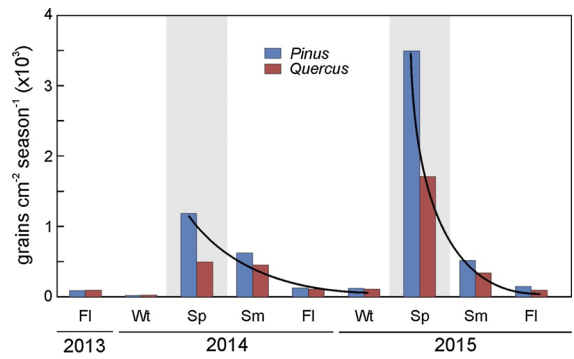


Fig. 6 Pollen influx values for *Pinus* and *Quercus* during the study period. *Sp* spring, *Sm* summer, *Fl* fall, *Wt* winter. (Color figure online)

seasonal pattern is less apparent, and the spring percentages were below 30% (Fig. 5). In both cases, the supply of pollen to the sediment traps was continuous throughout the year, although parent plants were no longer in bloom. This phenomenon, called pollen sedimentation lag (PSL), is better assessed using influx values (Fig. 6). A large fraction of *Pinus* and *Quercus* pollen settled onto the sediments during the spring, but a significant portion of pollen settled later, particularly between summer and winter. This negative exponential trend occurred in both years, with lower decreasing rates in 2014 than in 2015. In both cases, however, summer to winter values were very similar, indicating that, no matter the intensity of the spring peak, the background signal for the rest of the year was almost the same.

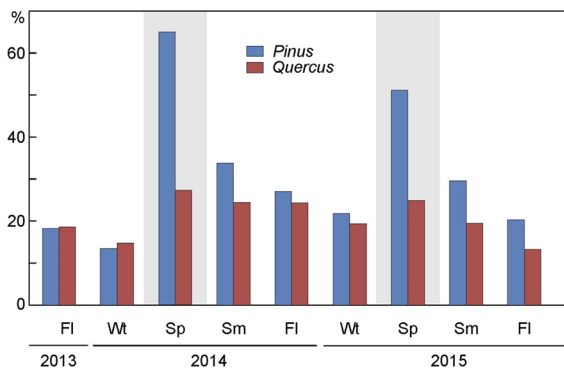


Fig. 5 Pollen percentages of *Pinus* and *Quercus*, the two major components of the pollen assemblages, throughout the year. Grey bands represent the flowering season of *Pinus* and *Quercus* species present in the Montocrtès region, according to Bolòs et al. (2000). *Sp* spring, *Sm* summer, *Fl* fall, *Wt* winter. (Color figure online)

The most important pollen taxa, in terms of abundance and seasonality (Fig. 7) illustrate that trees tend to bloom before (winter/spring) herbs (spring/fall), a trend that is also reflected in patterns of pollen sedimentation. In general, percentage pollen peaks coincide with the flowering season of each taxon, but almost all plant species exhibited PSL, expressed by the presence of pollen outside the flowering season. Taxa with a lower PSL, that is, with pollen sedimentation patterns that are very similar to flowering patterns, such as *Corylus*, *Fraxinus*, and *Artemisia*, are all represented by a single species in the lake (Mercadé et al. 2013). The genus *Olea* had the highest PSL. On the other hand, *Plantago* and *Chenopodium* had an intermediate PSL. Possible presence of several species from the adjacent flora with similar pollen morphology, but with different flowering seasons, might also explain these patterns. In families such as Poaceae and Cyperaceae, which include many genera and species, this is certainly the case. In general, aside from *Pinus* and *Quercus*, the most abundant pollen type belongs to *Cannabis*, especially during the fall, when it reaches values of 40% or more. Cluster analysis yielded two groups that represent two distinct pollen assemblages, the spring/summer assemblage and the fall/winter assemblage (Fig. 8). The only exception was the sample from fall 2014, which was more similar to the spring/summer samples.

Regarding the relationship between pollen and meteorological variables, a preliminary visual inspection showed that the influx of total pollen and of pollen from major types (i.e. *Pinus* and *Quercus*) roughly matched seasonal trends in temperature and precipitation (Fig. 9). Pollen maxima occurred during

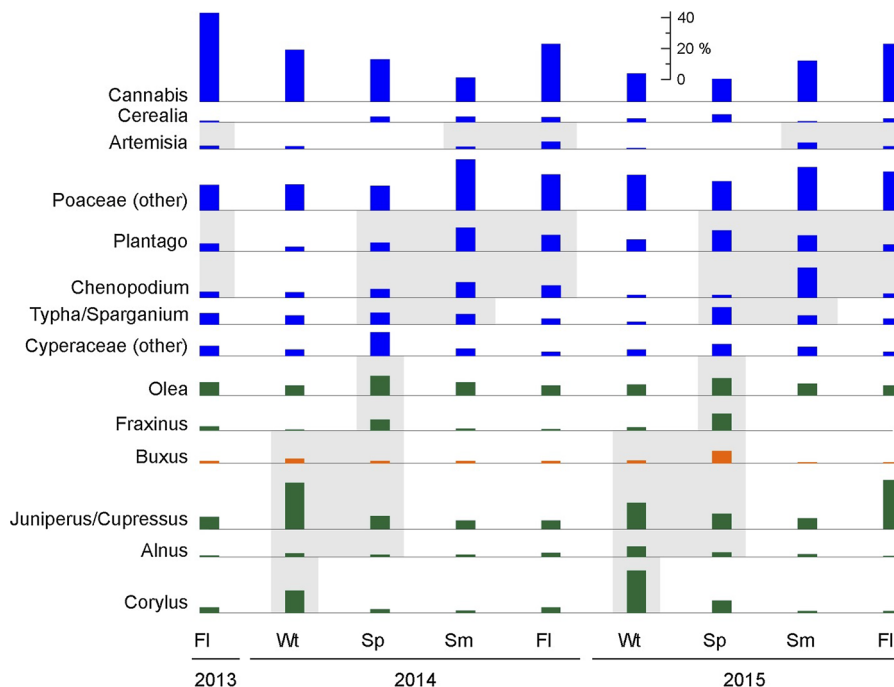


Fig. 7 Percentage diagram of the most relevant pollen taxa during the study period. Percentages were calculated, excluding the super-abundant *Pinus* and *Quercus* (Fig. 5). Taxa are ordered by their respective flowering seasons (grey bands) (Bolòs et al. 2000), from bottom to top and from left to right. The flowering season of all species of the different genera present in

the Montcortès region (Mercadé et al. 2013) was considered. 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. *Sp* spring, *Sm* summer, *FI* fall, *Wt* winter. (Color figure online)

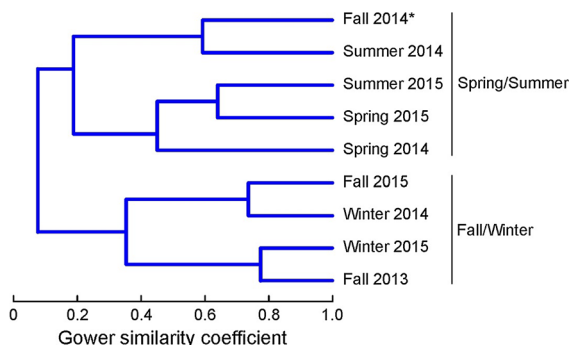


Fig. 8 Cluster analysis using the Gower (1971) similarity coefficient and the centroid clustering method. The asterisk indicates the only sample that does not follow the spring/summer versus fall/winter pattern. (Color figure online)

the flowering season of the involved taxa (spring), one season before temperature and precipitation maxima (summer). The relationship between pollen and relative humidity was inverse. Moreover, maxima of pollen influx coincided with moderate wind velocities

with a predominant SSE ($\sim 150^\circ$) direction, whereas pollen minima coincided with slower winds with a WSW ($\sim 250^\circ$) direction.

Individually, a number of pollen taxa exhibited significant correlations with meteorological variables, whereas others did not (Table 1). Some of the relationships are worthy of mention. The variables with greatest significant correlations were wind velocity, wind direction and relative humidity, whereas pressure did not show a significant correlation. Pollen taxa that lacked significant correlations with meteorological variables were *Alnus*, *Artemisia*, *Buxus*, *Cerealia*, *Corylus*, *Plantago* and *Poaceae* (others). *Olea*, *Pinus*, *Quercus* and *Cyperaceae* (others) were negatively associated with relative humidity and wind direction and positively correlated with wind velocity. *Chenopodium* and *Juniperus/Cupressus* were correlated with temperature (together with *Pinus*) and total precipitation. *Cannabis* was correlated only with wind velocity.

Fig. 9 Relationships between pollen influx and the most relevant meteorological variables. Average temperature (T_m), relative humidity (H_m) and wind velocity (W) are represented by *lines*. Total precipitation (PPT) is represented by *bars*. The predominant direction of the wind (W_d) is shown in *circles*. (Color figure online)

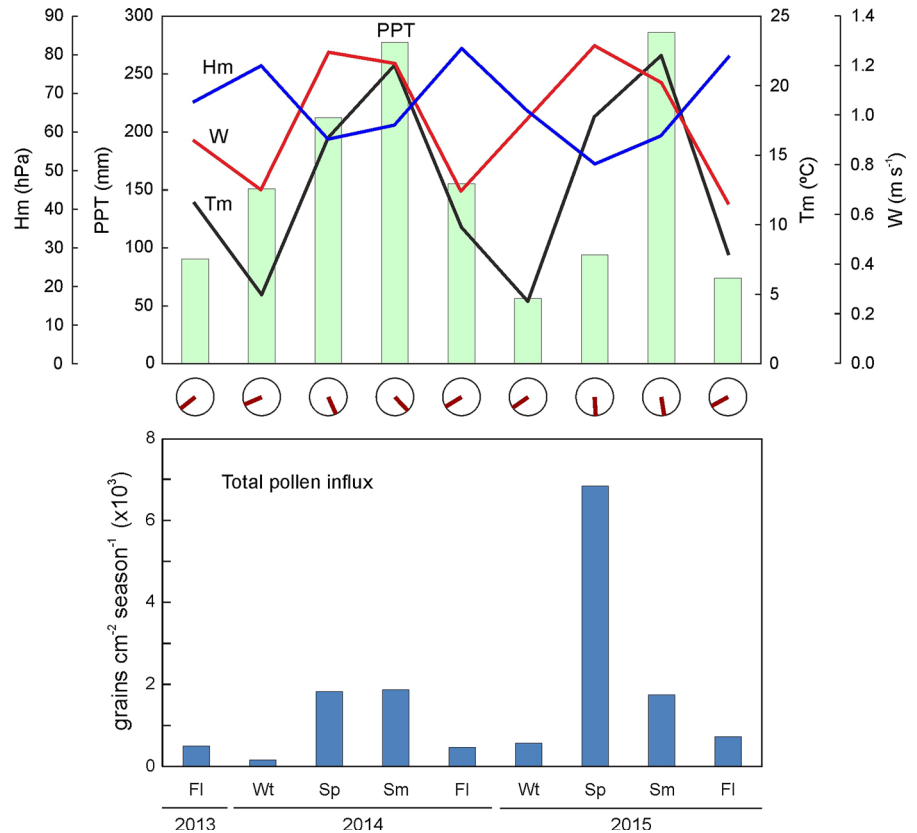


Table 1 Spearman-rank correlation coefficients between the most relevant pollen taxa and meteorological variables

See “Materials and methods” section for abbreviations

T_m , average temperature; H_m , average relative humidity; P_m , average pressure; PPT, total precipitation; W , average wind velocity; W_d , predominant wind direction
Significant correlations (* $\alpha = 0.05$; ** $\alpha = 0.01$) are in bold

Pollen taxa	T_m	H_m	P_m	PPT	W	W_d
<i>Alnus</i>	-0.200	-0.167	-0.217	-0.100	0.250	0.117
<i>Artemisia</i>	-0.008	0.661	0.025	0.226	-0.661	0.368
<i>Buxus</i>	-0.150	-0.383	-0.417	-0.100	0.500	-0.033
<i>Cannabis</i>	-0.383	0.750	0.150	-0.200	-0.767*	0.533
Cerealia	0.300	-0.500	0.000	0.067	0.617	-0.533
<i>Chenopodium</i>	0.683*	-0.033	-0.417	0.900**	0.083	-0.500
<i>Corylus</i>	-0.533	-0.033	-0.167	-0.467	0.117	0.317
Cyperaceae (others)	0.567	-0.883**	-0.017	0.233	0.850**	-0.717*
<i>Fraxinus</i>	0.117	-0.577	0.017	-0.176	0.678*	-0.427
<i>Juniperus/Cupressus</i>	-0.667*	0.150	0.267	-0.700*	-0.300	0.650
<i>Olea</i>	0.650	-0.850**	0.050	0.267	0.867**	-0.867**
<i>Pinus</i>	0.667*	-0.750*	-0.200	0.517	0.800**	-0.800**
<i>Plantago</i>	0.617	-0.433	-0.050	0.433	0.567	-0.650
Poaceae (others)	0.317	0.150	0.133	0.300	-0.150	-0.150
<i>Quercus</i>	0.600	-0.700*	-0.367	0.533	0.817**	-0.767*
<i>Typha/Sparganium</i>	0.567	-0.650	-0.183	0.333	0.700*	-0.533

A synthetic analysis was conducted using Canonical Correspondence Analysis (CCA). Figure 10 shows the scatter plot with the first two axes, which

accounted for 70.74% of the total variance. The strongest gradient corresponds to axis 1 (56.80% of the total variance), which was highly correlated with

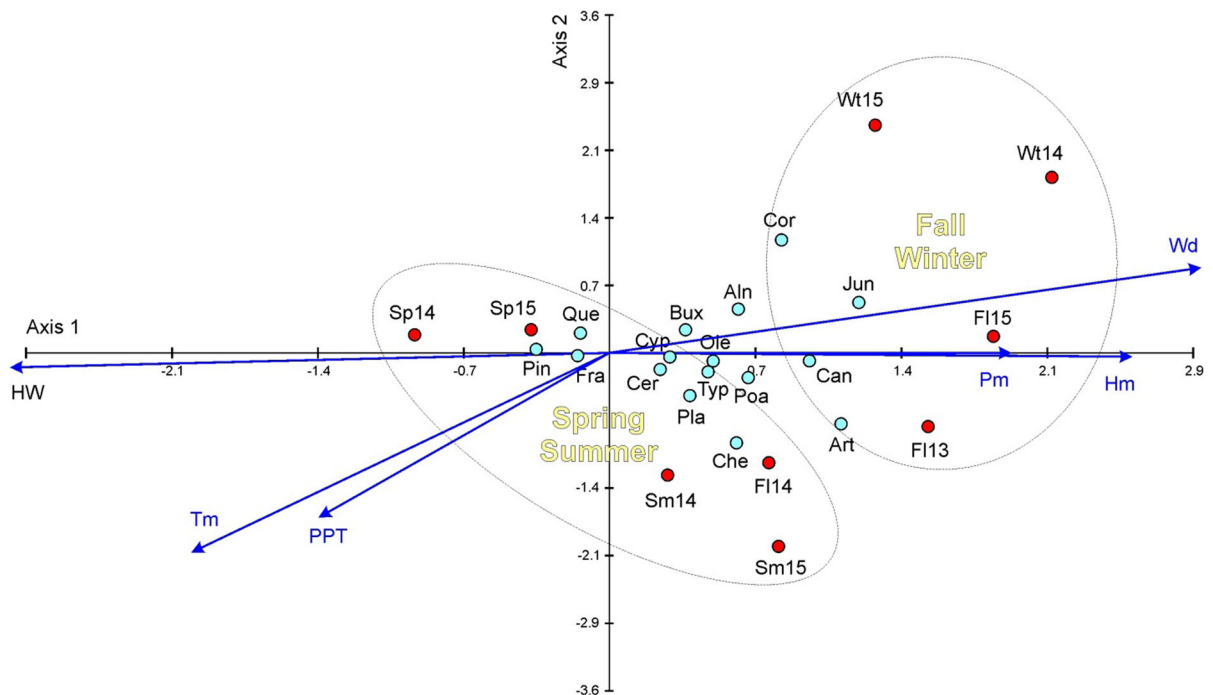


Fig. 10 CCA biplot using the scores of the first two axes accounting for 70.74% of the variance. Samples are represented by red dots and pollen taxa are represented by blue dots. (Color figure online)

relative humidity and pressure with its positive values, and with wind velocity with its negative values. Along this gradient, samples were ordered according to a seasonal gradient from spring (left) to winter (right), with summer and fall occupying intermediate positions. Pollen taxa were also ordered according to the same gradient: *Pinus*, *Quercus* and *Fraxinus* were close to spring samples and *Juniperus/Cupressus*, *Corylus*, *Cannabis* and *Artemisia* were located in the fall/winter cluster. The remaining taxa occupied an intermediate position. The spring group was highly correlated with temperature, precipitation and wind velocity. The fall group was also highly correlated with wind direction, which in this season comes from the WSW. This wind direction contrasts with the winds during the spring/summer, which blow from the SSE. Taxa most associated with WSW winds were *Juniperus* and *Cannabis*, which are typically fall taxa.

Discussion

In general, our results show a distinct seasonal pattern in pollen sedimentation that is reflected in

both total influx and taxonomic composition. In the spring/summer assemblage, *Pinus* had values of 50–65% of the total counts and *Quercus* had approximately 25%, whereas in the fall/winter, *Pinus* was below 30% and *Quercus* was lower than 20% (Fig. 5). Regarding the other plant species, the most significant differences were in *Plantago*, *Chenopodium*, *Typha/Sparganium*, *Cyperaceae*, *Fraxinus* and *Juniperus/Cupressus*, which were more abundant in the spring/summer assemblage, and *Cannabis* and *Corylus*, which were more abundant in the fall/winter assemblage (Table 2). This seasonal pattern appears to coincide with the varved pattern of the sediments, which are formed by two-layered couplets that correspond to the same seasons, as interpreted by Corella et al. (2012). Such a correspondence should be demonstrated by analyzing individual sediment layers, which may show that the spring/summer pollen assemblage coincides with the white layer and that the fall/winter assemblage coincides with the dark layer. Physico-chemical analyses of the bulk material collected in the other traps used in this study are in progress and can provide additional evidence for this correlation

Table 2 Composition of the pollen assemblages obtained in the cluster analysis (Fig. 7), using the average percentages of major pollen types (Fig. 6), excluding *Pinus* and *Quercus*

Pollen taxa	Spring/summer	Fall/winter
<i>Cannabis</i>	16.02	27.95
Cerealia	2.36	1.05
<i>Artemisia</i>	1.06	1.19
Poaceae	18.39	15.53
<i>Plantago</i>	8.66	3.92
<i>Chenopodium</i>	7.08	2.31
<i>Typha/Sparganium</i>	6.03	3.64
Cyperaceae	6.48	3.38
<i>Olea</i>	7.67	5.58
<i>Fraxinus</i>	3.57	0.95
<i>Buxus</i>	2.14	1.27
<i>Juniperus/Cupressus</i>	5.83	1.65
<i>Alnus</i>	1.44	2.00
<i>Corylus</i>	2.39	8.82

between pollen seasonality and the formation of white and dark seasonal sediment layers.

Specific aspects of the pollen sedimentation require further discussion. For example, there is a lag in pollen sedimentation (PSL), i.e. between production and deposition, throughout the year. The cause of this lag might be manifold. First, such lags may be explained by water dynamics in the lake (Punning et al. 2003). Second, PSL may arise because of re-suspension of sediments from the uppermost layers (Mieszcankin 1997; Mieszcankin and Noryskiewicz 2000). Third, PSL may stem from the fact that pollen deposited on catchment soils during the flowering season can be washed into the lake for several months (St. Jacques et al. 2008). The first potential explanation (internal water dynamics) is currently under study. This additional study should shed some light on the potential mechanics of PSL via thermal and other density stratification. Resuspension can be identified and measured using sediment traps at different depths, in combination with aerobiological samplers at the lake surface (Bloesch 1994; Mieszcankin and Noryskiewicz 2000; Giesecke and Fontana 2008). The same combination of techniques, along with aerobiological samplers distributed across the catchment soils, might be useful for distinguishing the different processes that participate in pollen dispersal and could provide

insights into the potential role of pollen washing into the lake.

The similarity in the sedimentation patterns between *Pinus* and *Quercus* pollen is also striking because the pollen grains of these two genera are different morphologically. *Pinus* pollen is inaperturate and bears two large empty sacchi, which confer unique “buoyancy” to this pollen in air. On the other hand, *Quercus* pollen is tricolporate/tricolporoidate (Erdtman 1952) and has no distinct morphological traits or ornamentation. In spite of these differences with respect to air suspension, once the pollen is in the waters of Lake Montcortès, the sedimentation of the pollen of *Pinus* and *Quercus* was quite similar, even during summer when the lake thermal stratification is very stable. This finding could suggest that internal lake dynamics are not as important for pollen sedimentation as resuspension or catchment runoff. Aerobiological studies, however, are needed to assess this hypothesis.

Pollen of *Cannabis* (hemp) was the most abundant during the fall; however, the parent plant was not reported in an intensive floristic study of the lake catchment (Mercadé et al. 2013), or in regional surveys (Carreras et al. 2005–2006). The pollen of *Cannabis* is similar to *Humulus*; however, the criteria that distinguish them in the Montcortès sediments have already been established (Rull and Vegas-Vilarrúbia 2014). *Cannabis* is a cultivated plant whose pollen has been present and fairly abundant around Montcortès for the last 1200 years. The exact source of the pollen, however, has been impossible to locate (Rull and Vegas-Vilarrúbia 2015; Rull et al. 2011). This plant is known to have been cultivated in the adjacent lowlands (Gerri de la Sal, La Pobla de Segur and La Pobleta de Bellveí; Fig. 2) during the nineteenth century. In addition, Lake Montcortès may have been used for hemp retting, especially between the fifteenth and eighteenth centuries, but no historical documents have been found to support this hypothesis. Currently, the source for the pollen of *Cannabis* is unknown. More studies will be required to identify the source of *Cannabis* pollen. The same is true for *Humulus*, which is very scarce in the wild and has only been observed near Gerri de la Sal (A. Mercadé, pers. commun. 28 April 2016).

The overall pollen influx patterns are consistent with the fact that, in anemophilous species, high temperature, low humidity and moderate winds favor

passive flower dehydration, thereby facilitating the opening and release of pollen from the anthers (Helbig et al. 2004). These meteorological variables, however, do not provide a clear explanation for the difference in intensity of the spring pollen peaks of 2014 and 2015. Although temperature and wind velocity show almost identical patterns across 2014 and 2015, precipitation and relative humidity do not. Indeed, precipitation was significantly higher before the spring of 2014 than in 2015, whereas relative humidity was lower in the spring of 2015. These differences might have affected the release of pollen, but this hypothesis remains speculative until more local aerobiological studies are conducted. Slight differences in the location of the pollen sources cannot be dismissed, as there was a slight variation in the direction of the predominant winds between the springs of 2014 and 2015.

Individual correlations also deserve further comment, especially in the case of relative humidity, wind direction and velocity, which primarily affected *Olea*, *Pinus* and *Quercus*. Cyperaceae will not be discussed here as it may contain several species with different flowering periods and pollen dispersion/sedimentation features. *Olea* is a lowland taxon that is not common around Montcortès, which is located near the boundary of lowland and montane biomes (Rull et al. 2011; Mercadé et al. 2013). In a previous study in the Central Pyrenees, Cañellas-Boltà et al. (2009) found that *Olea* pollen occurred consistently from the lowlands to the alpine zone above 2500 m elevation. The authors attributed this distribution of pollen to the effect of upward winds. This explanation is supported by our results from Montcortès, which show that dry and windy conditions favor the sedimentation of this pollen type in lake sediments. In addition, the significant negative correlation with wind direction, expressed in degrees, indicates that the source for this pollen should be from the SW ($\sim 225^\circ$), that is, in the southern lowlands, where the species grows. The same is true for the pollen of *Pinus* and *Quercus* forests, which are better represented in the southern part of the area under study (Fig. 2).

The CCA plot (Fig. 10) yielded the same groups as the cluster analysis, which strengthened the seasonal character of the pollen succession throughout the year and showed the clear separation of the spring/summer and the fall/winter assemblages. This analysis also provided the more relevant meteorological variables linked to seasonal pollen sedimentation as a whole.

The main environmental gradient resulted from the windy, rainy and warmer character of the spring/summer seasons, with winds from the SSE, and the high pressure and high relative humidity of the fall/winter seasons, with winds from the WSW. This gradient was strongly associated with the abundance of the main pollen taxa that are characteristic of each seasonal assemblage.

Conclusions

General patterns of pollen sedimentation in Lake Montcortès during the two study years were consistent with there being a strong seasonal signal. This signal permitted the spring/summer and fall/winter assemblages to be distinguished. These seasonal differences were expressed in terms of the amount of pollen sedimentation and also in the taxonomic composition of the pollen assemblages. In addition, the main meteorological variables that influence these seasonal features of pollen were identified. Pollen seasonality coincided with the same seasonal patterns previously identified in sedimentological (varve) studies. Therefore, seasonal pollen patterns described in this study appear to adhere to a pollen-varve model that is constrained by meteorology, and which can be extrapolated down-core to be used in high-resolution paleoecological investigations. This finding needs to be corroborated with a detailed palynological analysis of the assumed seasonal sediment layers and with physico-chemical analyses of the bulk content of sediment traps. By analogy, differences in the pollen content of past varves could be explained in terms of meteorological variability, which makes pollen a potentially powerful paleoenvironmental proxy in this particular lake. In addition, pollen analysis of down-core sediments can be used to identify intra-annual seasonal patterns and to date the sediments, even in the absence of varves or at depths where the varve record has been partially disturbed. The seasonal pollen model obtained here can be applied, at least, to the last 1200 years, as all pollen types have been present with reasonably similar abundances (Rull et al. 2011). The seasonal patterns described here are sufficiently well established for use in Lake Montcortès paleoenvironmental studies. Pollen analysis of trap sediments should continue in efforts to account for potential inter-annual variability. As a general observation, the

trends and relationships established in this paper should be considered empirical, with some causal relationships yet to be demonstrated. The present study can account for processes that occur after pollen has reached the lake surface. Therefore, our study can account only for factors such as the flowering season of each pollen type and internal lake processes involved in pollen sedimentation. Other factors such as pollen production, dispersal, diagenesis or other post-depositional phenomena should be addressed with further aerobiological and sedimentological studies. In summary, our data suggest that phenological traits (i.e. flowering season) of the plant taxa involved exert a dominant control on the seasonal patterns of pollen sedimentation and inter-annual meteorological variations cause minor quantitative shifts. Sedimentological processes linked to internal lake dynamics, mainly the mixing–stratification regime and sediment reworking/resuspension, may, however, modify the original expression of biological and meteorological seasonality, and should be taken into account to explain the final pollen sedimentation patterns.

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High-resolution (sub-decadal) pollen analysis of varved sediments from Lake Montcortès (southern Pyrenean flank): A fine-tuned record of landscape dynamics and human impact during the last 500 years

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ABSTRACT

A high-resolution (average 6 years/sampling interval) palaeoenvironmental reconstruction using pollen, charcoal and non-pollen palynomorphs was carried out on annually laminated sediments of Lake Montcortès (southern Pyrenean flank). The results were combined with historical data to better understand landscape evolution and human interaction during the last 500 years. Our results show that human activities (cropping, livestock breeding and hemp cultivation and retting) have been the most important factors responsible for vegetation changes with highest intensity between 1530 and 1900 CE. By means of a sub-decadal study we have been able to evaluate short-lasting events at local and regional scales related to climate (heavy rainfall events and, high-land forest fluctuations) or to historical and well-dated and documented socio-economic events (i.e., crop promotions (hemp) or land abandonment-population emigration). The temporal extent (400 years) and continuity of *Cannabis* pollen peak have been confirmed, and new evidence of water quality changes, likely as a consequence of hemp retting practices between the mid-17th to late 19th century, are provided. This is the first high-resolution palaeoenvironmental study carried out in a varved lake on the Iberian Peninsula so far. With these data we hope to contribute to filling the gap in high-resolution palaeoenvironmental data.

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1. Introduction

Lake and peatlands sediments are natural archives that store information on limnological, biological, geochemical and anthropogenic processes occurring in the water body and in the catchment area (Smol et al., 2002; Veski et al., 2005). Understanding the process that leads to the recent evolution of landscapes and discerning between natural and anthropogenic causes is a challenging question that can best be empirically addressed with palaeoecological data. Changes in the spatial structure of a landscape result from natural process such as climate variability and/or soil development combined with human activity driven by socio-economic and cultural factors (Veski et al., 2005). Thus, to fully understand the changes and their drivers, it is necessary to combine data from different sources and disciplines such as archaeology, documentary sources, ecology, palaeoclimatology and

palaeoenvironmental data, although such a task may not be easy. For instance, the lack of enough spatial and temporal resolution of the different data sources sometimes does not permit to obtain a complete and accurate image of the past (Jones et al., 2009; Rull, 2014; Sadori et al., 2015; Contreras et al., 2018). Archaeology, palaeoclimatology and palaeoenvironmental science often strive to achieve regional and long-term relevance, resulting in a coarse resolution of multi-decadal to multi-millennial scales (Rull, 2014; Contreras et al., 2018). In contrast, ecological, and historical data provide more constrained spatial and temporal resolutions (sub-decadal/annual/seasonal) (Rull, 2014; Contreras et al., 2018). In palaeoecology, a solution for this issue is to work with varved sediments that allow annual to seasonal time-resolutions or with sediment records with very high sediment accumulation rates (Veski et al., 2005; Ojala et al., 2012; Rull 2014).

Within the sedimentary archive, the last millennium is especially interesting for studying landscape and human environment interactions due to the availability of good quality and well preserved historical records (Dearing, 2013; Zolitschka et al., 2015) and because it has been a key period of the development of modern vegetation types and the formation of cultural landscapes (Rull et al., 2011; Wacnik et al., 2016). Furthermore, significant climatic variations occurred in relatively short periods of time (Medieval Climatic Anomaly (MCA), Little Ice Age

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(LIA), and the onset of Global Warming) that might drive short-lasting vegetation disturbances only detectable by high-resolution analyses (Wacnik et al., 2016).

Several high-resolution studies from lake sediments and some from varved records are already available for Europe using both physico-chemical and biological proxies. Most of them are focused on palaeoclimate and are aimed to perform quantitative reconstructions (some examples: Feurdean et al., 2008 (pollen); Trachsel et al., 2010 (biogenic silica and chironomids); Lotter et al., 2012 (chironomid and pollen); de Jong et al., 2013 (chrysophyte stomatocyst). Palaeo-environmental high-resolution studies are less frequent but equally important as they are the only available tool to evaluate past biodiversity losses or to identify past key periods that can help to set conservation targets and to visualise realistic future scenarios (Ekblom and Gillson, 2017).

For the Iberian Peninsula, a considerable number of pollen records are already available. They are mainly performed at a low resolution and cover several millennia, although some exceptions at moderate resolution and covering the last millennium exist (i.e., Riera et al., 2004; Morellón et al., 2009; Ejarque et al., 2009; Rull et al., 2011; Garcés-Pastor et al., 2016). The available pollen records from the Pyrenean range mostly belong to high altitude lakes and peatlands. High altitude mountain areas have traditionally been viewed as pristine environments with low human population density where more severe climatic conditions might hamper human occupation. But, for the case of the Pyrenees and Pre-Pyrenees, it has been demonstrated that substantial human pressure and considerable exploitation of natural resources have taken place since the Mesolithic: farming, mining, logging and fire impact (Gassiot and Jiménez, 2006; Palet et al., 2007; Sancho and Planas, 2009; Ejarque et al., 2010, 2012; Bal et al., 2011; Cunill et al., 2013; Corella et al., 2013). Therefore, lower altitudes on the Pyrenean-montane stage (ranging from 800 to 1600 m a.s.l.)- which are very favourable for human occupation and consequently sensitive to be higher human impacted, arise as an interesting area to study human occupation history and human-landscape evolution relationships.

Lake Mont cortès, which is located in the pre-Pyrenean range (1026 m a.s.l.) is a very suitable place to reconstruct past human-environment relationships. The exceptional scientific value of this lake is due to its strategic location and to the varved nature of its sediments that are ideal for performing high-resolution studies with accurate time control (Corella et al., 2011, 2012). This feature, which is uncommon among Iberian lakes, supports several studies which have demonstrated the potential to reconstruct the ecological dynamics of the lake communities and vegetation dynamics related to climate and human activities at a sub-centennial scale (Scussolini et al., 2011; Rull et al., 2011; Rull and Vegas-Vilarrúbia, 2015; Montoya et al., 2018). Reconstructions of past climate and past shifts in oxygenation regime at annual and sub-decadal resolutions have also been performed in this lake (Corella et al., 2014, 2016; Vegas-Vilarrúbia et al., 2018). Moreover, modern sedimentary analogue studies and a detailed floristic inventory of lake surroundings have been published recently, both, are very useful tools that helped to interpret the sediment record (Mercadé et al., 2013; Rull et al., 2017; Trapote et al., 2018). Among the key results found, the Lake Montcortès pollen record contains large amounts of hemp (*Cannabis* sp.) (Rull et al., 2011). Historically, *Cannabis* has been a fundamental plant for the development of human societies then, the identification of *Cannabis* pollen is a very useful tool to track and identify human activities and their impacts (van Zant et al., 1979; Mercuri et al., 2013; Peglar, 1993).

Here, we present for the first time in the Iberian Peninsula, a high-resolution and continuous palynological reconstruction (6 years/sample on average) carried out in a Pre-Pyrenean varved lake. We perform a multiproxy reconstruction using pollen, charcoal, non-pollen palynomorphs (NPPs) and historical documents for the last 500 years adding unique high-resolution palaeoenvironmental and palaeoecological studies covering modern period. Our main aim is to

perform a detailed, and accurate palaeoenvironmental reconstruction to investigate vegetation history, land-use and human impact around Lake Montcortès at the highest detail achieved so far. This study provides new data on historical land-use and management and on the potential use of Lake for hemp retting. Data covering the last century and therefore, the climatic instrumental record, is presented for the first time. Our data, together with the varved nature of the Lake Montcortès record and the already available palaeoenvironmental information for the Lake Montcortès, combined with historical and paleoecological data available for the Pyrenees have made possible to perform a thorough picture at local and regional scales of human-vegetation interactions around the lake. The data obtained with this work may contribute to develop and test computational models of interactions between climate, landscape evolution and land-use as well as to constrain and decrease the uncertainties in future environmental projections (Dearing, 2006, 2013; Hegerl et al., 2006).

1.1. Study area

Lake Montcortès (42° 19' N; 0° 59' E and 1027 m a.s.l.) is a small karstic lake situated on the south-central pre-Pyrenees in the Pallars region (Fig. 1). It was formed by karstic processes of dissolution and collapse on Triassic evaporates. The lake's catchment is small and is emplaced in Oligocene carbonate conglomerates, Triassic limestones, marls and evaporites (Rosell, 1994). The lake is fed mainly by ground-water and lake level is controlled by an outlet stream located along the northern shore and water evaporation (Corella et al., 2016). It has a maximum water depth of 30 m. According to the nearest meteorological station, total annual mean precipitation is 669 mm, with February being the driest month and May being the wettest. Annual average air temperature is 12.8 °C, with maximum and minimum mean temperatures of 23.3 °C (July) and 2.9 °C (January) respectively (reference period 1961–1990). Lake alternating meromictic and holomictic conditions as has been demonstrated by Trapote et al. (2018) and Vegas-Vilarrúbia et al. (2018), remaining stratified most of the year and mixing during winter. It is an oligotrophic lake with very low nutrient content particularly for phosphorous, well buffered waters and maximum phytoplankton productivity occurring during late summer and early autumn (see Trapote et al. (2018) for more detail).

The lake lies near the altitudinal boundary corresponding to the sub-montane belt, which in the Pyrenees is situated around 800–1000 m a.s.l. elevation, depending on local conditions (Vigo and Ninot, 1987). Three major forest formations occur at the lake region reflecting this boundary condition: (1) Evergreen oak forest dominated by *Quercus rotundifolia* L. (representative of the Mediterranean lowlands); (2) Deciduous oak forest dominated by *Q. pubescens* L. and *Q. pyrenaica* L. (representative of the middle montane belt with higher precipitation); and (3) Conifer forest of *Pinus nigra* L. at lower and southern regions (probably secondary replacing the deciduous oak forest) and *Pinus sylvestris* L. at higher elevations (making the transition between Sub-montane and Montane belt) (Folch, 1981; Rull et al., 2011; Mercadé et al., 2013) (Fig. 1A). The lake is surrounded by a dense littoral vegetation belt dominated by *Phragmites*, *Cladium mariscus* L. and *Typha* and, to a lesser extent, represented by *Juncus* and *Scirpus* (Mercadé et al., 2013). Hay meadows, pastures (mostly for cattle and horses) and cereal crops are the most important rural anthropic habitats around the lake. Besides farming, since 1970's the most important human activity around the lake and in the area is rural tourism.

2. Methods

2.1. Coring and age modelling

In July 2013, a 114 cm long sediment core named MONT-0713-G05 was retrieved from the deepest distal lake basin (~30 m water depth) using a UWITEC 60 mm diameter gravity corer. It was kept at the lake

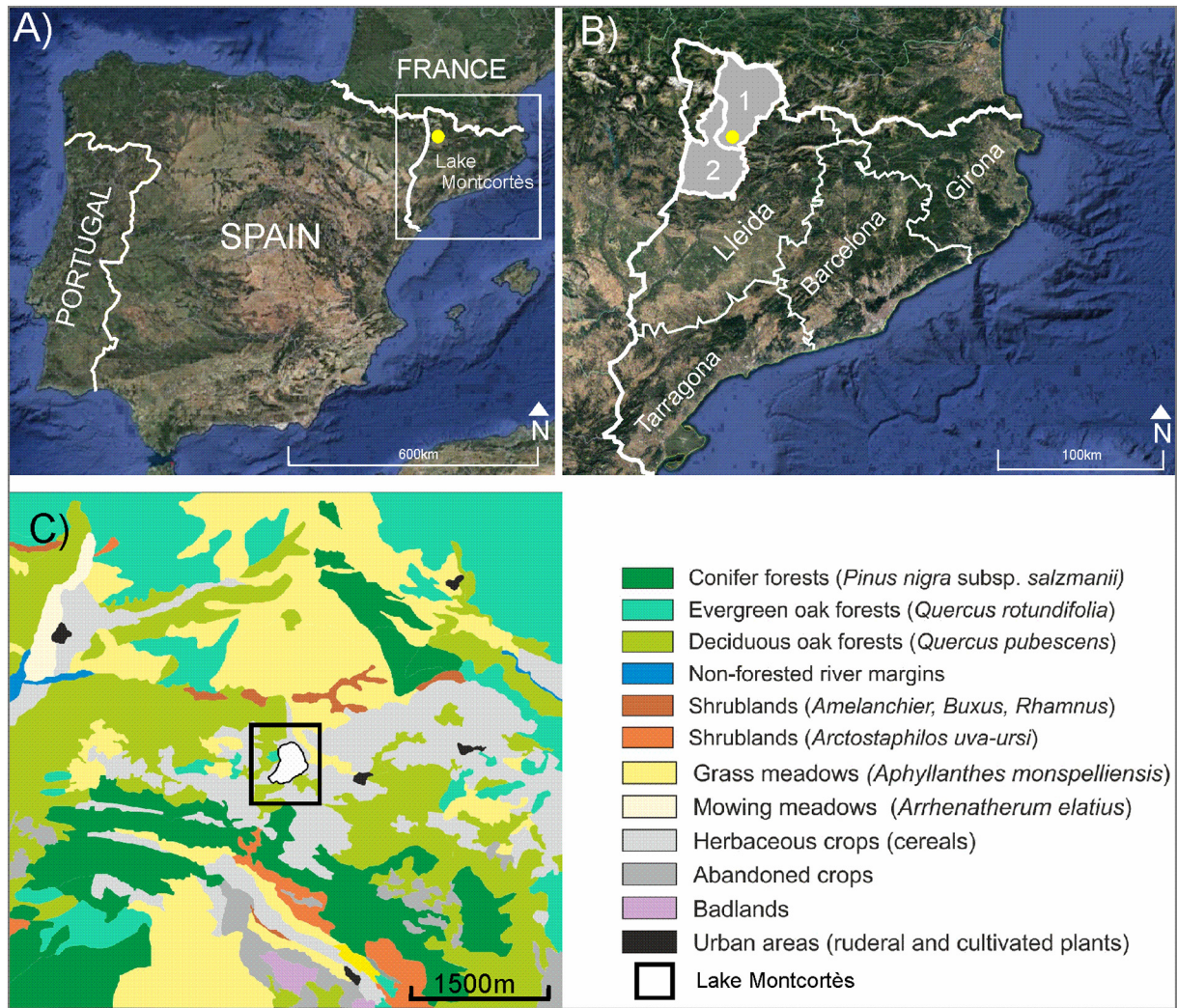


Fig. 1. (A) Location of Catalonia (squared area) and Lake Montcortès (yellow point). (B) Catalonia map with its provinces and Pallars region (green shaded areas): Numbers refer to two different administrative boundaries within the Pallars region: 1- Pallars Sobirà (Montcortès location) and 2- Pallars Jussà. (C) Vegetation map modified from Rull et al. (2015).

shore during 3 days to allow consolidation and then transported to the core repository at the Institute of Earth Sciences Jaume Almera (Spanish Research Council). In previous studies, Corella et al., (2011, 2014) built an age-depth model for the last six centuries, which is based on independent varve counting and ^{210}Pb and ^{14}C radiometric dating. Varve counting was performed on a composite sequence obtained from cores MON12-3A-1G and MON12-2A-1G and by double counting in 14 overlapping thin sections. Less than 1% of varves were interpolated using annual sedimentation rates from well-preserved adjacent varve sections. Further details of this age-depth model are provided in Corella et al., (2014). Stratigraphic correlation between core MON12-3A-1G and MONT-0713-G05 was obtained based on a detailed inspection of sedimentary structures, varve thickness patterns and characteristic features seen in specific varves that allowed the identification of 96 marker horizons (i.e., flood layers and/or distinct sub-layering in calcite layers).

2.2. Core sampling, pollen, charcoal and NPPs

The varved part of MONT-0713-G05 was sampled continuously every 0.5 mm using a syringe to obtain volumetric samples, which was the highest resolution possible that allowed us to obtain enough sedimentary material for pollen analysis. Turbidites were avoided following the sampling procedure described in Corella et al. (2017) since

these sediment-laden layers represent allochthonous material eroded from the lake catchment and deposited within hours/days (Corella et al., 2015). A set of 96 samples were processed using standard palynological methods (Moore et al., 1991; Vigo and Ninot, 1987), including KOH, HCl, HF digestions and acetolysis. Two *Lycopodium* tablets (batch n° 483,216; 18,583 spores/tablet) were added to each sample before chemical processing. Residues were suspended in liquid glycerine and microscopic slides were mounted in the same medium. Pollen was identified according to Moore et al. (1991) and Reille (1992-1998) and following previous Montcortès studies (Rull et al., 2011; Rull and Vegas-Vilarrúbia, 2014, 2015). All samples were counted until diversity saturation (Rull, 1987) with a minimum of 300 pollen grains excluding *Cannabis-type pollen*, which was superabundant in some samples (40–85% respect to the total terrestrial pollen sum). Algal remains were also identified and counted to genus level. Charcoal particles were counted and classified into two groups based on size: charcoal I (<100 μm) as indicator of regional fires and charcoal II (between 100 and 500 μm) as indicator of more local fires (Whitlock and Larsen, 2001). Fungal spores were identified following van van Geel and Aptroot (2006), Van Geel et al. (2003), and López-Vila et al. (2014). The pollen sum included all pollen types except those from aquatic and semi-aquatic taxa: Cyperaceae, *Myriophyllum*, *Scirpus*, *Potamogeton* and *Typha-Sparganium*, hereafter referred as *Typha* according to local vegetation surveys (Mercadé et al., 2013). Pollen and spores below 3%

of the pollen sum were not shown in the pollen diagram. Pollen accumulation rates (PAR) and charcoal influx in $\text{cm}^{-2} \text{yr}^{-1}$ were calculated. Diagrams were plotted and zoned using the software Psimpoll 4.27 (Bennett, 2002) and the method of optimal splitting by information content (Bennett, 1996) considering only pollen types. Percentages for NPPs (algal remains and fungi spores) were referred to the pollen sum. Pollen groups were defined according to the present day vegetation types as previously presented for Montcortès in Rull et al. (2011) and Mercadé et al. (2013). Table 1 presents each group and the corresponding taxa included in it for the sediment record presented in this work.

3. Results & interpretation

3.1. Age model

The three different lithostratigraphic units previously defined in Corella et al. (2014) were also clearly identified in core MON-0713-G05 (Fig. 2) (unit 1, 0–15 cm, 2013–1902 CE; unit 2, 15–59 cm, 1901–1844 CE; unit 3, 59–100 cm 1844–1423 CE). Several marker horizons have also been detected and correlated between cores (Fig. 2). Sedimentation rates (SR) in cores MON12-3A-1G and MON-0713-G05 display similar values except for unit 2, where SR was 26% higher in core MON12-3A-1G than in core MON-0713-G05 due to the thicker detrital layers deposited during the 19th century.

3.2. Vegetation and landscape changes

Results are expressed in both percentage – for pollen and NPPs (Figs. 3 and 4) and PAR (Fig. 5). The interpretation, in terms of vegetation shifts, is based on the percentage diagram (including *Cannabis* as a component of the regional landscape), with reference to PAR values to follow the behaviour of the more significant taxa and/or vegetation groups. A summary pollen diagram excluding *Cannabis* has been added to assess vegetation changes in zones where hemp pollen attain more than 40% of relative abundance and therefore pollen signal could have been adulterated. Vegetation classification and interpretation of pollen spectra follow previous palynological studies: Cañellas-Boltà et al. (2009), Mercadé et al. (2013), Rull and Vegas-Vilarrúbia (2015) and Rull et al. (2011, 2017). This section is concerned only with vegetation dynamics and the potential processes involved that can be directly inferred from the evidence obtained in this work (pollen, charcoal, algae, and fungi spores). Other aspects needing additional independent evidence, such as the potential influence of climatic shifts, historical

Table 1
Pollen groups according to the present day vegetation types, based on Rull et al. (2011).

Vegetation type	Pollen taxa
Conifer forest	<i>Pinus</i> , <i>Abies</i>
Evergreen oak forest	<i>Quercus</i> - evergreen-type
Deciduous oak forest	<i>Cornus</i> , <i>Carpinus</i> , <i>Fagus</i> , <i>Fraxinus</i> , <i>Tilia</i> , <i>Betula</i> , <i>Quercus</i> deciduous- type
Riverine forest	<i>Alnus</i> , <i>Populus</i> , <i>Salix</i> , <i>Ulmus</i>
Shrubs	<i>Buxus</i> , <i>Erica</i> -type, <i>Ilex aquifolium</i> , <i>Juniperus/Cupressus</i> , <i>Phillyrea</i> , <i>Pistacia</i>
Low shrubs	<i>Ephedra</i> , <i>Hedysarum</i> , <i>Helianthemum</i>
Meadows/pastures	<i>Plantago</i> , Poaceae (others)
Cultivated trees	<i>Corylus</i> , <i>Juglans</i> , <i>Olea</i> , <i>Prunus</i>
Herbaceous crops	Cerealia (others), <i>Secale</i> , <i>Cannabis</i> -type
Ruderal/weeds	<i>Artemisia</i> , <i>Centaurea</i> , <i>Chenopodium</i> , <i>Echium</i> , <i>Rumex</i> , <i>Urtica</i> -type
Other	Apiaceae, Asteraceae (others), Asteraceae (fenestrate), <i>Campanula</i> , <i>Euphorbia</i> , <i>Castanea</i> , <i>Cerastium</i> , <i>Galium</i> , <i>Morus</i> , <i>Potentilla</i> , <i>Sanguisorba minor</i> , <i>Thymus</i> , <i>Veronica</i> -type, <i>Scabiosa</i> , <i>Sedum</i> -type
Aquatic plants	<i>Alisma</i> , <i>Claudium</i> , <i>Typha</i> , Cyperaceae (others), <i>Mentha</i> -type, <i>Myriophyllum</i> , <i>Scirpus</i> , <i>Potamogeton</i> , <i>Ranunculus</i>

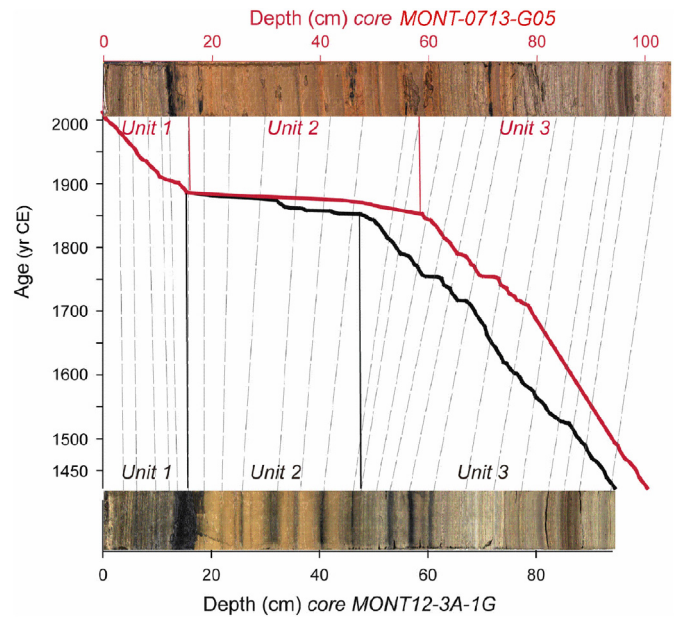


Fig. 2. Age–depth model for the sediment cores MONT-0713-G05 (present study) and MONT12-3A-1G (Corella et al., 2014) based on varve counting for the last 500 years. Core correlation of the main sedimentary units and marker horizons are also shown.

events or comparisons with previous works and similar regional reconstructions, are addressed in the discussion.

Overall, the percentage diagram (Fig. 3) is dominated by conifers (*Pinus*), evergreen and deciduous oaks (*Quercus*) and herbaceous crops, notably the *Cannabis*-type (thereafter *Cannabis*). These pollen types show significant abundance changes, especially at the base and the top of the diagram. Some autochthonous and cultivated trees (*Betula*, *Olea*), *Juniperus/Cupressus* type (likely corresponding to *Juniperus communis* L., which is abundant in the present vegetation), herbs (mainly Poaceae, *Plantago* and *Artemisia*) and aquatic taxa (*Typha*, Cyperaceae other than *Scirpus*) are also well-represented and exhibit meaningful shifts throughout the diagram. The sequence has been subdivided into six significant pollen zones, named MC-1 to MC-6, which are described as follows.

3.2.1. Zone MC-1: 100.5–95.5 cm, 1423–1481 CE (58 years; 11 samples; average resolution: 5.2 years/sampling interval)

This zone is dominated by trees – notably evergreen *Quercus*, the main representative taxon of the evergreen oak forests- and herbs, mainly *Artemisia* and Poaceae (others), belonging to the ruderal/weeds and the meadows/pastures groups, respectively (Fig. 3). These two herbaceous groups attain up to 50% of the pollen assemblage in this zone. Other trees (*Pinus*, deciduous *Quercus* and *Olea*), shrubs (*Juniperus*) and herbs (*Plantago*) show intermediate values. Among minor components, *Betula*, *Corylus*, *Fagus* and *Alnus* are below 10% but they attain their maximum abundances as compared to other zones. Trees such as *Quercus* (evergreen), *Pinus* and *Olea* experience an increasing trend while some of the main herbaceous pollen types, notably *Artemisia* and *Plantago*, decrease towards the top of the zone. Aquatic taxa are at their minimum values, with a slight decrease in *Typha*, which almost disappears at the end of the zone. PAR values are very low, showing an increasing trend in all vegetation types except low shrubs, herbaceous crops and aquatic plants (Fig. 5). Charcoal I (indicative of regional fires) reaches its lowest values spiked by a conspicuous peak near the base of the zone (99.5 cm; 1434 CE), whereas charcoal II (indicative of fires occurring in a more local scale) is present only in the form of two small peaks. Among algal remains, *Botryococcus* is the most abundant, showing an increasing trend towards the top of the zone that coincides with a small *Tetraedron* peak, which was almost absent before

Lake Montcortès pollen diagram (core MONT-0713-G05)

Analyst: M.C. Trapote

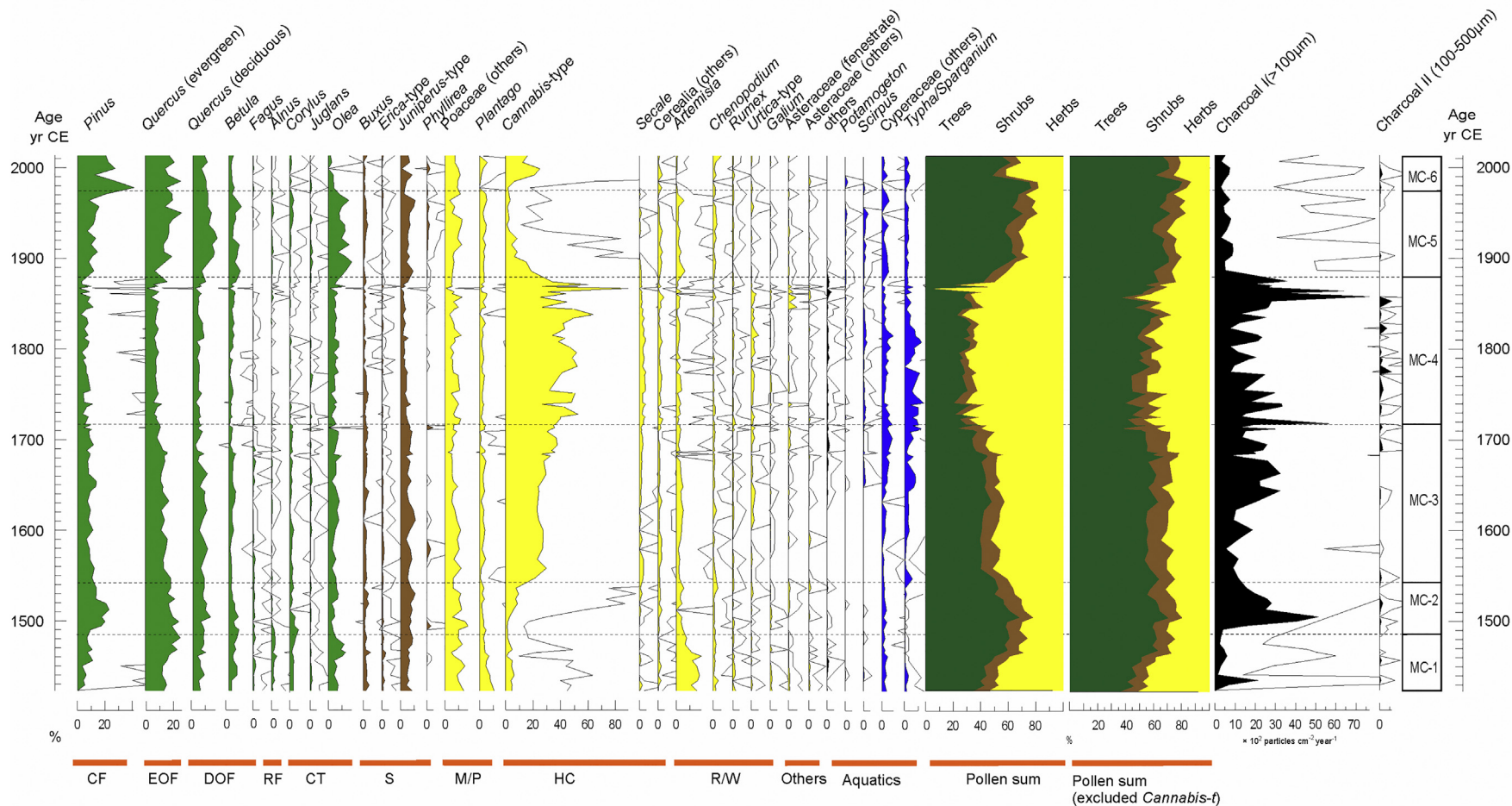


Fig. 3. Percentage sporomorph diagram including the total pollen sum (%). Elements included in the pollen sum: CF, conifer forests; EOF, evergreen oak forests; DOF, deciduous oak forests; RF, riverine forests; CT, cultivated trees; S, shrublands; M/P, meadows/pastures; HC, herbaceous crops and R/W, ruderal/weeds. Elements outside the pollen sum: aquatic plants. An additional pollen sum diagram excluding *Cannabis* pollen is also shown. Charcoal curves are expressed in fluxes for two categories of particle size. The horizontal lines correspond to statistically significant pollen zones (Bennett, 1996). Solid lines indicate ($\times 10$) exaggeration.

Lake Montcortès NPP diagram (core MONT-0713-G05)

Analyst: M.C. Trapote

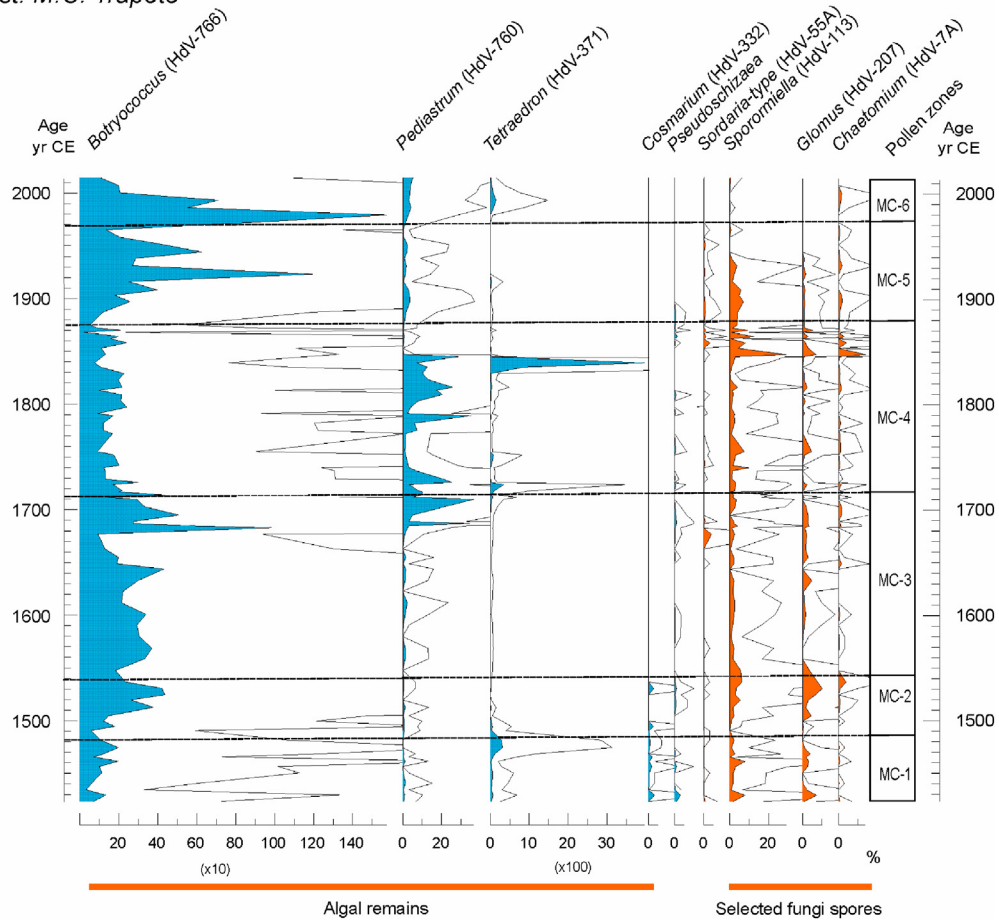


Fig. 4. Percentage diagram for non-pollen palynomorphs (NPP) with respect to the pollen sum. The scales of *Tetraedron* and *Botryococcus* have been reduced for more clarity. NPP nomenclature is based on the original publications (van Geel 1978; van Geel et al., 1981; 1989; 2003; Montoya et al., 2018; Bakker and van Smeerdijk 1982). Solid lines indicate ($\times 10$) exaggeration. Zonation as in Fig. 3.

(Fig. 4). *Cosmarium* and *Pseudoschizaea* are present only as small and scattered peaks. The most abundant fungal spores are *Sporormiella* and *Glomus*, always below 10%, and showing a similar trend between them.

During the time interval represented by this zone (1420–1490 CE), the landscape of the Montcortès catchment and its surroundings was characterised by the presence of forests, mostly evergreen oak forests, meadows, pastures and herbaceous crops, with the corresponding ruderal plants and weeds. This was not a static landscape state as oak forests were expanding at the expense of the rest of vegetation types, especially ruderal plants and weeds. Fire incidence was very low, except for a distinct burning event around 1434 CE. The presence of *Sporormiella*, a coprophilous fungi living in the dung of herbivorous animals, suggests the presence of livestock around the lake (van Geel and Aptroot, 2006). The occurrence of *Glomus* indicates that erosion of catchment soils was ongoing (Anderson et al., 1984). *Pseudoschizaea*, that also has been used as indicator of soil erosion (van Geel et al., 1989, 2003), might be indicative of cattle trampling around the lake (Ruiz-Zapata et al., 2006). The whole picture suggests a humanised landscape where anthropogenic impact was declining and wild oak forests were in expansion. *Olea* is a low-elevation tree but its pollen can be easily transported long-distance and to higher elevations than the parent plant (Cañellas-Boltà et al., 2009; Bell and Fletcher, 2016); hence, its peak at the end this zone could be due to causes related with events occurring at the adjacent lowlands, which will be discussed later.

3.2.2. Zone MC-2: 95–89 cm, 1490–1536 CE (46 years; 9 samples; average resolution: 5.1 years/sampling interval)

This zone is dominated by arboreal taxa from both conifer forests (*Pinus*) and evergreen oak forests (*Quercus*), reaching overall abundances above 60% at the middle of the zone, with a decline to almost 45% at the top (Fig. 3). Shrubs do not change but herbs of the meadows/pastures and ruderal/weeds groups (mainly *Plantago* and *Artemisia*) significantly decrease, as compared to zone MC-1. The peak of arboreal pollen is due to the increase of *Pinus*, as other trees from other evergreen and deciduous forests either decrease (evergreen *Quercus*, *Betula*, *Alnus*, *Corylus*) or remain at values similar to zone MC-1 (deciduous *Quercus*, *Fagus*). Concerning cultivated plants, *Cannabis* (hemp) is insignificant at the base of the zone but progressively increase to ~20% at the top. *Secale* (rye) also experiences a slight increase, whereas *Olea* (olive tree) decrease with respect to the former zone. No remarkable shifts are observed in aquatics. PAR values (Fig. 5) experience a general increase but declined to minimal values at the top of the zone. Charcoal I undergoes an abrupt increase at the base to progressively decrease through the top, whereas charcoal II do not change with respect to the former zone (Fig. 3). Regarding algae, *Botryococcus* continues to increase and *Tetraedron* almost disappear, whereas other types do not experience significant changes, except *Cosmarium*, which is much less frequent. Fungal spores (*Sporormiella*, *Glomus* and *Chaetomium*) undergo a general increase (Fig. 4).

Lake Montcortès PAR diagram (core MONT-0713-G05)

Analyst: M.C. Trapote

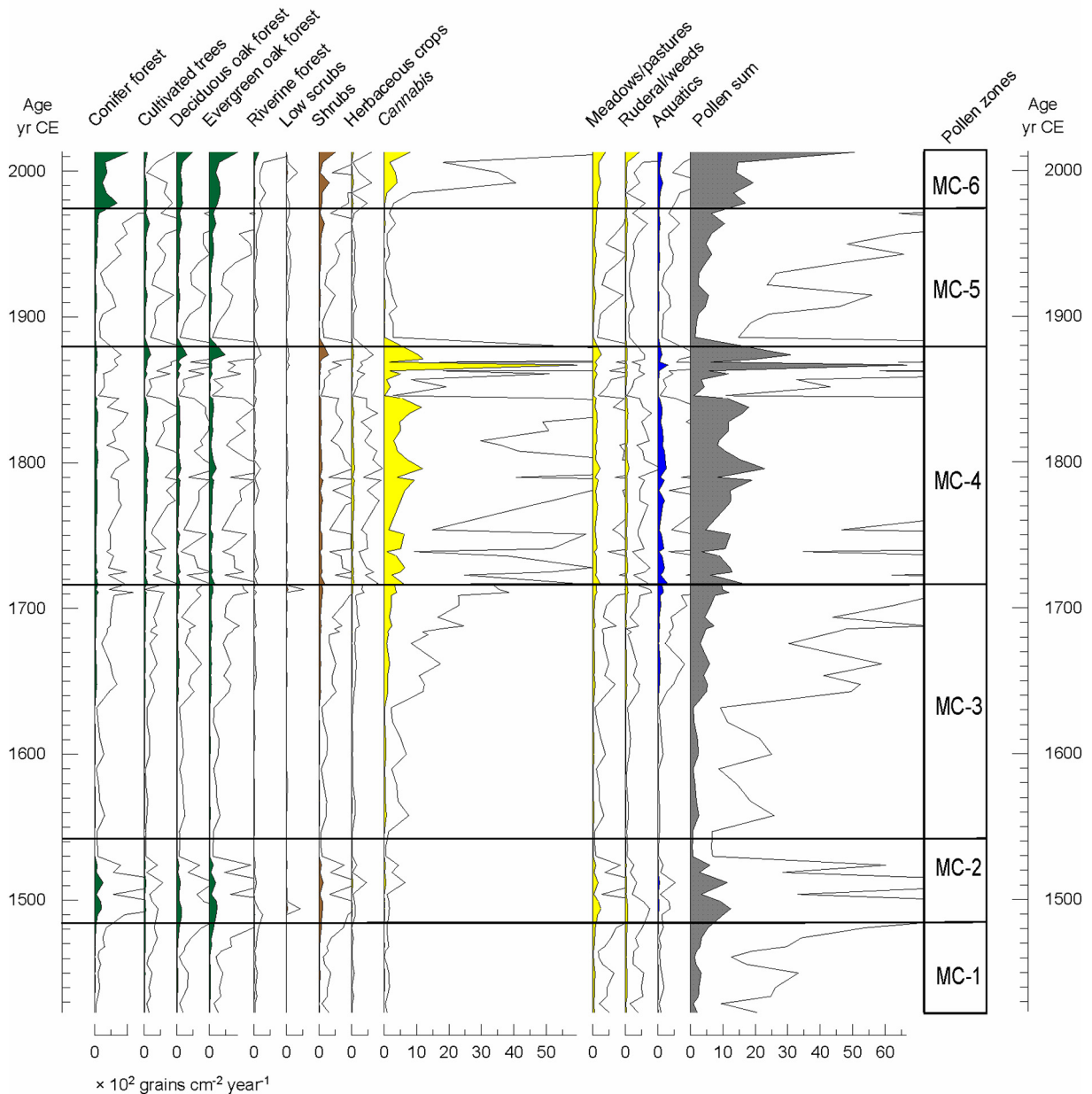


Fig. 5. Pollen Accumulation Rates (PAR) diagram grouped by the vegetation groups detailed in Table 1. Solid lines indicate ($\times 10$) exaggeration. Zonation as in Fig. 3.

Between 1490 and 1540 CE, evergreen and deciduous forests around the lake retracted and herbaceous crops, mainly hemp, and to a lower extent rye, expanded. The increase of *Glomus* indicates enhanced erosion, likely due to forest cover reduction, and the higher abundance of coprophilous fungi compared to former zone suggests grazing intensification. This, together with the sudden increase of charcoal, is compatible with an intensification of human impact both on the catchment area and at regional scale, probably by using slash-and-burn practices for forest clearance and the enhancement of arable lands. The decline of *Artemisia* and *Plantago*, indicators of grazing, suggests the decline of pastoral activity, in which case, coprophilous fungi would indicate the presence of domestic animals associated with agriculture and/or transport activities. Pine forests are characteristic of higher elevations; therefore, their expansion would be independent of increased human activity around the lake, and will be discussed later.

3.2.3. Zone MC-3: 88.5–76.5 cm 1547–1717 CE (170 years; 25 samples; average resolution: 6.8 years/sampling interval)

This zone is characterised by a sharp increase of *Cannabis*, attaining values of almost 40%, and a general decrease of trees, including *Pinus*, with the exception of *Olea*, whose percentages remain stable. PAR values show that this is not a percentage artefact, as tree pollen – as well as most pollen types – actually decrease whereas *Cannabis* increases, with respect to the former zones (Fig. 5). Shrubs also stay unchanged. The most significant herbs, including those cultivated, also show rather stable percentages with only minor variations. Aquatic plants (*Typha* and Cyperaceae) experience a general increase at about the middle of the zone (84 cm; 1643 CE), coinciding with the appearance of *Scirpus* and a general increase of PAR values (Figs. 3 and 5). Charcoal I increases about the middle of the zone (ca 1643 CE, 84 cm) and peaks at the top, coinciding with the increasing trend in aquatic plants

(Fig. 3). Among algae, *Botryococcus* stabilises in values attained at the end of the former zone but sharply peaks towards the end of this zone, coinciding with a remarkable increase of *Pediastrum*, which is very scarce earlier. *Cosmarium* is absent in this zone and for the rest of the sequence. In this zone, fungi spores experience a general decline (Fig. 4).

Between 1540 and 1720 CE, a dramatic shift occurred in the Montcortès landscape due to the general forest retraction and the onset of intensive and/or extensive hemp cultivation. Other crops (olive trees and cereals) remained in a situation similar to former times. Pastoral practices, might experience a slightly decrease as indicated by the modest reduction of *Poaceae* and coprophilous fungi although *Artemisia* remained similar to the former zone. It seems that most agricultural activity was centered on hemp. Within this general scenario, the aquatic ecosystem (aquatic plants and the algal remains) changed around the middle of the zone (ca 1643 CE) probably indicating changes in lake water quality.

3.2.4. Zone MC-4: 76–44 cm; 1723–1874 CE (151 years; 32 samples; average resolution: 4.7 years/sampling interval)

The most distinguishing traits of this zone are the acme of *Cannabis*, reaching values of 60% to 80%, and the reduction of all trees and shrubs with no exception even *Cannabis* pollen is excluded of the pollen sum (Fig. 3). PAR values show that tree pollen do not decrease and their lower percentages are due to the comparatively higher rates of *Cannabis* increase (Fig. 5). Cereal crops, including *Secale*, slightly increase and the ruderal/weeds group remains unchanged. Some herbs that are scarce or sporadic in former zones appear more constantly and with slightly higher values in this zone. This is the case of *Urtica*-type and *Galium*. Charcoal I slightly declines and peaks near the top. Charcoal II increases its frequency and abundance, as compared to former zones. Regarding aquatic plants *Typha* and *Scirpus* attain their maximum percentages in this zone and decrease towards the top (Fig. 3). *Potamogeton*, almost absent in former zones, starts to be present in a continuous fashion but with low values. Among algae, *Botryococcus* declines and *Pediastrum* increases. *Tetraedron*, almost absent in the former zone, reappears in the form of two peaks, at the base and nearly the top of the zone. Concerning fungal spores, *Sporormiella* and *Glomus* show similar values to the former zone including two peaks and *Chaetomium* exhibits a similar trend but only relate to/in regard to the upper peak (Fig. 4). A peculiar feature of these zones is an interval located in its uppermost part (61–45 cm; 1838–1869 CE), where *Cannabis* pollen, as well as some algae (*Tetraedron*) and fungi spores (*Sporormiella*), show large and sharp peaks coinciding with a conspicuous charcoal acme (Figs. 3 and 4). This feature is also evident in PAR values of almost all pollen groups, except for the conifer forest (Fig. 5).

Contrary to zone MC-3, in this time interval (1720–1880 CE), the increase in *Cannabis* pollen, suggesting its cultivation around the lake, was not paralleled by forest reduction. If hemp was actually cultivated this did not occur at the expense of forests or other vegetation types, as none of them seem to have reduced their cover (see PAR values). Therefore, the increase of *Cannabis* suggests an extra source for this pollen (likely hemp retting), which might have increased pollen release to the sediments. It cannot be ignored that a proportion of this significant *Cannabis* pollen increment can also indicate increases on cultivation at local and also at regional scale attending the great distances that *Cannabis* pollen can travel away from parental plant (Cabezudo et al., 1997; Giner et al., 2002). Increases in aquatic plants and algal remains suggest that the limnological shifts initiated in the former zone (1640 CE) were maintained, possibly exacerbated. Lake-level changes cannot be dismissed as increases in aquatic plants could be related with increases of the flooded area. Independent data (i.e. geochemical data) able to record lake-level changes, would be necessary to support this interpretation. Relative high values of charcoal I and increases in charcoal II (indicatives of more regional and local fires respectively) together with increases in cereal crops and nitrophilous plants (such as *Urtica*-

type and *Galium*) support human impact intensification on the area. This seems to be the phase of maximum anthropogenic influence, not only on the catchment and regional landscape but also on the aquatic ecosystem.

3.2.5. Zone MC-5: 15–3 cm 1886–1971 CE (85 years; 13 samples; 6.5 years/sampling interval)

This zone is characterised by the rapid decrease of *Cannabis* and a general increase of trees (up to 60%, as in zone MC-2) and shrubs, notably *Juniperus*. *Fagus* is an exception as its pollen is almost absent (Fig. 3). PAR values confirm that tree and shrub pollen is generally increasing and *Cannabis* pollen almost disappears (Fig. 5). Notably, *Quercus* (deciduous) and *Olea* attain their maximum values in this zone. *Secale* and the ruderals/weeds group (especially *Urtica* and *Galium*) also decline while other cereals remain stable but decreased at the end of the zone. Charcoal I decreases notably and charcoal II almost vanishes in this zone (Fig. 3). Aquatic plants remain with values similar to former zones, except *Typha*, which is significantly reduced from this zone onwards. Regarding algae, *Botryococcus* sharply increases, peaking at the middle of the zone, whereas *Pediastrum* significantly declines and *Tetraedron* almost disappears (Fig. 4). Fungal spores, with *Sporormiella* as the most abundant type, initiate a decreasing trend, almost disappearing at the top of the zone (Fig. 4).

The results indicate that, between 1880 and 1970 CE, the *Cannabis* industry (i.e., cultivation and retting) was virtually non-existent around Lake Montcortès and forests recovered the same importance of former times, as for example, between 1490 and 1540 CE. In general, it could be stated that the landscape and the aquatic ecosystem returned to conditions similar to those observed on MC-1 before the *Cannabis* peak, with enhanced forest cover, less fire incidence and a similar trophic state of the lake. The conspicuous increase of *Olea* suggests that its cultivation in the adjacent lowlands increased.

3.2.6. Zone MC-6: 2.5–0 cm; 1978–2013 CE (35 years; 6 samples; average resolution 5.8 years/sampling interval)

This zone is defined by a new increase of *Cannabis* to values of 20–30% and some trees, chiefly *Pinus* (attaining its maximum values throughout the diagram) and *Quercus* (deciduous), reaching values similar to zones MC-1 and MC-2. Other trees and shrubs decline, as is the case of *Quercus* (deciduous), *Betula*, *Corylus*, *Olea* and *Juniperus*. Herbaceous crops and ruderals/weeds attain their minimum values and aquatic plants remain stable with respect to the former zone, except for *Scirpus* and *Potamogeton* that disappear towards the top. PAR values show a general increase of all vegetation types except low shrubs (Fig. 5). Charcoal also shows values similar to zone MC-5 (Fig. 3). *Botryococcus* attains its maximum in this zone, whereas *Pediastrum* and *Tetraedron* remain at lower values. Fungal spores are very scarce, almost absent (Fig. 4).

During the time interval represented in this zone, conifer forests and evergreen oak forests expanded and deciduous forests receded. The significant increase in *Cannabis* contrasts with the general decline of farming and grazing activity, which requires explanation. Data from other disciplines are needed for a sound interpretation of this zone. Fortunately, historical documentation is abundant for this time period, but this will be addressed in the discussion.

4. Discussion

Fig. 6 shows a comparison between a previous pollen study carried out in Lake Montcortès (Rull et al., 2011) and the present study. With this contribution, we have notably enhanced the average time resolution from 52 to 6 years/sampling interval which means an improvement from 9 to 79 samples for the overlapping time period. Additionally, we have analysed the recent vegetation history corresponding to 20th and 21st centuries for the first time in Lake Montcortès. The multiproxy study of the sediment record (pollen, charcoal and NPPs), the continuity

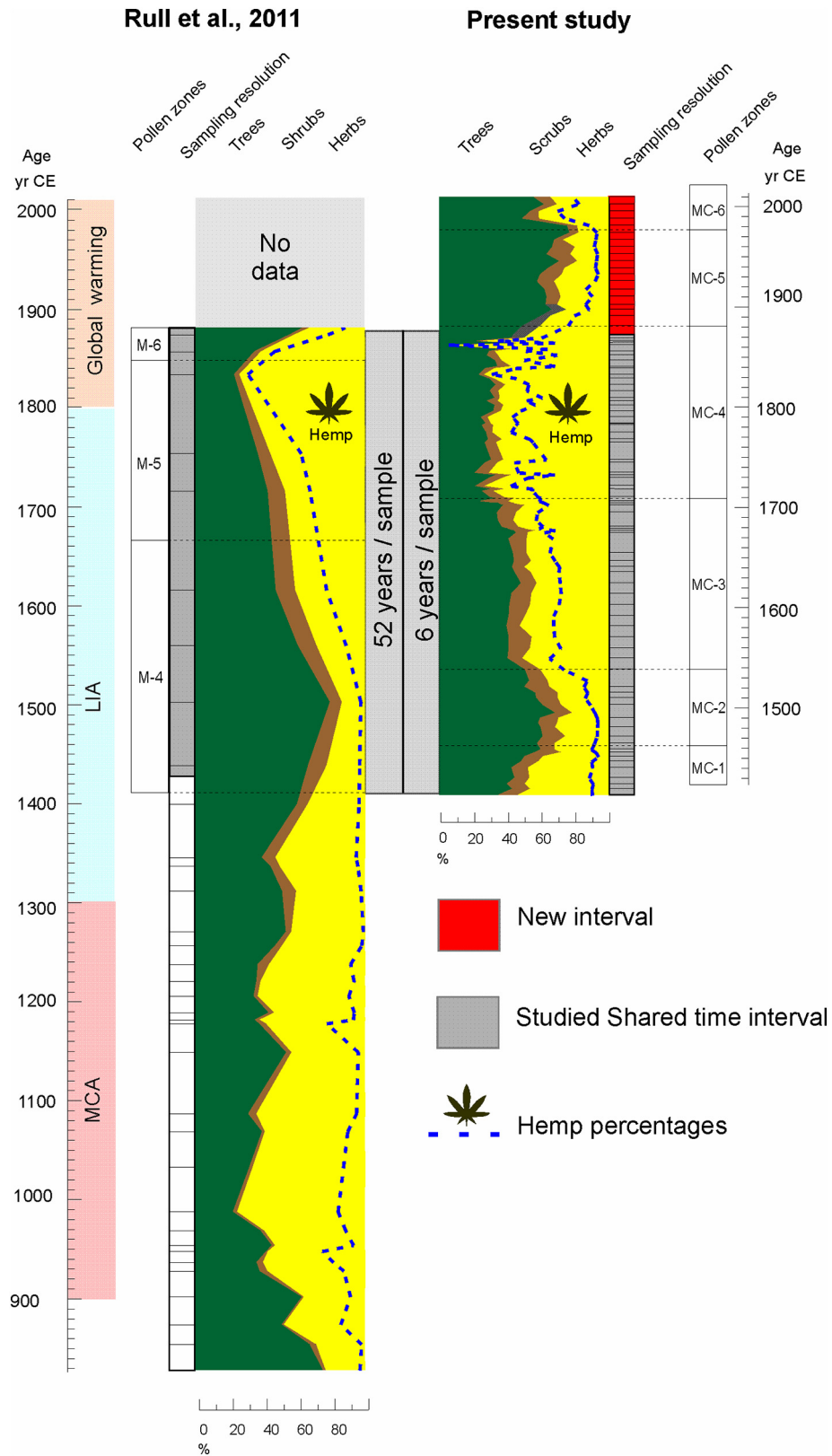


Fig. 6. Comparison between the palynological results obtained in Rull et al. (2011) and the present work. Grey coloured correspond to shared time interval. Red coloured correspond to new time interval presented in this work. Note horizontal lines as indicators of sampling resolution.

between samples and the high temporal resolution covering the last 500 years have provided new insights about vegetation and the environmental history of the Lake Montcortès catchment that were

previously unnoticed. These new data allowed us to perform more precise comparisons with the available historical records and to disentangle local from regional events. Correlations with other sequences at

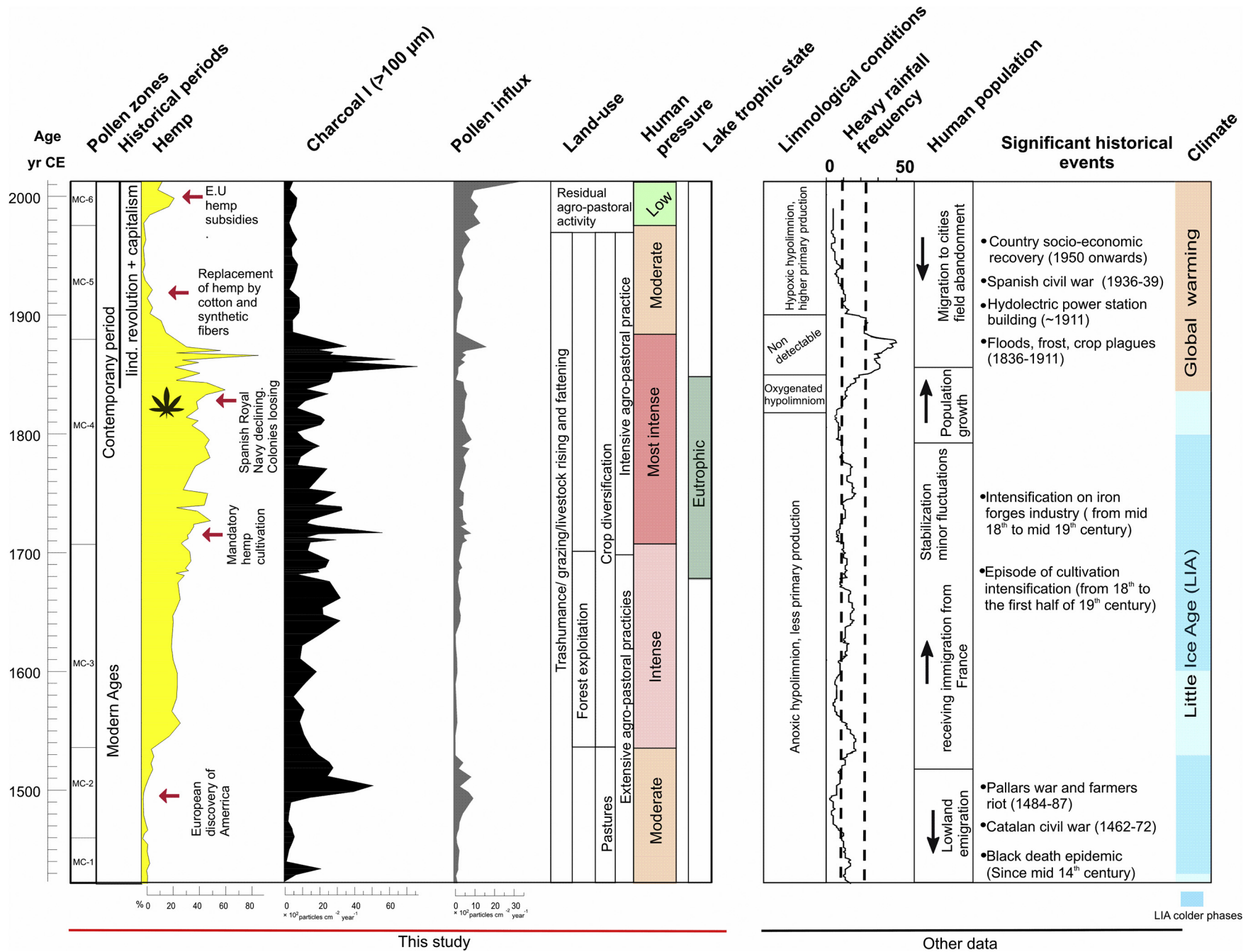


Fig. 7. Summary figure including the main findings of this study compared with previous studies of Lake Montcortès (Corella et al., 2014; Vegas-Vilarrúbia 2018), historical data referring to main historical events (Díaz Ordoñez, 2016; Rotés 2011; Reventós 2004; Bringué 2005; Pérez-Sanz et al., 2011; Simó 1985; Rotés 2011; Gorchs and Lloveras, 2003; Karus and Kaup, 2002) and climatic data (Oliva et al., 2018; Morellón et al. 2012; Mateo and Gómez 2004).

local and regional scales are not easy to perform because of the lack of high-resolution reconstructions covering the same period. Nevertheless, some similarities are found. Fig. 7 shows a summary of the main findings of the present work and comparison with other studies developed in Lake Montcortès. Due to the variable human pressure on the landscape, discussion has been organised according to different degrees of anthropogenic impact.

4.1. Moderate human pressure (Pollen zones MC-1 and MC-2; from ~1423 to ~1536 CE)

Our record begins in the 15th century when pollen percentages suggest a humanised landscape where anthropogenic activity was progressively declining. This decline can be appreciated by forest increases at expense of pastures, meadows and ruderal taxa (Fig. 3). The end of the 14th century was a turbulent socio-economic moment for all of the Western Europe devastated by the “black death” epidemic. In Catalonia (Spain) and the Pallars region (where Montcortès is located) (Fig. 1), this moment was especially severe due to the Catalan Civil War, the contemporary farmer rebellion (1462–1472 CE) and the Pallars War (1481–1487 CE). As a consequence, the population decreased and emigrated to the lowlands (see Rull and Vegas-Vilarrúbia, 2015 and literature therein for more detail). This is consistent with the Lake Montcortès pollen record that showed evergreen oak forest increases and pine forest expansions in the higher-mountain areas, likely as a result of field abandonment. *Olea* expansion during this period was also in concordance (Fig. 3). Olive is a lowland crop, probably promoted due to lowland emigration. Its expansion, together with cereal cultivation, was recorded during the same period in other lowland lakes at that time (Estanya lake; 670 m a.s.l. (Riera et al., 2004)) and in high-mountain records (Garcés-Pastor et al., 2016; Ejarque et al., 2009, 2010; Pérez-Sanz et al., 2011) owing to its high pollen dispersion capacity (Cañellas-Boltà et al., 2009; Bell and Fletcher, 2016). However, human activity was still ongoing around Lake Montcortès, which is indicated by the continuous presence of coprophilous fungi and soil erosion indicators (Fig. 4). As recorded in historical records, the movement of large amounts of livestock through the Pyrenees was a regular practice. Farmers tried to keep livestock moving to avoid them being stolen due to poverty, famine and social instability (Bringué, 2005). Lake Montcortès, which is located on the way to the North, was likely used as a water source and rest area for livestock on the journey to the high-mountain areas, where livestock was hidden during social instability periods. Later on, from 16th century onwards, Lake Montcortès was likely used as a rest area for transhumance livestock. Lake level increases might also took place giving rise to marshy environments, as *Pseudoschizaea* is often related with humid environments (Scott, 1992), and *Cosmarium* has been related to lake level changes and increased turbidity (Reynolds, 2006; Casco et al., 2009). These conditions were probably promoted by cattle trampling near the lake shore. This is also in agreement with the lake level rises recorded for the same period in the nearby karstic Lake Estanya (Riera et al., 2004; Morellón et al., 2011). However, no changes in aquatic taxa such Cyperaceae, *Typha* or submerged vegetation were recorded in the Lake Montcortès sequence at that time.

4.2. Intensification of human related activities: Cannabis pollen peak and water quality changes (Pollen zones MC-3 and MC-4; from ~1547 to ~1874 CE)

At the end of the 16th century, oak and riverine forests started to decrease locally, charcoal notably increased and herbaceous crops (hemp and cereals) expanded, meanwhile pine forest increased between 1490 and 1524 CE (Fig. 3). The recovery of the human population after the crisis was fast due to immigration from France (Bringué, 2005; Rull et al., 2011; Rull and Vegas-Vilarrúbia, 2015). The conifer forest expanded, which contrasts with the progressive decrease of oak and

riverine forest around Lake Montcortès. The conifer forest expansion that took place in Lake Montcortès record has been observed in other Pyrenean records (Ejarque et al., 2009, 2010) and it is in agreement with a period of farming decrease recorded in the Pyrenean high-mountain areas between 1430 and 1530 CE (Ejarque et al., 2009). This was one of the colder phases of the Little Ice Age recorded nearby (Mateo and Gómez, 2004), which could have determined unfavourable for human life in the area (Ejarque et al., 2009; Mazier et al., 2009). The farming declining episode recorded in the Pyrenean high-mountain areas seem to have not affected Lake Montcortès catchment where the riverine and oak forest decreased and anthropogenic activities persisted probably because its lower mountain elevation (González-Sampériz et al., 2017). Actually, during the Little Ice Age (from ~14th to 19th centuries), and even during its colder phases (Fig. 7) and when droughts and floods occurred (Corella et al., 2014, 2011; Morellón et al., 2012; Oliva et al., 2018), intense human impact is recorded from 1550 to 1900 CE in the Montcortès record (Figs. 3 and 4). Increases in hemp, cereals, ruderal and nitrophilous taxa are the main vegetation changes recorded in the Montcortès pollen record from approximately 1500 to 1900 CE (Fig. 3). Charcoal also increased while forest retreated. This period was marked by a diversification and intensification in the exploitation of natural resources in the Pyrenees (crops, cattle and forest exploitation) (Raventós and Marés, 2004; Bringué, 2005). Forest, mainly pines, oak and beech, were used to obtain coal to feed a rising and increasing demand for iron industry (iron forges) and for domestic use (Madoz, 1845–1850; Pèlach et al., 2009; Ferrer Alòs, 2017). The Lake Montcortès pollen record showed the diversification of land uses in concordance with historical sources that documented slash-and-burn agriculture practices to obtain fields for cropping and cattle from 1500 to 1700 CE (Bringué, 2005; Ferrer Alòs, 2017). The consistent presence of coprophilous fungi and soil erosion indicators (*Glomus*) confirm the presence of livestock around Lake Montcortès (Fig. 4). Transhumance practices and iron forge activities were also very important activities between 1550 and 1700 CE and caused the intensification of forest exploitation to obtain fields for grazing and charcoal to fuel iron industry activities (Pèlach et al., 2009; Ejarque et al., 2009). A rising intensity of charcoal production was recorded near Montcortès, in the Vallferrera Valley (Pèlach et al., 2009), coinciding with increases in the charcoal influx in the Lake Montcortès record.

The high hemp pollen percentages and the dramatic increase from 1720 to 1880 CE in the Montcortès record is coincident with one of the most important socio-economic and political moments throughout the Iberian Peninsula. Since the European discovery of America in 1492, the Spanish Royal Navy intensified its activity and hemp became a highly demanded product, mostly for supplying rigging and sails, and became strategic for commercial purposes (Díaz-Ordóñez, 2016). In this context, hemp cultivation was mandatory in Spain (for more detail, see Riera et al., 2004; Rull and Vegas-Vilarrúbia, 2014 and the literature therein). Catalonia was the second most important region for hemp production in Spain, just behind Valencia, and the one that produced the highest quality hemp fibres of the Iberian Peninsula (Sanz, 1995; Raventós and Marés, 2004). Lleida (Lerida) province (the present administrative limit where the Pallars region and Montcortès are located) (Fig. 1), was an important area for hemp production and fibre manufacturing (Ferrer Alòs, 2017). The detailed proxy-data obtained and the historical data reviewed in the present work attest that the Pallars was a renowned region for hemp production. Inhabitants of the area were well-known by their specialised skills in hemp manufacturing and by the diversity of peculiar tools for hemp manipulation characteristic of the region (Violant I Simorra, 1934). This is the case of la Pobleta de Bellví at less than 6 km from Lake Montcortès, where inhabitants were highly appreciated by their outstanding skills for hemp combing (Violant I Simorra, 1934). This can explain the implication of Lake Montcortès in hemp related activities.

Two main shifts can be observed in the hemp pollen curve coinciding with pollen zones MC-3 and MC-4 where percentages of 30% and

more than 40% are the trend, respectively, and hemp peaked twice in 1838 and 1867 CE. The question of whether these percentages are due to hemp cultivation around Lake Montcortès or the lake was used for hemp retting has been raised in former low resolution studies (30–50 years/sampling interval, in average) (Rull et al., 2011; Rull and Vegas-Vilarrúbia, 2014). The present study presents more independent data (proxies and historical data sources), and higher resolution data required to fully address the question. Water changes are recorded in Lake Montcortès from the mid 17th century onwards. Cyperaceae, and more notably *Typha*, increased coinciding with the increases of hemp pollen that overcome the threshold of more or less constant frequencies of 30% that lasted ~200 years. At the same time, *Pediastrum* increased notably and *Tetraedron* peaked in 1838 CE, coinciding with one of the maximum hemp values (~63%). Increases in Cyperaceae and *Typha* have been related to fluctuations in water levels but also with water nutrient enrichment. *Typha* species thrives in areas of high nutrient input (i.e., nitrogen and phosphorous) mainly because of their fast growth rates and ability to take up nutrients rapidly (Newman et al., 1996; Miao and Sklar, 1998). The notable increase in *Pediastrum* 1680 to 1850 CE and *Tetraedron* peak that coincided with hemp maxima might also be related with eutrophication processes as it has been observed in other similar lakes with hemp retting (Riera et al., 2006). Furthermore, increases in both types of algal remains might be an indicator of the differential growth response to different nutrient species supply. *Tetraedron* spp. grow better in the presence of phosphorous compounds, whereas *Pediastrum* spp. responds more effectively in exploiting nitrogen sources (Berman et al., 1991; Berman and Chava, 1999). The process of hemp water retting pollutes water bodies and induces significant changes in water quality, eutrophication and oxygen depletion (Paridah et al., 2011; Clarke and Merlin, 2013). Therefore, the changes recorded in the aquatic communities in Lake Montcortès might be a response to disturbances produced by the hemp retting process. Other proposed proxies that can provide additional arguments to corroborate retting in a water body are lithological changes (increases on sedimentation rates, detrital material or shore reworked sediments) (Cox et al., 2001), plant fibres and seeds (that indicate the physical presence of the plant in the water body) (Clarke and Merlin, 2013), diatoms and cyanobacteria (responding to water quality changes) (Lotter, 2001; Bradshaw et al., 2006; Miras et al., 2015), biomarkers (unique to *Cannabis* plants, i.e. Cannabinol) (Lavrieux et al., 2013) and the presence of *Potamogeton* (Bradshaw et al., 1981; Riera et al., 2004, 2006). This latter case is fulfilled for Lake Montcortès as *Potamogeton* is absent in the former zones until the *Cannabis* acme, when appeared (Fig. 3). *Potamogeton* species grow well in eutrophic and mesotrophic waters and seem to be a good competitor regarding other submerged taxa in turbid waters (Sidorkewicz et al., 1996; Van den Berg et al., 1999). This is in concordance with Vegas-Vilarrúbia et al. (2018), who, by analysing sedimentary pigments and physicochemical parameters of the sediment record, inferred anoxic water conditions, increased nutrient supply and turbidity during this period in Lake Montcortès. Despite the notable increase of hemp and cereals crops in MC-4, forest and/or other vegetation types did not decrease as might be expected (Fig. 5). This fact reinforces the idea of hemp retting as an extra source of *Cannabis* pollen into the lake. Another explanation for this surprising hemp pollen increase lies in the episode of cultivation intensification that took place during this period (from 18th century to the first half 19th century) due to technical advances and enhancement on irrigation techniques that made it possible to increase land productivity by obtaining more than one harvest per year (commonly two) (Sanz, 1995; Reventós, 2004). Cultivation intensification has also been recorded in other Pyrenean lakes and peatlands (Riera et al., 2004; Ejarque et al., 2009, 2010; González-Sampériz et al., 2017), but their lower resolution prevents detailed comparisons with Lake Montcortès and precise assessments of eventual time offsets and/or regional trends. Decreases in smaller charcoal influx (charcoal I) are also consistent with forest recovery observed in PAR values during this period (1700–1880

CE) although the frequency of bigger charcoal particles (type II) increased. These increases could be related to some agrarian techniques applied on fields surrounding Lake Montcortès as a consequence of production intensification. To maintain the intense productivity, farmers needed to fertilise the field to assure soil properties suitable for cropping. The most well-known and used method consisted of spreading manure in the fields, but it was too expensive, and depending on the crop type, the cost of manuring was higher than the profit of the corresponding harvest. Alternatively, farmers burned vegetal biomass mixed with soil to later spread and fertilise the field. Curiously, hemp was one on the most commonly used biomass sources for burning mixed with other vegetation types (Reventós, 2004). With technical advances and cropping intensification, it was possible to keep harvested products in extra stock and feed animals at home. Consequently, transhumance practices were notably reduced (Reventós, 2004). The reduction of transhumance and the increase of field productivity also helped to make forest recovery possible nearby Lake Montcortès.

From 1850 to 1870 CE (pollen zone MC-4), a saw-tooth trend is mostly recorded by the hemp pollen curve (exceeding 80%) but also by other taxa and charcoal and fungal spores. Such trends are much more evident looking at PAR values (Fig. 5). The saw-tooth trend is recorded at the same time that extreme precipitation events were observed in the Lake Montcortès record (Corella et al., 2014, 2016). The clastic microfacies resulted from the increased runoff during flood events that took place at the sub-decadal scale and were cross-correlated with documented extreme floods occurred in most rivers in the NE Iberian Peninsula (see Corella et al., 2014, 2016 for more detail) (Fig. 7). The increased input of external detrital material from the watershed is clearly recorded in pollen sedimentation rates and other terrestrial proxies (charcoal and fungi). The only vegetation type that did not record the saw-tooth trend was the conifer forest, which mostly comes from high-mountain areas, giving a weak signal resulting from the watershed weathering. Therefore, the high-resolution obtained for the pollen record, in this case, gives us information about sub-decadal scale periods of runoff increases from the immediate surroundings of Lake Montcortès.

4.3. Low human pressure: field abandonment (Pollen zones MC-5 and MC-6; from ~1875 to ~2013)

The notable decrease in *Cannabis* pollen, as well as the decrease of other human presence indicators during 20th century in Lake Montcortès record, indicates the decrease in human pressure intensity: *Secale*, *Urtica*-type decreases and the near disappearance of coprophilous fungi at the end of the century together with the considerable reduction of fire and forest recovery indicate the reduction of agropastoral activities and field abandonment. The dramatic and sharp hemp reduction occurred during the 20th century coincided with the dismantlement of the Spanish Royal Navy and also with important socio-political changes in the country. The need for materials for rigging, sails, clothes or trading was sharply reduced. Moreover, the appearance of new fibres (synthetic fibres and imported cotton from USA, Brazil, Mexico, Syria and Turkey) provoked almost the total disappearance of hemp fibre used for clothing in Spain (Simó, 1985). The only crop that seemed to increase during the 20th century was olive, but it could be an artefact of pollen percentages since increases in *Olea* at that moment were not recorded in PAR values (Cultivated trees) (Fig. 5). A remarkable human population increase took place from 1860 onwards in the Pyrenees, which provoked a crisis at the end of the 19th century due to the lack of enough resources to maintain the increasing population. This situation forced the population to emigrate to industrialised areas (Guirado, 2011; Farràs, 2005). Agro-pastoral activities in the Pyrenees were reduced to fewer and richer houses that were able to adapt to a high demanding market and change to a more intensive production model. The economic activities in the area changed with the advent of capitalism and the most important economic activity in the Pyrenees

was the implantation and exploitation of hydroelectric power stations (Rotés, 2011). Furthermore, the Spanish Civil War (1936–1939 CE) interrupted industrialisation and socio-economic activities. It was not until the 1950s when the country again started with socio-economic recovery and modernization (Guirado, 2011). The effects of emigration can be appreciated in the Montcortès record with the declining of human indicators. Aquatic plants and algal remains also experienced changes during this period. *Typha* decreased and *Botryococcus* (a low nitrogen and phosphorous tolerant alga (Reynolds, 2006)) increased. This is coherent with a declining human pressure scenario. It seems that Lake Montcortès returned to similar states prior to *Cannabis* pollen maxima, likely more oligotrophic conditions that lasted until present day (Trapote et al., 2018).

From the 1970s onwards, forest continued to expand around Lake Montcortès. Coprophilous fungi disappeared, and hemp was the only cultivated plant increasing its percentages. Currently, there is no hemp cultivation known close to Lake Montcortès and, according to local people, the lake has not been used for hemp retting during the last decades. Since 1992, Montcortès is considered a site of natural heritage and it is protected by means of different administrative measures (Gentcat, 2006; Xarxa Natura, 2000). The hemp maxima during the 21st century in Lake Montcortès is recorded from the mid to late 1990s. At the beginning of the 1970s, a renewed interest in hemp, mainly as a source for paper pulp manufacturing, took place in Spain (Gorchs and Lloveras, 2003). The European Union started to economically support hemp cultivation from the late 1980s. In the middle 1990s, EU hemp subsidy amounts reached their maxima and farmers have since been interested in growing hemp for its economic benefits (Gorchs and Lloveras, 2003; Karus and Kaup, 2002). Consequently, in just a couple of decades, areas of hemp cultivation expanded again and reached their climax in Spain around 1998 (Karus and Kaup, 2002), coinciding with maximum hemp production in Catalonia (Gorchs and Lloveras, 2003). From the 2000s onwards, EU hemp cultivation subsidy amounts were notably reduced, and therefore, hemp production became less economically profitable and cultivation was reduced. Currently, hemp is still used for clothing, animal feeding, oil (seeds), pulp paper, building materials and water treatment (hemp dust) among other uses (Gorchs and Lloveras, 2003; Clarke and Merlin, 2013). The abrupt hemp peak observed during the 21st century lasted less than 20 years after almost a century of very low hemp pollen frequencies, then, decreased again until the present. The presence of this peak is related to a very specific and short-term duration historical event that can only be detected by means of high-resolution observations.

Despite a notable hemp reduction at the beginning of the 2000s, at present, Lake Montcortès still records significant amounts of hemp pollen. It was observed by Rull et al., (2017), who carried out a two-year study of seasonal sediment trapping in Lake Montcortès from 2013 to 2015 CE. *Cannabis* pollen was continually recorded in the trapped material during the whole studied period at about 5% of total abundance and greatly increased during fall seasons when reached 40%. The current presence of hemp pollen in Lake Montcortès can be explained by its great air dispersion. Hemp pollen can travel far away from the parent plant (i.e., from North Africa to southern Spain, as found by Cabezudo et al., 1997; Giner et al., 2002). The mentioned trap study also found that hemp pollen was positively correlated with wind velocity supporting this idea. Another explanation could be due to an involve increasing cultivation near Lake Montcortès, as currently, there is a growing interest on hemp cultivation to recover natural and environmental friendly fibres. Moreover, the pre-Pyrenean areas have been identified as suitable for hemp cultivation due its humidity and because hemp has been reintroduced as a rotation crop together with wheat (Gorchs Altarriba et al., 2006). During the trapping study period, no hemp retting was carried out in Lake Montcortès and inhabitants of the area had no notions about the effect of hemp fields on the surroundings.

5. Conclusions

We have evaluated a 500 year-long varve record from Lake Montcortès at the highest resolution achieved so far for a varved lake in the Iberian Peninsula. We have notably improved our knowledge of the recent history of Lake Montcortès and its surroundings in several terms. We have improved the temporal resolution (~6 years /sampling interval) and improved the historical and palaeoecological precision, as well as the spatial scope, with the new studied time interval (20th and 21st centuries). By means of a multiproxy analysis of biological indicators combined with independent evidence from historical sources and comparison with previous studies carried out in Lake Montcortès, we have reconstructed human-landscape dynamics in detail. The present work also helped to answer some questions that arose with former studies and to go deeply into one of the most striking features of the Montcortès pollen record: the outstanding hemp pollen percentages. We shed more light on potential consequences of human impact on the aquatic system derived from hemp retting practices.

Human activity around the lake during the last 500 years has had a greater influence on vegetation community changes than climatic factors, and only increases in the frequency of flood events could have been inferred from the studied record from the mid to the end of the 19th century. Cropping, livestock breeding, and hemp related activities have been the most important factors responsible for landscape modulation. Even during harsher climate conditions (LIA), human activities remained significant in the area. The high-resolution study provided enough data to evaluate short-lasting events at local and regional scale that otherwise would not be possible to identify related to climate (sub-decadal frequency of floods and high-land forest recovery) or to historical and socio-economic events (i.e., crop promotions (hemp) or land abandonment).

The temporal extent of the *Cannabis* pollen peak (400 years) and its temporal continuity have been confirmed. The revision of new historical sources available combined with pollen and NPP indicators from Lake Montcortès provided further and detailed evidence of the local use of hemp, implying cultivation and manufacturing, as well as potential effects of retting hemp in the lake, on the aquatic communities between the mid 17th to late 19th centuries, which was not possible to confirm in previous studies at lower resolution. Further investigations using aquatic proxies at high-resolution are necessary in this sense to better assess the eutrophication degree and water community disturbance. Geochemical analyses of sedimentary cannabiniol or other hemp specific biomarker would unequivocally confirm the use of Lake Montcortès for hemp retting.

More work is needed to take advantage of the great scientific potential of the Montcortès sediment record, and also to exploit the unusual and very valuable availability of modern sedimentary pollen analogues as a tool to better interpreting the fossil signal. Studies that include and combine lake and modern analogue monitoring with high-resolution palaeoenvironmental reconstructions in varved sediments are very scarce, but it opens a range of possibilities to exploit the potential of palaeodata contained in the sediment record, i.e. it is possible to assess the yearly flux of pollen sediment rates and define rates of palaeoenvironmental changes at decadal and even at sub-decadal scales (Birks and Birks, 2006).

The present study is the first that combines all of the explained above advantages for a varved lake in the Iberian Peninsula and the Mediterranean region. The value of the data obtained in this study lies in the potential to be used to calibrate and validate future model scenarios, perform quantitative climatic or environmental reconstructions and use it as a tool to apply in the conservation and restoration of cultural landscapes. Further work should focus on improving sampling techniques to obtain higher temporal resolution for biological proxies and to obtain modern analogues for the variety of potential environmental and climatic proxies.

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