High mountain palaeoecology and palaeolimnology fo Central Pyrenees, based on pollen and diatom analyses

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High mountain palaeoecology and palaeolimnology of Central Pyrenees, based on pollen and diatom analyses

Memòria presentada per Sandra Garcés Pastor
per optar al grau de Doctor per la Universitat de Barcelona

Sandra Garcés Pastor
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Amb el vist-i-plau de la directora i tutora de tesi:

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Als meus pares, als avis, a l'Owen

Al que hi eren, als que hi són i als que vindran

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Agraïments:

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Nolla, amb qui tantes diatomees hem comptat. Als companys del departament d'Ecologia, els de la sala del galliner, la Txell, la Sílvia, l'Alba, l'Àstrid, el Lluís, la Rebeca i la Verónica. A la Claudia i els missatges d'ànims, i San Cucufato. Les valuoses converses vespreres amb la Marta Tobella. Les trobades amb la Neus Sanmartí pel passadís i les converses dels serveis amb tantes persones. Al Cesc Múrria i la Montse Vidal pel recolzament moral. Als botànics que vaig acompanyar durant el mostreig de torberes als Pirineus, a l'Aaron, el Marc, el Jaume, el Juan Carlos, l'Efrem, la Claudia, la Cèlia, l'Elena i el Jose. Va ser genial mostrejar amb vosaltres. Especialment gràcies a l'Elena i al Jose per ajudar-me en aquell tram on jo sola no podia. Sense vosaltres encara estaria enganxada a aquell roc!

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Informe de la directora

La Dra. Teresa Vegas Vilarrúbia, Professora Agregada del Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals de la Universitat de Barcelona, directora i tutor, de la Tesi Doctoral elaborada per la candidata Sandra Garcés Pastor, i que porta per títol "High mountain palaeoecology and palaeolimnology of Central Pyrenees, based on pollen and diatom analyses"

INFORMA

Que els treballs de recerca portats a terme per la Sra. Sandra Garcés Pastor com a part de la seva formació pre-doctoral i inclosos a la seva Tesi Doctoral han donat lloc a quatre capítols, dos dels quals ja estan publicats, un està en procés de revisió i el darrer manuscrit està a punt per ser enviat a una revista d'àmbit internacional. A continuació es detalla la llista d'articles, així com els índex d'impacte (segons la ISI Web of Knowledge) de les revistes on han estat publicats o bé s'han enviat els capítols, i el quartil a que correspon segons el tema

1. Garcés-Pastor, S., Cañellas-Boltà, N., Clavaguera, A., Calero, M. A., and Vegas-Vilarrúbia, T. 2017. Vegetation shifts, human impact and peat bog development in Bassa Nera pond (Central Pyrenees) during the last millennium. *The Holocene*, 27(4), 553-565.

   L' índex d'impacte de la revista *The Holocene* l'any 2016 va ser de 2.324. Aquesta revista està situada en el primer quartil de la categoria "Ecology". Aquesta revista ocupa el lloc 55è de un total de 311 revistes considerades en aquesta categoria.

2. Garcés-Pastor, S., Cañellas-Boltà, N., Pèlachs, A., Soriano, J. M., Pérez-Obiol, R., Pérez Haase, A., Calero, M.M., Andreu, O., Escolà, N., and Vegas-Vilarrúbia, T. (2017). Environmental history and vegetation dynamics in response to climate variations and human pressure during the Holocene in Bassa Nera,

Central Pyrenees. Palaeogeography, Palaeoclimatology, Palaeoecology, 479, 48-60.

L' índex d'impacte de la revista *Paleo3* l'any 2016 va ser de 2.578. Aquesta revista està situada en el primer quartil de la categoria "Ecology, Evolution, Behavior and Systematics Aquesta revista ocupa el lloc 81è de un total de 547 revistes considerades en aquesta categoria.

3. Garcés-Pastor, S., Gutiérrez, E., Martínez-Sancho, E., Dorado-Liñán, I., Camarero, J.J., Cañellas- Boltà, N., Vegas-Vilarrúbia, T. Subalpine forest dynamics reconstructed throughout the last 700 years in the Central Pyrenees by means of pollen and tree rings. (enviat a la revista *The Holocene*).

   L' índex d'impacte de la revista *The Holocene* l'any 2016 va ser de 2.324. Aquesta revista està situada en el primer quartil de la categoria "Ecology". Aquesta revista ocupa el lloc 55è de un total de 311 revistes considerades en aquesta categoria.

4. Garcés-Pastor, S., Wangensteen, O.S., Pérez-Haase, A., Pèlachs, A., Pérez-Obiol, R., Cañellas-Boltà, N., Mariani, S., Vegas-Vilarrúbia, T. Genetic characterization of modern and past communities of a high mountain peat bog system using eukaryotic metabarcoding. (enviat a la revista *Journal of Paleolimnology*).

   L' índex d'impacte de la revista *Journal of Paleolimnology* l'any 2016 va ser de 2.017. Aquesta revista està situada en el primer quartil de la categoria "Earth-Surface Processes". Aquesta revista ocupa el lloc 29è de un total de 139 revistes considerades en aquesta categoria.

Alhora CERTIFICA

Que la Sandra Garcés Pastor 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 cadascun dels articles ha estat la següent:

- Participació en el plantejament inicial dels objectius de cadascun dels treballs.
- Mostreig de camp a l'Estany de Sant Maurici, Bassa Nera, Lòssa i Basturs
- Mostrejos de entrenament a Montcortès i a Ecuador amb el curs X-PERT
- Obtenció i anàlisi de dades
- Síntesi, elaboració i difusió dels resultats
- Presentació oral a les Jornades de Recerca d'Aigüestortes i al ASLO Honolulu 2017.
- Póster al congrés PAGES 2017 a Saragossa.
- Redacció dels articles i seguiment del procés de revisió dels mateixos.

Part d'aquestes tasques es van realitzar durant dues estades de recerca al Ecosystems and Environment Research Centre, School of Environment & Life Sciences, University of Salford, Greater Manchester, UK (equip del Dr. Stefano Mariani).

Finalment, certifico que cap dels coautors dels articles abans esmentats i que formen part de la Tesi Doctoral de la Sandra Garcés Pastor ha utilitzat ni té previst utilitzar implícita o explícitament aquests treballs per a l'elaboració d'una altra Tesi Doctoral.

Barcelona, 13 de Juliol de 2017

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Abstract

High-mountain ranges are suitable ecosystems for studying local environmental shifts driven by large-scale climate changes. The sedimentary records obtained from those regions contain information that allows to understand past environmental changes that would help to predict the influence of the ongoing climate warming. However, the precise features in the response of species assemblages are not fully understood, and human pressure may often hide climatic signals.

This thesis is aimed at the study of the palaeoecology and palaeolimnology of Central Pyrenees along the Holocene. For this purpose, sedimentary sequences were extracted from the peat bog that surrounds Bassa Nera pond. In order to understand the ecosystem responses to past environmental changes and climate, those sediments have been analysed using several biological and inorganic proxies. On the one hand, we studied biological indicators such as pollen, diatoms, chrysophytes, non-pollen palynomorphs, microscopic charcoal particles, macroremains, tree-rings and sedimentary DNA metabarcoding. On the other hand, the inorganic proxies studied were Loss-on-ingnition and chemical elements. This work comprises diverse parts according to the proposed questions and the proxies that were used to assess them.

Combining pollen and diatom analyses at multidecadal resolution, we inferred vegetation shifts and peat bog development during the past millennium. A montane pollen ratio was introduced as a new palaeoecological indicator of altitudinal shifts in vegetation. Our results emphasize the sensitivity of the montane ratio to detect upward migrations of deciduous forest and the presence of the montane belt close to Bassa Nera pond during the Medieval Climate Anomaly. Changes in aquatic taxa allowed to date the onset of the surrounding peat bog which appeared and infilled the coring site around AD 1565. Overall, results suggest that Bassa Nera had a low-intensity human pressure. During the last millennium people changed the management of natural resources. From the Medieval Climate Anomaly until AD 1500 farming was the main activity. However, with the Little Ice Age people turned to highland livestock raising.

Shortly after, we reconstructed the vegetation and lacustrine dynamics during the last 10,000 years using pollen, plant macroremains, charcoal, chemical elements

and loss-on-ignition at centennial to decadal resolution. We also applied the montane pollen ratio to track altitudinal shifts in high mountain vegetation and we compared this ratio to the ice-rafted debris index (IRD), a proxy that summarizes the climatic influence of the North Atlantic Circulation. Our results revealed upward shifts of deciduous forest and its presence in Bassa Nera from the onset of the Holocene until 4200 cal yr BP, when it was replaced by coniferous taxa. On the other hand, the montane ratio showed a link between vegetation and North Atlantic influence, while changes in *Sphagnum* macroremains and aquatic taxa allowed the description of local ontogenic changes from the initial pond to the present peatland. The loss-on-ignition record showed some flood events at Bassa Nera between 4500 and 3900 cal yr BP. The first inferences concerning anthropic pressure in Bassa Nera date by 7300 cal yr BP and were grazing activities, while cereal agriculture appeared around 5190 cal yr BP. Along the studied record, the periods of highest human pressure occurred in the late Bronze Age, Roman Period and Middle Ages.

To assess the past dynamics of subalpine forests of Central Pyrenees for the last 700 years, we studied the relationships between the sedimentary pollen and tree-ring records, as well as their link with climatic drivers. We compared the sedimentary pollen and montane pollen ratio from Bassa Nera with nearby tree-ring width data from old *Pinus uncinata* subalpine forests. To study the climate-growth associations, we related the dendrochronological data with instrumental meteorological records of the 1901-2010 period and with temperature reconstructions for the Pyrenees and Northern Hemisphere for the last 700 years. Few robust associations were found between any specific arboreal pollen taxa and the tree-ring width of the surrounding forests. However, a significant correlation was found between the integrative montane pollen ratio and the pine growth of nearby (less than 10 km apart) subalpine forests. On the other hand, our results suggest that the sensitivity of pine growth to climate has varied over the last 700 years. We also observed that tree-growth variability at high elevations is more constrained by low than by high temperatures, although we also noted a relaxation of this constrain in recent decades.

To explore the diversity of eukaryotic communities of Bassa Nera, we performed a metabarcoding study using universal 18S and COI genetic markers. We assessed the molecular diversity of four different micro-habitats and five sedimentary depths. The

resulting palaeoecological communities recovered from sedimentary DNA were compared to those from the modern communities. We also compared the information provided by the ancient DNA to the environmental reconstruction from morphological proxies such as pollen and macroremains from the same record. We successfully amplified ancient DNA with both universal markers from all the sedimentary samples, including the deepest one (10200 years old). Even though 18S could amplify a broader group of organisms, the taxonomic resolution was lower than that obtained from COI, and typically reached the family or genus levels. On the other hand, the taxonomic assignment of the detected COI sequences yielded mostly metazoans, probably due to gaps in reference databases. This first molecular approach has allowed to prove that the diversity of modern and past eukaryotic peat bog communities can be assessed using universal metabarcoding markers.

Resumen

Las cordilleras de alta montaña son ecosistemas idóneos para estudiar los cambios ambientales locales promovidos por cambios climáticos a gran escala. Los registros sedimentarios obtenidos de esas regiones contienen información que permite entender los cambios ambientales ocurridos en el pasado que ayudarían a predecir la influencia del calentamiento climático actual. Sin embargo, las características concretas de la respuesta entre las comunidades de especies no se entienden completamente, y a menudo la presión humana puede ocultar las señales climáticas.

Esta tesis se centra en el estudio de la paleoecología y paleolimnología de los Pirineos Centrales a lo largo del Holoceno. Para ello, se extrajeron dos secuencias sedimentarias de la turbera que rodea el estanque de la Bassa Nera. Con el fin de comprender las respuestas de los ecosistemas a los cambios ambientales y climáticos ocurridos en el pasado, estos sedimentos has sido analizados utilizando varios indicadores biológicos e inorgánicos. Por un lado estudiamos indicadores biológicos como el polen, las diatomeas, los crisófitos, palinomorfos no polínicos, partículas microscópicas de carbón vegetal, macrofóssiles, anillos de árboles y ADN sedimentario a través del metabarcoding. Mientras que los proxis inorgánicos fueron "pérdida por calcinación" y elementos químicos. Este trabajo comprende diversas partes en función de las preguntas propuestas y los proxis que se han utilizado para responderlas.

Combinando el análisis de polen y de diatomeas a una resolución multidecadal inferimos los cambios de la vegetación y el desarrollo de la turbera durante el último milenio. Se introdujo un índice de polen montano como un nuevo indicador paleoecológico para resaltar cambios altitudinales en la vegetación. Nuestros resultados enfatizan en la sensibilidad del índice para detectar migraciones ascendentes de los bosques caducifolios, así como la presencia del piso montano cerca del estanque de Bassa Nera durante la Anomalía Climática Medieval. Los cambios en los taxones acuáticos permitieron datar cuando la turbera circundante se desarrolló y colmató el lugar donde se extrajo el sondeo, siendo esta en 1565 AD. En general, los resultados sugieren que Bassa Nera tuvo una presión humana de baja intensidad. Durante el último milenio los habitantes de la zona cambiaron su gestión de los recursos naturales. Desde la Anomalía Climática Medieval hasta el año 1500 AD los

cultivos fueron la actividad principal. Sin embargo, con la Pequeña Edad de Hielo los habitantes pasaron a la actividad ganadera en tierras altas.

Posteriormente reconstruimos la vegetación y la dinámica lacustre durante los últimos 10.000 años mediante polen, macrofósiles de plantas, carbones, elementos químicos y pérdida de materia orgánica a una resolución centenaria a decadal. También se aplicó el índice montano de polen para trazar los cambios altitudinales en la vegetación de alta montaña y se comparó esta relación con el índice de detritos rocosos transportados por el hielo (IRD), un proxy que resume la influencia climática de la Circulación del Atlántico Norte. Nuestros resultados mostraron cambios en el ascenso de los bosques caducifolios y su presencia en Bassa Nera desde el inicio del Holoceno hasta el año 4200 años BP, período en que fueron reemplazados por taxones de coníferas. Por otra parte, el índice montano mostró una relación entre la vegetación y la influencia del Atlántico Norte, mientras que los cambios en los macrofósiles de *Sphagnum* y los taxones acuáticos permitieron la descripción de los cambios ontogénicos locales desde el estanque inicial hasta la turbera actual. El registro de pérdida de materia orgánica mostró algunos eventos de inundación en Bassa Nera entre 4500 y 3900 años BP. Las primeras inferencias de presión antrópica en Bassa Nera datan de 7300 años BP y fueron actividades de pastoreo, mientras que los cereales aparecieron alrededor de 5190 años BP. A lo largo del registro estudiado, los periodos de mayor presión humana ocurrieron en la Edad del Bronce tardía, el Período Romano y la Edad Media.

Para evaluar la dinámica de los bosques subalpinos de los Pirineos centrales durante los últimos 700 años, se estudió la relación entre el polen sedimentario y los registros de los anillos de los árboles, así como su relación con los factores climáticos. Comparamos la proporción de polen sedimentario y el índice montano de Bassa Nera con los datos de los anillos de los árboles cercanos de los bosques subalpinos de *Pinus uncinata*. Para estudiar las asociaciones clima-crecimiento, relacionamos los datos dendrocronológicos con registros meteorológicos instrumentales del período 1901-2010 y con reconstrucciones de temperatura para los Pirineos y Hemisferio Norte durante los últimos 700 años. Se encontraron pocas asociaciones significativas entre los taxones específicos de polen y el grosor de los anillos de los bosques circundantes. Sin embargo, se encontró una correlación significativa entre el índice de polen montano y el crecimiento del pino de bosques subalpinos cercanos (menos de 10 km). Por otro lado, nuestros resultados sugieren que la sensibilidad del crecimiento del pino al clima ha variado en los últimos 700 años. También observamos que la variabilidad del crecimiento arbóreo en localidades altas está más limitada por las temperaturas bajas que por altas, aunque también observamos una relajación de esta restricción en las últimas décadas. .

Para explorar la diversidad de las comunidades eucariotas de Bassa Nera, se realizó un estudio de metabarcoding utilizando los marcadores genéticos universales 18S y COI. Se evaluó la diversidad molecular de cuatro micro-hábitats diferentes y cinco profundidades sedimentarias. Las comunidades paleoecológicas resultantes recuperadas del ADN sedimentario se compararon con las de las comunidades modernas. También comparamos la información proporcionada por el ADN antiguo con la reconstrucción ambiental a partir de proxies morfológicos como polen y macrofósiles del mismo registro. Amplificamos con éxito el ADN antiguo de todas las muestras sedimentarias con los dos marcadores universales, incluyendo la más profunda (10.200 años de edad). A pesar de que 18S pudo amplificar un grupo más amplio de organismos, la resolución taxonómica fue menor que la obtenida de COI, y por lo general alcanzó los niveles de la familia o género. Por otro lado, la asignación taxonómica de las secuencias de COI detectadas fueron principalmente metazoos, probablemente debido a lagunas en las bases de datos de referencia. Este primer enfoque molecular ha permitido demostrar que la diversidad de comunidades de turberas eucariotas modernas y pasadas pueden ser evaluadas usando marcadores genéticos universales.

Thesis structure

This thesis is presented as a compendium of publications. It is composed of an introduction, four chapters compending the publications, a general discussion and main conclusions. This work is aimed at the study of the paleoecology of Central Pyrenees. Chapters 2 to 5 are the articles that have been published, accepted or submitted in scientific journals cited in the Journal Citation Reports. All chapters have been written in English. At the beginning of each chapter, the current status of the related paper is indicated.

Chapter 1. **Introduction**. This work comprises a general overview of the main issues related to the purposes of each chapter, including the study area and the main objectives.

Chapter 2. **Vegetation shifts, human impact and peat bog development in Bassa Nera pond (Central Pyrenees) during the last millennium**. This chapter assess past dynamics of Bassa Nera aquatic system and surrounding vegetation and discerns between climate and anthropic pressures. It is a high resolution multiproxy study using pollen, charcoal and diatoms. It also performs a montane ratio to infer information about past altitudinal shifts of the montane-subalpine vegetation ecotone.

Chapter 3. **Environmental history and vegetation dynamics in response to climate variations and human pressure during the Holocene in Bassa Nera, Central Pyrenees**. This study uses the montane ratio introduced in the former chapter and applies it to investigate the vegetation shifts during the last 10.200 years. It also relates the ratio with global climatic signals of the Northern hemisphere to study the montane vegetation sensitivity. The multiproxy analysis of two cores using pollen, charcoal, macrorremains, LOI and chemical elements has allowed to reconstruct changes in vegetation and the lentic system at local scale.

Chapter 4. **Pollen and tree-rings relationships along the last 700 years in the Central Pyrenees**. This chapter is focused on the relationship between the sedimentary pollen, montane ratio of Bassa Nera and the tree-ring growth of regional forests of *Pinus mugo* var *uncinata* since the Little Ice Age. It also studies how climatic variables affected the pine populations during the 21th century.

Chapter 5. **Genetic characterization of modern and past communities of a high mountain peat bog system using eukaryotic metabarcoding**. This chapter applies DNA high-thoughput sequencing techniques to provide a description of current and past eukaryotic communities in the peat bog that surrounds Bassa Nera

Chapter 6. **Discussion**. The main results presented in the preceding chapters are discussed together, indicating whats is new and how this compares to or reinforces what is already known. Possible directions to future work are also proposed.

Chapter 7. **Conclusions**. A list of the main conclusions of the thesis based on the conclusions from each chapter and grouped according to the main objectives described in Chapter 1.

Annex 1. This annex contains the supplementary material used in the Chapters 2 to 5.

Annex 2. Publications. This annex includes the chapters that have been already published.

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List of Abbreviations

MCA: Medieval Climate Anomaly

LIA Little Ice Age

IR: Industrial Revolution

CGW: Global Climate Warming

PNAESM: Aigüestortes i Estany de Sant Maurici National Park

IRD: ice-rafted debris index

NAO: North Atlantic Oscillation

LOI: Loss-on-ignition

NHTA: Northern Hemisphere Temperature Anomaly

PTA: Pyrenean Temperature Anomaly

aDNA: ancient DNA

nDNA: nuclear DNA

rRNA: ribosomal DNA

COI: mitochondrial gene cytochrome c oxidase I.

18S: 18S ribosomal RNA

***Trn*L**: chloroplast *Trn*L intron

rbcL: L*arge-chain* gene of the chloroplast ribulose-bisphosphate carboxylase

MatK: Maturase K, plastid plant gene

MOTUs: sequences belonging to the same molecular operational taxonomic units

Chapter 1

General introduction and objectives

"*Somehow, when I am counting pollen at the microscope, everything is fine*"

\- Conversations with Katja -

1.1 Why Palaeoecology?

Changes in the atmosphere, land, ocean and cryosphere evidence that the current climate is changing across our planet, largely as a result of human activities (IPCC 2013). As a consequence, many species may not be able to acclimate to the speed of changes of climate variables, especially temperature. Many ecosystems will be transformed at high rates and over relatively short timescales. However, there are still huge knowledge gaps about the ecological processes that occur over multi-decadal and longer timescales (Dorado Liñán et al., 2011), partly due to the short length of instrumental records and biotic proxis. A better understanding of the dynamics of past climate changes would be very valuable in determining to what extent the current rates of climate change are unusual, as well as to improve the reliability of climate prediction models. The IPCC assessments have compared their projections of future climate change with climatic and biotic observations in order to develop more sophisticated models with less uncertainty. Since instrumental climate records prior to the 20th century are scarce, there is a need to document past biotic and abiotic responses to climatic shifts. These information is obtained from indirect indicators or proxy, which consist on natural archives or documentary records prior to the use of instrumental climatic data, that by their biological or physical nature record some kind of climatic or environmental information. Palaeoclimatic reconstructions resulting from the study of such proxies offer a means for placing the current climate changes in the perspective of natural climatic variability (Smol et al., 2001). On the other hand, environmental reconstructions provide information of ecosystem sensitivity and past environmental responses to climate variability. This knowledge has been used to test the ability of models to simulate the magnitude and large-scale patterns of past changes. Palaeoenvironmental and palaeoecological studies are crucial to improve our comprehension of long-term ecosystem dynamics and present composition of vegetal communities, and try to distinguish between the effects of climatic and anthropogenic forcings (Berglund, 1987; Birks and Birks, 1980; Warner, 1990). Past environmental and ecological assessments are appropriate tools that provide guidelines to predict how forthcoming climate shifts will affect natural ecosystems and allow for the design of appropriate land management measures (Willis et al., 2007).

High mountain ecosystems in temperate mid-latitude areas are well-suited places to study past environmental changes. Their harsh environmental conditions make them less prone to intensive direct human influence and they will most likely be among the first ecosystems to exhibit a response to current climate changes (Engler et al., 2011). Evidence exists already, i.e. Gottfried et al. (2012) reported elevated replacement rates of cold-adapted plants by thermophilic species in several European mountain regions due to the current changes in the climate, and Thuiller et al. (2005) predicted high rates of vegetal species loss in the same areas. Lakes and peat bogs located in high-mountain areas are excellent sources of sediments containing aquatic and terrestrial environmental components, accumulated over time and preserved in robust stratigraphic contexts and anoxic conditions (Smol et al., 2001). These features make them suitable palaeoenvironmental archives for assessing the potential consequences of climate fluctuations on mountain biota and aquatic systems (Cohen, 2003)□. In this context, aquatic systems of the Pyrenees can be considered paradigmatic case studies, as the Mediterranean area is one of the most vulnerable regions on Earth to the Climate Global Warming (Christensen et al., 2007; IPCC 2013).

1.2 Multi-proxy analyses

Palaeoecological techniques use diverse proxy indicators to reconstruct how organisms and communities responded to past environmental changes (Mann, 2002). Below, we introduce some of these proxies which have been relevant for the present study. Palaeoecology is based in the principle of uniformitarianism created by James Hutton and Charles Lyell in the late 18th century (see Simpson, 1970), which states that laws of nature are constant across time and space. This means that the physical, chemical and biological processes that link the present environment with present proxy variations are the same processes that operated in the past, though the rates and intensity of such processes might have varied in time (Delcourt and Delcourt, 1991). Therefore, we can use present-day information to make inferences about the past (Birks and Birks, 1980).

1.2.1 Pollen and spores

The analysis of pollen and spores (palynology) from sedimentary samples is the principal technique for determining vegetation responses to past terrestrial environmental changes and is a well established discipline (Erdtman, 1943). Among other uses, it has also been used to assess the anthropic impacts in vegetation and to document successional changes (Birks and Birks, 1980; Davis, 1963; Delcourt and Delcourt, 1980). The pollen is produced by angiosperms and gymnosperms, while spores are mainly from ferns and mosses, but also fungi. The outer walls of pollen grains and spores are formed by sporopollenin, a very stable and resistant component to most forms of chemical and physical degradation, except oxidation (Traverse, 1988). This favours pollen to be maintained in anaerobic environments and allows to perform strong chemical digestions to remove the organic matter of the sediments in order to concentrate the pollen and spores for their subsequent analysis. In addition, the outer wall of pollen and spores (exine) has a very variable appearance, with different shapes and sculpture in species-specific patterns that makes possible to identify them to various taxonomic levels under the optical microscope (Faegri and Iversen, 1989; Hesse et al., 2009; Moore et al., 1991).

Plants have diverse strategies of pollination, where pollen dispersion can be mediated by the wind (anemophilous) or by insects (entomophilous). The pollen production will considerably differ in each case and plants with wind-dispersed pollen will produce higher amounts than those with insect-dispersed pollen. In general, pollen and spores are produced in abundance and liberated into the environment, mixing in the atmosphere and transported until they fall to the ground in form of pollen rain (Faegri and Iversen, 1989). As a result, some of them will accumulate in the sediments of non-oxidising environments such as bogs, lakes, fens or the sea (Smol et al., 2001). The proportion of each pollen type in the pollen rain depends of the abundance of its parent plants, providing a picture of the vegetation composition in that area and time. However, it is important to note that pollen recovered from lake and terrestrial sediments is largely from plants with wind-dispersed pollen, mainly from trees and shrubs. Although local individual taxa will be better represented than those farther away, pollen records may suffer an overrepresentation of wind-dispersed taxa from a variety of local and distant sources while others insect-dispersed taxa could be underrepresented (Birks and Birks, 2000). Hence, it is important to perform local and regional studies with modern vegetation analogs before performing palaeoenvironmental pollen reconstructions (Jackson and Williams, 2004; Rull, 2006). Pollen grains represent a clearly identifiable evidence of plant species which can be preserved for a long time in suitable environments, whereas changes in pollen frequencies along diverse sedimentary samples from the same record will inform about changes in a distance-weighted integration of vegetation through time.

1.2.2 Macrorremains and charcoal

In contrast with pollen, which generally represent the regional flora, plant macrorremains (seeds, buds or vegetative tissues) provide a local record of the vegetation of the past, as they are large and have low dispersal and transport capacity (Delcourt et al., 1986; Delcourt and Delcourt, 1980). In studies of lake sediments, the macrofossil record is often dominated by the aquatic and wetland plants growing in and around the lake, while species preferring dry environments or even terrestrial taxa are drastically underrepresented or entirely absent (Birks, 2003).

Charcoal found in pollen slides will allow to reconstruct long-term variations in fire occurrence as well as to examine the linkages among climate, vegetation, fire and, in some instances, anthropogenic activities in the past (Carcaillet et al., 2001; Thinon, 1978).

1.2.3 Diatoms

Diatoms are unicellular algae from the division Bacillariophyta. These organisms are characterized by their siliceous wall, the frustule, which is composed by two valves that fit perfectly forming a box-like structure. The taxonomy of this group is based on the shape and ornamentation of the frustule, which is taxonomically distinct to specific level (Krammer and Lange-Bertalot, 2004a, 2004b, 1999a, 1999b).

The preservation of the frustule allows diatom remains to be maintained along time and makes them a useful indicator of present and past ecological conditions. Diatoms can live in almost all aquatic environments and most species are cosmopolitan. Although they can be found in many habitats, the most relevant are the planktonic and benthic. The planktonic diatoms live either permanently in the water column (holoplankton) or just during a part of their life-cycle (meroplankton, tychoplankton). On the other hand, benthic diatoms are associated with substrates and can be attached to stones in the margin of water bodies (epilithon) or even aquatic plants (epiphyton) (Smol et al., 2001). These organisms present relatively strict preferences for physical, chemical and biological conditions in the water column and quickly react to environmental changes (Dam et al., 1994). In sedimentary samples, diatom frustules are numerous, diverse and frequently well-preserved. They allow a reliable taxonomic determination at specific level and given that they often have well-known ecological preferences, changes in their frequencies can provide valuable information about changes in the aquatic ecosystem conditions (Smol et al., 2001).

1.2.4 Tree-rings

The study of the growth of tree-rings (dendrochronology) allows to date tree-rings to the exact year when they were formed and to obtain information on the diverse factors that influenced their growth. The tree-rings are formed with the division of vascular cambium cells that lay between the wood and the bark and are active only during a part of the year. In each growth season, the new ring reflects the weather conditions of that growth season (Fritts, 1976). Tree-rings result from the change in growth rates through the seasons of the year. The inner portion of a growth ring is formed by xylem cells divided at the beginning of the growing season. In this period, growth is comparatively rapid and cells tend to be large and thinned-walled, producing a less dense wood, known as “earlywood” or “springwood”. As the growing season progresses and conditions become less favourable, cambial activity slows and the xylem produces smaller cells with thicker walls and denser wood, giving rise to the outer portion of the annual ring, which is referred as “summerwood” or “latewood”. In temperate climates it is often produced in the summer and the cells stop dividing when temperatures are colder. The rings are thus easier to discern in temperate zones, where seasonal conditions differ more markedly and there is a higher difference between earlywood and latewood cells (Fritts, 1976). Climate influence tree development across space and time, resulting in a common variability expressed in the pattern of annual growth rings in trees across broad regions (Douglass, 1919; Hughes et al., 1982). Dendrochronology is an invaluable tool for dating events and for providing robust annually-resolved palaeoenvironmental insights. When all trees are affected by a common environmental factor, such as climate, crossdating provides an accurate chronological record that can be used to date events or describe variations in environmental conditions (Cook and Kairiukstis, 1990; Schweingruber, 1988). The chronologies with the highest degree of strength in their common signal are from regions where trees are mostly limited by climatic factors such as temperature or drought, normally at the limit of their ecological range and with minimum influences of competition and disturbance (Gornitz, 2008). Tree-ring analyses provide both reliable and ubiquitous archives for palaoenvironmental reconstruction at local to hemispheric scales (Elias, 2006; Gutiérrez, 2009).

1.2.5 DNA and metabarcoding

In some instances, a large proportion of the remains from the ancient flora and fauna are so damaged that they cannot be properly identified at a taxonomical level, but they still can leave some DNA traces in the sediments which could be detected and analysed. As a result, the DNA preserved in a small amount of sediment can provide valuable information about the biodiversity of past ecosystems and communities, even when no macroscopically identifiable remains are present (Coolen and Overmann, 1998; Willerslev et al., 2003). After an organism dies, several enzymatic, hydrolitic and oxidative processes damage the DNA, leading to the fragmentation of DNA molecules (Parducci et al., 2017). Environments with limited bacterial abundance such as frozen, anoxic or arid areas, with lower proportion of nucleases that damage nucleic acids, will present a better preserved DNA (Hofreiter et al., 2001). During the last decade, the development of DNA metabarcoding techniques (Hajibabaei et al., 2011; Taberlet et al., 2012b) has revolutionized the concept of biodiversity assessment and has allowed to perform broader ecological studies. This technique can be used for biodiversity screening of modern samples and has also the potential to be used as a palaeoecological tool (Epp et al., 2012). Using a metabarcoding approach, thousands of species present in a sample can be detected by high-throughput DNA sequencing and automatically identified using molecular taxonomy databases for limited cost. These techniques are sensitive to detect not only DNA from organisms that were alive when sample was taken ("community DNA") (Creer et al., 2016), but they can also detect an array of different types of "extra-cellular DNA", originated from dead remains (Barnes and Turner, 2016). On the other hand, environmental DNA refers to community DNA and extracellular DNA that can be extracted from environmental samples (such as soil, water or air), without first isolating any target organisms (Taberlet et al., 2012a).

There are several eukaryotic short standardized DNA regions markers that can be amplified by PCR, such as the mitochondrial cytochrome *c* oxidase I gene for animals (COI), the plastid ribulose 1,5-bisphosphate carboxylase gene (rbcL) and the maturase K gene (matK) for plants, nuclear ribosomal RNA (18S) genes and the internal transcribed spacer (ITS) for fungi (Thomsen and Willerslev, 2015). The taxonomic resolution can vary among Phyla depending on the natural variability of the used marker. In some cases the marker allows identifications at species-level, while in other cases it can only identify higher taxonomic levels such as genera, families or orders (Taberlet et al., 2012a).

1.2.6 Modern analogs as palaeoecological tools

The study of ecological patterns and processes occurring on present environments gives us information about the communities and ecosystems that can be applied for reconstructions of past ecosystems (Jackson and Williams, 2004). Studies of modern pollen assemblages and their relationships with vegetation and environmental factors are necessary to improve our knowledge on palaeoenvironmental interpretations (Birks and Birks, 1980; Huntley, 2001). Altitudinal gradients are well suited places for modern-analog studies as they encompass significant ecological and environmental variations in a relatively small study area (Rull, 2006). On the other hand, the study of modern analogs allows to establish a baseline of knowledge of the current communities, needed to monitor environmental shifts and responses to the ongoing climate variability.

1.2. Study area

Peat bogs are wetland environments in areas with excess water, where vegetation is mainly composed of hygrophilous species (*Sphagnum*, mosses, sedges and grasses). When those plants die, the vegetal matter does not decompose easily, but much of it accumulates, ultimately becoming peat (Minelli, 2004). These ecosystems have a remarkable degree of structuring, with a high spatial heterogeneity and characteristic assemblages such as water pools alternating with moss carpets and *Sphagnum* hummocks. In the Pyrenees, *Sphagnum* peat bogs cover small areas and are usually restricted to lake shores or wet soils located between 1500 and 2700 m a.s.l (Casas et al., 1994; Pérez-Haase et al., 2010; Pérez Haase, 2016). The present study was carried out in Bassa Nera, a small lacustrine system of the Central Pyrenees mountain range. Future scenarios predict a 5°C increase in temperature for this mountain range by 2100 (A2, IPCC, 2007), while precipitation will decrease and extreme hydric events will be more frequent (Barrera-Escoda and Cunillera, 2011; Brunet et al., 2009; López-Moreno and Beniston, 2009).

The Bassa Nera (BSN) (42°38′18.5″ N, 0°55′27.6″ E, 1891 m) is a small lentic system from glacial origin located close to the montane-subalpine boundary, in the peripheral zone of Aigüestortes i Estany de Sant Maurici National Park (PNAESM). It is placed in the Aiguamòg Valley (Aran Valley), on the Northern slope of the Central Pyrenees (Figure 1). Until the protection of this area by the National Park in 1990, the main anthropogenic activities were extensive cattle husbandry, forest exploitation and hydroelectric power generation. Nowadays, tourism has displaced all those activities, although pasturing and hydroelectric exploitation are still authorized. The pond has an area of 2.01 ha and 5 m maximum depth and receives mineral salts only from precipitation and runoff. It has a small outlet that drains into the Garonne River. The BSN basin lies on a granodiorite bedrock from the Maladeta batholith, which dates from the Carboniferous-Permic age (Roca i Adrover et al., 2010). The climate is subalpine with Atlantic influence and the annual average precipitation reaches 1152 mm and is evenly distributed over the seasons. The mean annual temperature is 4.25°C, with January being the coldest month (−3°C on average) and July the warmest month (14°C on average) (Ninyerola et al., 2003). Bassa Nera is currently in the final stages of infilling, surrounded by a complex of marshes and mixed *Sphagnum* peat bogs (Carrillo et al., 2008; Pérez-Haase and Ninot Sugrañes, 2006). Seven plant community types have been distinguished in this wetland mosaic of fens, carpets and bogs, depending of the water table variation. From all, the most abundant are geogenous fens (*Scheuchzerio-Caricetea fuscae*) and ombrogenous bogs (*Oxycocco-Sphagnetea*) (Pérez-Haase et al., 2010; Pérez-Haase A. and Ninot Sugrañes J., 2017). The vegetation of Bassa Nera catchment is composed of a conifer forest of *Pinus mugo* ssp. *uncinata* (Ramon) Domin. and *Abies alba* Mill. with *Rhododendron ferrugineum* L. in the understory and some Poaceae meadows surrounding the pond.

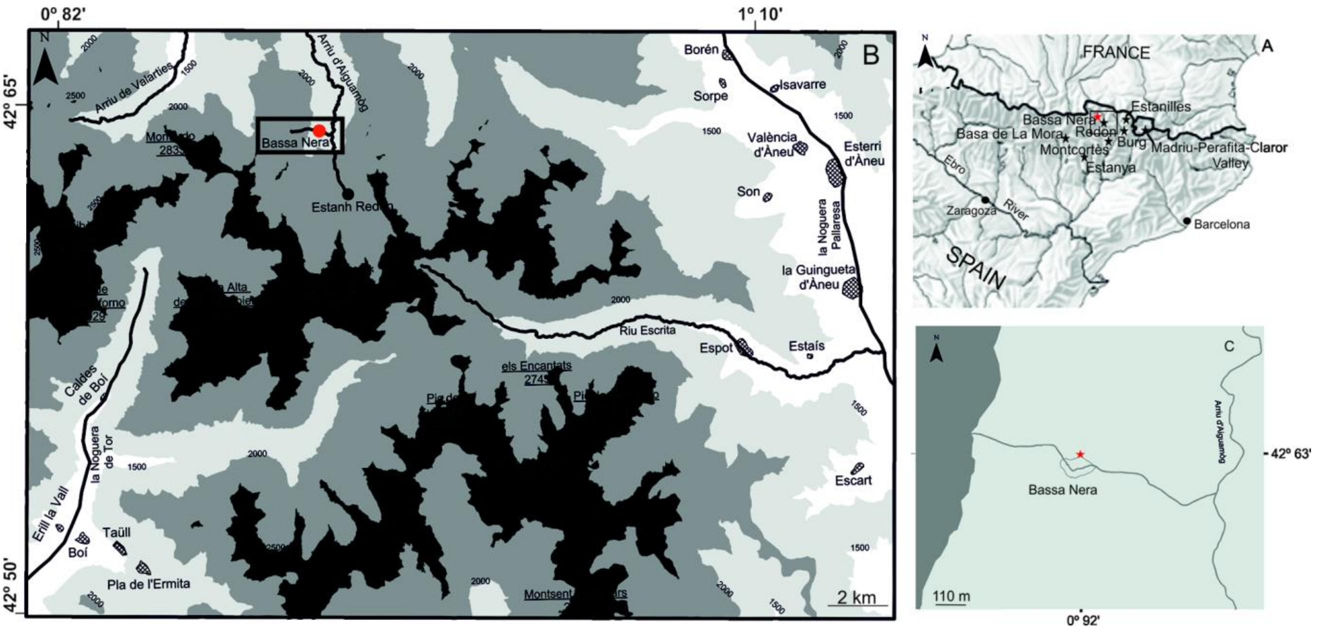

Figure 1.1. Location of the study area: (a) map indicating the relative location of Bassa Nera Pond (red point) and other palaeoenvironmental sequences mentioned in the text (black points), (b) topographic map of the terrain surrounding Bassa Nera (red point) and (c) coring site (red star).

Biogeographically, Aiguamòg Valley lies within the Boreo-Alpine and Eurosiberian zones. Cañellas-Boltà et al. (2009) and López-Vila et al. (2014) described three altitudinal vegetation belts: the montane belt (<1600 m) is composed of deciduous oak forests of *Quercus petraea* (Mattuschka) Liebl. with *Betula pendula*

Roth., riverine forests (*Alnus glutinosa* L., *Fraxinus excelsior* L. and *Salix* spp.), forests with *Tilia platyphyllos* Scop., *Prunus avium* L. and *Corylus avellana* L. and mixed forests of *B. pendula* with *Pinus sylvestris* L. The subalpine belt (1600–2250 m) is dominated by coniferous forests of *A. alba* and *R. ferrugineum* at the lowest altitudes and *Pinus mugo* ssp. *uncinata* with *R. ferrugineum* at higher altitudes. Wetlands are characterized by *Scirpus cespitosus* L. communities, assemblages of *Juncus balticus* Willd. ssp. *pyrenaeus*, *Carex rostrata* Stokes and *Caltha palustris* L. with *Epilobium palustre* L. and *Sphagnum* peat bogs. The alpine belt (>2250 m) is formed by open and sparse patches of *Nardus stricta* L. and *Festuca eskia* Ramond ex DC. meadows with *Carex* spp.

1.3. Palaeoecology of the Central Pyrenees during the Holocene

1.3.1 Climate, vegetation and anthropic influence

The Lateglacial and early Holocene period (broadly 15,000–10,000 cal yr BP) was a period of large and rapid climatic changes covering the transition from glacial to interglacial conditions. After the Younger Dryas (~11.500 cal yr BP), relatively warmer temperatures and an increase in humidity marked the onset of the Holocene in Europe (Walker, 1995). The new environmental conditions of the "Holocene climate optimum" prompted a rapid expansion of deciduous forests in southwest European mountains (Jalut et al., 2009; Pérez-Obiol et al., 2011; Vescovi et al., 2010) and the Pyrenees (Benito et al., 2008; Jalut et al., 1992; Montserrat, 1992; Reille and Lowe, 1993). Around 8.200 cal yr BP, the most sixiignificant crisis of the Holocene occurred, which was globally identified as an abrupt cold and arid event (Alley and Ágústsdóttir, 2005; Rohling and Pälike, 2005) and was recorded in the Pyrenees (González-Sampériz et al., 2006; Pérez-Sanz et al., 2013). By the Middle-Holocene, a southward shift of the North Atlantic westerly jet led to a change in precipitation seasonality (Bond et al., 2001; Pla and Catalan, 2005) and a transition from a significant Atlantic influence into a Mediterranean-influenced climate. This change prompted a drastic decline in deciduous taxa and a progressive consolidation of conifers in the Pyrenees (González-Sampériz et al., 2006; Pèlachs et al., 2011). Some aridification phases inferred from pollen occurred in the Mediterranean Iberia, established at c. 8400-7600, 5300-4200, 4300-340, 2850-1730 and 1300-750 cal yr BP (Jalut et al., 2000). Such changes in plant community composition suggest that the North Atlantic climatic variability had sufficient magnitude and duration to affect the Pyrenean ecosystems and force them to cross a threshold into a different state. However, the precise features in the response of deciduous and conifer species to climatic shifts are not yet fully understood, and their study becomes even more complicated when anthropogenic influence is taken into account. The past millennium can be considered a crucial period with significant climatic variations, and different climatic phases can be distinguished in the Northern Hemisphere (Mann et al., 2009): the relatively warm and arid Medieval Climate Anomaly (MCA), the cold Little Ice Age (LIA), with increased and irregular rainfall, and the current global warming (CGW), with an increase in temperatures caused by human activities (IPCC, 2007), starting with the Industrial Revolution (IR) (Seager et al., 2007). Reconstructing pre-industrial environmental conditions helps to discriminate anthropogenic mechanisms from natural forcings (Jansen et al., 2007) and thus to assess the extent of human impact and to provide insight into how anthropized ecosystems will respond to current climate change.

Several palaeoecological studies cover from the beginning of the Holocene to the 20th century in the Central Pyrenees. The northern slope of the Pyrenees has been intensively studied by Jalut et al. (1992) and Reille and Lowe (1993). In the southern slope, studies of Pla and Catalan (2005), González-Sampériz et al. (2006) and Pérez-Sanz et al. (2013) recorded the rapid climate variability with well-defined arid and cold events during the Holocene, highlighting an efficient translation of climate variability from the North Atlantic to the mid-latitudes. Pèlachs et al. (2011) also found a close coupling between regional climatic patterns from the North Atlantic, inferred from the ice-rafted debris index, IRD (Bond et al., 2001) and the accumulation of organic matter in a mountain wetland system in the Central Pyrenees. This evidence indicates that the Pyrenean landscape has undergone important changes during the Holocene. For example, the vegetation response to Holocene abrupt climate changes were studied through altitudinal variations in the treeline by Cunill et al (2012, 2013), who showed that the edge of the forest and montane vegetation reached higher altitudes in past and warmer times, above the current line. The role of fire was also important in the configuration of the landscape. During the Early Holocene, fires were prompted by the interaction of climate and large amounts of deciduous biomass (Gil-Romera and González-Sampériz, 2014; Pérez-Sanz et al., 2013). Shortly after, Neolithic societies occupied and exploited Central Pyrenees through fire (Cunill et al., 2013; Ejarque et al., 2010; Gassiot et al., 2014) and complex land use management (Bal et al., 2010; Pérez-Obiol et al., 2012). Phases of higher anthropogenic pressure in high mountain areas are known since the Early and Middle Neolithic, with an important threshold in the Early Bronze Age (Gassiot E, Jiménez J, 2006; Miras et al., 2007). Since then, it has been widely assumed that the configuration of high mountain landscapes has been influenced by humans (Bal et al., 2011; Cunill et al., 2013; Ejarque et al., 2010), or at least that humans have accentuated the effects of climatic constraints on vegetation during the late Holocene (Jalut et al., 2009) through mining activities, farming or cattle raising (Cunill et al., 2013; Albert Pèlachs et al., 2009). The vegetation changes during the last millennium associated with the MCA and the LIA in Central Pyrenees has been studied at centennial resolution by Cunill et al. (2013) and Pérez-Sanz et al. (2013). During that millennium, human exploitation of mountainous resources has also played a substantial effect on landscape (Ejarque et al., 2010; Miras et al., 2010; A Pèlachs et al., 2009). Over the 20th century, the Pyrenean forests showed an enhancement of tree recruitment and densification of the alpine ecotones as a response to the increase of temperature and land use abandonment (Batllori and Gutiérrez, 2008; Camarero and Gutiérrez, 2004).

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1.5. Objectives

The general objective of this study was to study the environmental changes of Bassa Nera catchment along the Holocene to the present and disentangle the climatic or anthropogenic origin. To this end, a multiproxy analysis of pollen and spores, charcoal, diatoms, tree-rings and DNA has been performed. In order to achieve this main objective, the following specific objectives have been proposed along four chapters:

Chapter 2: **Vegetation shifts, human impact and peat bog development in Bassa Nera pond (Central Pyrenees) during the last millennium**.

This part aims to disentangle the nature of ecological changes in Bassa Nera lacustrine system and its surrounding vegetation during the last millennium. As well as to develop and validate specific palaeoecological indicators that are useful for measuring potential altitudinal shifts in vegetation.

Chapter 3: **Environmental history and vegetation dynamics in response to climate variations and human pressure during the Holocene in Bassa Nera, Central Pyrenees.**

The main purpose is to reconstruct and evaluate the ecosystem response of the area to climate forcings and North Atlantic influence, describing the main arboreal dynamics at the local level as well as identifying thresholds in vegetation communities and their possible causes during the Holocene. As well as to test the response of organic matter indicators such as LOI in front of North-Atlantic regional climatic patterns (IRD), comparing the results from BSN to those obtained from other Pyrenean systems. Finally, this part aims to assess the human influence on the BSN region, determining the point at which this influence became strong enough to be detectable and how it was affected by the climatic patterns.

Chapter 4: **Pollen and tree-rings relationships along the last 700 years in the Central Pyrenees.**

This chapter aims to understand how the subalpine forests have changed in response to past climate forcings in Central Pyrenees at century scales. For this purpose, we aimed to find the relationship between pollen and Mountain pine tree-ring proxies that describes the forest dynamics along the last 700 years. As well as to quantify how climate change can affect trees growth at local level, studying the climate-tree growth relationships at different temporal scales.

Chapter 5. **Genetic characterization of modern and past communities of a high mountain peat bog system using eukaryotic metabarcoding.**

This study focuses on establishing occurrence and abundance baselines for a wide array of taxa, necessary for characterising the current diversity of peat bogs and for detecting and monitoring future changes in these communities. In order to apply new markers, we desired to check the suitability of COI and 18S markers to assess taxonomic and ecological eukaryotic diversity in peat bog communities. Finally, we compared the knowledge about past communities inferred from ancient DNA with the palaeoenvironmental reconstructions based on morphological methods.

Chapter 2

Vegetation shifts, human impact and peat bog development in Bassa Nera pond (Central Pyrenees) during the last millennium.

Original publication (*Appendix 2 in the supplementary material*):

Garcés-Pastor, S., Cañellas-Boltà, N., Clavaguera, A., Calero, M. A., and Vegas-Vilarrúbia, T. 2017. Vegetation shifts, human impact and peat bog development in Bassa Nera pond (Central Pyrenees) during the last millennium. *The Holocene*, *27* (4), 553-565.

Abstract

High-mountain lakes are suitable ecosystems for studying local environmental shifts driven by large-scale climate changes, with potential applications to predict future scenarios. The precise features in the response of species assemblages are not fully understood, and human pressure may often hide climatic signals. To investigate the origin and impact of past environmental changes in high-mountain ecosystems and apply this palaeoecological knowledge to anticipate future changes, we performed a multi-proxy study of a sediment core from Bassa Nera, a pond located close to montane–subalpine ecotone in the southern central Pyrenees. Combining pollen and diatom analysis at multidecadal resolution, we inferred vegetation shifts and peat bog development during the past millennium. We introduced a montane pollen ratio as a new palaeoecological indicator of altitudinal shifts in vegetation. Our results emphasize the sensitivity of the montane ratio to detect upward migrations of deciduous forest and the presence of the montane belt close to Bassa Nera pond during the Medieval Climate Anomaly. Changes in aquatic taxa allowed to date the onset of the surrounding peat bog which appeared and infilled the coring site around AD 1565. Overall, our results suggest a low-intensity human pressure and changes in management of natural resources during the last millennium, where farming was the main activity from the Medieval Climate Anomaly until AD 1500. Afterwards, people turned to highland livestock raising coinciding with the 'Little Ice Age'.

Keywords: diatoms, montane–subalpine shifts, palaeoecological indicators, peat bog, pollen, Pyrenees

2.1 Introduction

Ecosystem reconstructions for the past millennium are crucial for understanding past environmental variability and predicting future changes. During this interval, different climatic phases are distinguished in the Northern Hemisphere (Mann et al., 2009): the relatively warm and arid Medieval Climate Anomaly (MCA), the cold Little Ice Age (LIA) with increased irregular rainfall and the current global warming (CGW), with an increase in temperatures caused by human activities (IPCC, 2007), starting with the Industrial Revolution (IR) (Seager et al., 2007). However, due to large spatial and temporal heterogeneities, the precise features of climatic variability during these periods and the responses of the ecosystems to this variability at regional or local levels are not yet fully understood. In many cases, strong human pressure greatly influenced the ecosystems, hiding climatic signals even when cli-mate was the dominant driver (Bal et al., 2011). Reconstructing pre-industrial environmental conditions helps to discriminate anthropogenic mechanisms from natural forcings (Jansen et al., 2007) and thus to assess human impact and to predict how anthropized ecosystems will respond to current climate change. Lakes located in high-mountain ranges in temperate mid-latitude areas are especially suitable for assessing the potential consequences of climate fluctuations on mountain biota because they will most likely be among the first ecosystems to exhibit a response to current climate changes (Engler et al., 2011). Gottfried et al. (2012) have reported elevated replacement rates of cold-adapted plants by thermophilic species in several European mountain regions due to the current changes in the climate, and Thuiller et al. (2005) predicted high rates of vegetal species loss in the same areas. In this context, the Pyrenees are an interesting region to study, as the Mediterranean area is one of the most vulnerable regions on Earth to the CGW (Christensen et al., 2007). Several studies have furnished evidence that the Pyrenees have acted as a glacial refuge for forest species during past climate changes (Benito et al., 2008) and might contribute to buffer the effects of climate variability in the future (Alba-Sánchez et al., 2010). Although most of the currently described changes in the Pyrenees are related to land use (Améztegui et al., 2010), some Pyrenean forests have already experienced an enhancement of tree recruitment and growth during warm periods of the last century

(Camarero et al., 2006; Camarero and Gutiérrez, 2004). The palaeoecological history of the northern Pyrenean slope throughout the Holocene has been intensively studied (e.g. Jalut et al., 1992; Reille and Lowe, 1993). Working on the southern slope of the Central Pyrenees, González-Sampériz et al. (2006) emphasized the occurrence of abrupt climate changes during the Holocene and the response of vegetation and lake systems to such changes, implying an efficient translation of climate variability from the North Atlantic to the mid-latitudes. Using a centennial resolution, Cunill et al. (2013) and Pérez-Sanz et al. (2013) were able to describe vegetation changes associated with the MCA and the 'LIA' in the same area. During the last millennium, human exploitation of mountainous resources has also played a substantial effect on landscape (Ejarque et al., 2010; Miras et al., 2010; Pèlachs et al., 2009).

The main purpose of our work is to integrate palaeoecological research from the Pyrenees into the ongoing efforts to estimate the future ecosystem dynamics of European high-mountain environments in the face of global warming by trying to unravel human from climatic influences. The site of our case study is located in the Central Pyrenees of Catalonia, where future scenarios predict a 5°C increase in temperature by 2100 (A2, IPCC, 2007), while precipitation will decrease and extreme hydric events will be more frequent (Barrera-Escoda and Cunillera, 2011; Brunet et al., 2009; López-Moreno and Beniston, 2009).

With this aim, we present a multi-proxy study combining high resolution (multidecadal) of palaeobotanical (pollen, stomata) and palaeolimnological data (diatoms, freshwater sponges and chrysophyte cysts) to disentangle the nature of ecological changes in a lacustrine system and its surrounding vegetation during the last millennium. We also aim to develop and validate specific palaeoecological indicators that are useful for measuring potential altitudinal shifts in vegetation. Bassa Nera Pond is a location well suited for this purpose in view of its relative proximity to the montane–subalpine ecotonal boundary, a feature that is highly sensitive to altitudinal vegetation shifts in response to climate-driven changes (Luckman and Kearney, 1986). Moreover, the Bassa Nera is located in Aiguamòg Valley, where potential indicator taxa have been described (Cañellas-Boltà et al., 2009; López-Vila et al., 2014), providing information essential for properly interpreting palaeoclimatic and palaeoecological records.

2.1.1 Study area

The Bassa Nera (42°38′18.5″N, 0°55′27.6″E, 1891 m) is a small lacustrine system located in the peripheral zone of Aigüestortes i Estany de Sant Maurici National Park. It is situated in the Aiguamòg Valley (Aran Valley), on the Northern slope of the Central Pyrenees (Figure 2.1). Nowadays, this pond is surrounded by a complex of marshes and a peat bog formed by *Sphagnum* spp. and *Carex lasiocarpa* Her., with abundant *Molinia caerulea* (L). Moench, *Drosera longifolia* L., *Menyanthes trifoliata* L. and *Parnassia palustris* L (Carrillo et al., 2008). A conifer forest of *Pinus mugo* ssp. *uncinata* (Ramon) Domin. and *Abies alba* Mill. with *Rhododendron ferrugineum* L. in the understory and some Poaceae meadows surrounds the catchment. The pond has an area of 2.01 ha and 5 m maximum depth, with a small outlet that drains into the Garonne River. The water receives mineral salts only from precipitation and runoff. The pond's watershed bedrock is composed of Carboniferous–Permian granite rocks (Roca i Adrover et al., 2010). The annual average precipitation reaches 1152 mm and is evenly distributed over the seasons. The mean annual temperature is 4.25°C, with January being the coldest month (−3°C on average) and July the warmest month (14°C on average) (Ninyerola et al., 2003).

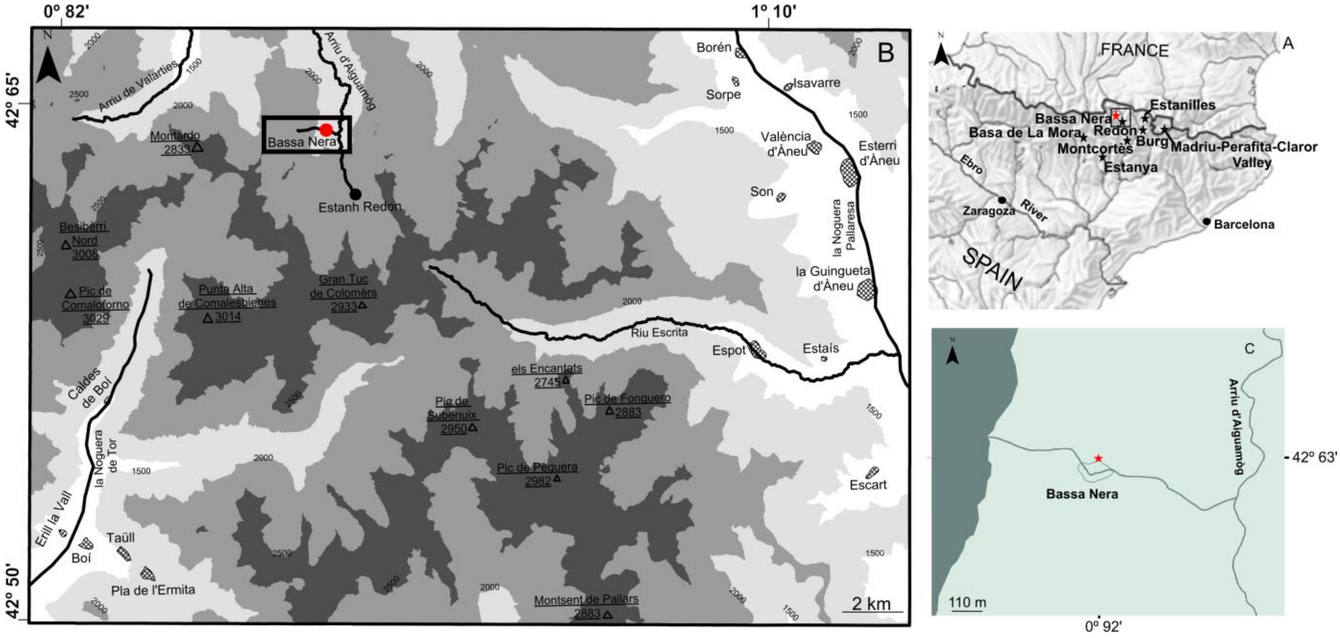


Figure 2.1. Location of the study area: (a) map indicating the relative location of Bassa Nera Pond (red point) and other palaeoenvironmental sequences mentioned in the text (black points), (b) topographic map of the terrain surrounding Bassa Nera (red point) and (c) coring site (red star).

Biogeographically, Aiguamòg Valley lies within the Boreo-Alpine and Eurosiberian zones. Cañellas-Boltà et al. (2009) and López-Vila et al. (2014) described three altitudinal vegetation belts: the montane belt (<1600 m) is composed of deciduous oak forests of *Quercus petraea* (Mattuschka) Liebl. with *Betula pendula* Roth., riverine forests (*Alnus glutinosa* L., *Fraxinus excelsior* L. and *Salix* spp.), forests with *Tilia platyphyllos* Scop., *Prunus avium* L. and *Corylus avellana* L. and mixed forests of *B. pendula* with *Pinus sylvestris* L. The subalpine belt (1600–2250 m) is dominated by coniferous forests of *A. alba* and *R. ferrugineum* at the lowest altitudes and *Pinus mugo* ssp. *uncinata* with *R. ferrugineum* at higher altitudes. Wetlands are characterized by *Scirpus cespitosus* L. communities, assemblages of *Juncus balticus* Willd. ssp. *pyrenaeus*, *Carex rostrata* Stokes and *Caltha palustris* L. with *Epilobium palustre* L. and *Sphagnum* peat bogs. The alpine belt (>2250 m) is formed by the open and sparse vegetation of *Nardus stricta* L. and *Festuca eskia* Ramond ex DC. meadows with *Carex* spp. The main anthropogenic activities until the creation of the National Park in 1955 and the demarcation of a peripheral protection area by 1990 were extensive cattle husbandry, forest exploitation and hydroelectric power generation. Tourism has displaced those activities, although pasturing and hydroelectric exploitation are still authorized.

2.2 Methods

2.2.1 Coring, sampling, dating and sedimentology

A sediment core 706 cm long (PATAM12) was collected in 2007 using a 'Russian' corer (Jowsey, 1966) on the peat bog that surrounds Bassa Nera and was sliced every 3–5 cm. This study is focused on the uppermost 330 cm. In total, 10 radiocarbon dates (Table 2.1) were obtained from wood and seed macroremains along the entire core by the accelerator mass spectrometry (AMS) method at the Beta Analytic Radiocarbon Dating laboratory (Miami, FL, USA) or Keck Carbon Cycle AMS Laboratory (Irvine, CA, USA). Seven radiocarbon dates fall within the interval analysed here. Ages were calibrated with the IntCal13.14C curve (Reimer et al., 2013), and the age–depth model was obtained by using smoothing spline interpolation in R package Clam 2.2 (Blaauw, 2010). The sedimentological description was performed following Schnurrenberger et al. (2003).

Table 2.1. Results of 14C radiocarbon dates for different depths at the Bassa Nera. Sample Beta-247298 was excluded of the age–depth model as stratigraphically incongruent (marked with an asterisk (*)).

Depth (cm)	Laboratory code	Dated material	AMS ^{14}C years BP
97.5	Beta-247296	Wood	220±40
127.5	Beta-251879	Wood	190±40
192.5	Beta-247297	Wood	270±40
222.5	Beta-251880	Wood	490±40
261.5 *	Beta-247298	Wood	250±40
304.5	Beta-251881	Wood	880±40
428.5	Beta-247300	Wood	2380±40
517	Beta-247301	Seeds	3570±40
604.5	Beta-251883	Wood	4530±40
698.5	UCI-43704	Wood	6410±20

2.2.2 Pollen analysis

A total of 51 samples were processed at the Catalan Institute of Human Paleoecology and Social Evolution, using standard palynological methods (Moore et al., 1991) with NaOH, HCl, HF and mineral separation in Thoulet solution (density 2.0 g/cm3). Microscopic slides were mounted in glycerine. Pollen grains were counted until diversity saturation (Rull, 1987) and identified according to Faegri et al. (1989), Reille (1992) and the reference pollen collection of IBB-CSIC. Given that most slides had superabundant Pinus, which could conceal the vegetation dynamics, counts were increased to obtain a representative sample (200–481 pollen grains without *Pinus*). Data are presented as a percentage of the pollen sum, excluding *Pinus*, Cyperaceae, aquatic plant pollen and spores. Diagrams were plotted using Psimpoll 4.27 software (Bennett, 2002), and statistically significant zones were based on changes in percentages of taxa showing abundances >1%. The method of Optimal Splitting by Information Content (Bennett, 1996) was used for this purpose. Stomata, *Botryococcus* algae and sedimentary charred particles (<100 µm; 100–500 µm) on the same pollen slides were also counted.

A new montane pollen ratio was calculated to infer past altitudinal variations in the montane–subalpine belt in the study site (see supplementary data, available online). To obtain this montane ratio, we used several pollen indicator types identified in the area by Cañellas-Boltà et al. (2009). Montane pollen types included *Alnus*, *Betula*, *Buxus*, *Corylus*, *Fraxinus*, deciduous *Quercus*, *Tilia* and *Salix*, while subalpine–alpine indicators included Asteraceae, *Calluna*, *Campanula*, Ericaceae, *Plantago* and Poaceae. These genera were selected as indicators because the local occurrence and abundance of both pollen and parent taxa show the same or similar altitudinal patterns. The percentages of the montane pollen were summed and divided by the sum of the percentages of subalpine pollen. This ratio was proved with 33 surface moss samples from the altitudinal transect studied by Cañellas-Boltà et al. (2009) in the Aiguamòg Valley. The usefulness of this ratio for discriminating both vegetation belts was assessed by calculating its values and their 95% confidence intervals for the 33 moss samples from the altitudinal transect studied by Cañellas-Boltà et al. (2009). According to the modern analogues, values of 2.5 indicate the close presence of the montane belt, while higher values imply the upward montane migration of the latter within Bassa Nera basin.

2.2.3 Diatom analysis

A total of 0.1 g of dry sediment from 35 samples was treated with H_2O_2, and the frustules were mounted in Naphrax (R.I. = 1.7). Valve concentrations (valves g sediment−1) were estimated through the addition of a known number of latex microspheres (Battarbee, 1986). A minimum of 500 valves per sample were counted with a Polyvar light microscope at 1000× magnification and identified at the lowest taxonomic level according to Krammer and Lange-Bertalot (1999a, 1999b, 2004a, 2004b), Cejudo-Figueiras et al. (2011), Buczkó et al. (2010), Bey and Ector (2013) and Morales (2005). Observed chrysophycean stomatocysts and sponge spicules were also counted. The centric-to-pennate (Ce/Pe) ratio was calculated as an indicator of the relative abundance of planktonic to benthic habitat availability. The diatom dissolution index (DDI) was computed as the percentage of valves showing dissolution and/or breakage signals. The Shannon–Wiener diversity index (H′) was used

(Shannon, 1963). The planktonic-to-fragilarioid (P/F) species ratio was calculated as an indicator of the duration and extent of ice cover (Douglas and Smol, 1999; Lotter and Bigler, 2000). The chrysophytes-to-diatoms (Cr/Di) ratio was calculated as a means for estimating trends in algal succession, nutrient content and the length of the growing season (Smol, 1985). Diatom diagrams were plotted using Psimpoll 4.27 software (Bennett, 2002), and zonation was based on changes in percentages of taxa showing abundances >3% according to the method of Optimal Splitting by Information Content (Bennett, 1996).

2.3 Results

2.3.1 Chronology and sedimentology

Seven radiocarbon dates were used to construct the age–depth model that covers the last 2500 cal. yr BP (Table 2.1; Figure 2.2). One date (Beta-247298) was rejected as stratigraphically incongruent, probably because the roots might have dragged down the wood macrorest where dating was performed. The x-ray radiograph of a core retrieved by other researchers working close to PATAM12 reveals that the depth of extracting our macrorest coincides with a root-rich level (Pelachs, 2015, personal communication), reinforcing this rejection. According to the age–depth model, the entire core extends from ca. 7464 cal. yr BP to the present, with an average confidence interval error of ca. 150 years. The age– depth pattern for the last millennium discussed here showed a sedimentation rate of 0.36 ± 0.15 cm yr^{-1} (mean ± SD) from 0.09 to 0.7 cm yr^{-1}, with an average interval of 14–22 years between samples (Figure 2.2). Three sedimentary facies have been described (Figures 2.3 and 2.4): (1) massive brown sandy silt with medium to large granulometry and scarce vegetal organic matter (0–50 cm); (2) massive brown-red clay and abundant vegetal organic matter (50–195 cm); and (3) massive brown-dark clay with abundant vegetal organic matter (195–330 cm).

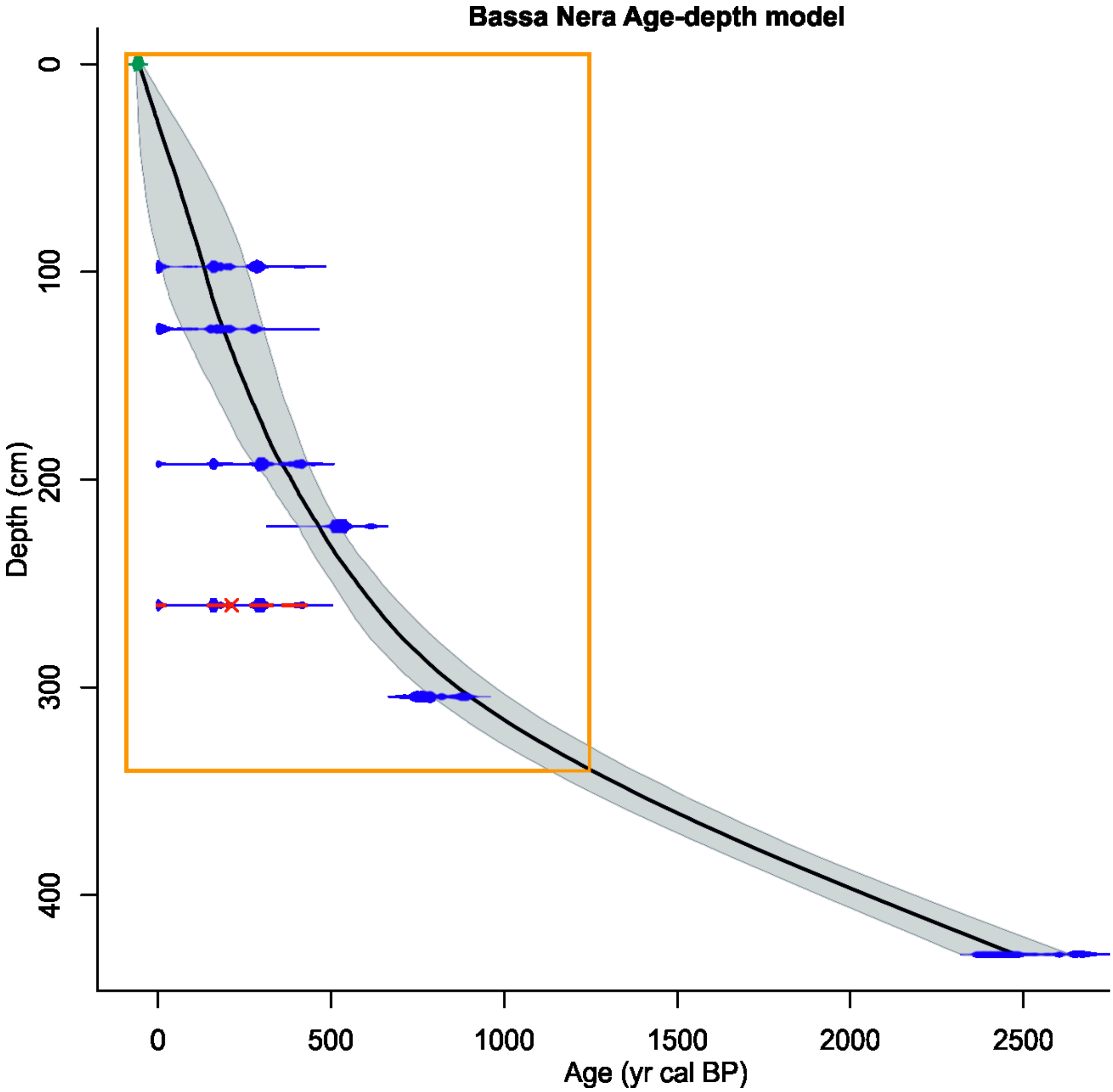


Figure 2.2. Age–depth model for the last 2500 years based on radiocarbon dating of Bassa Nera pond and performed with Clam 2.2 software. The box marks the studied period, the last 1000 years. Sample in red was considered as outlier.

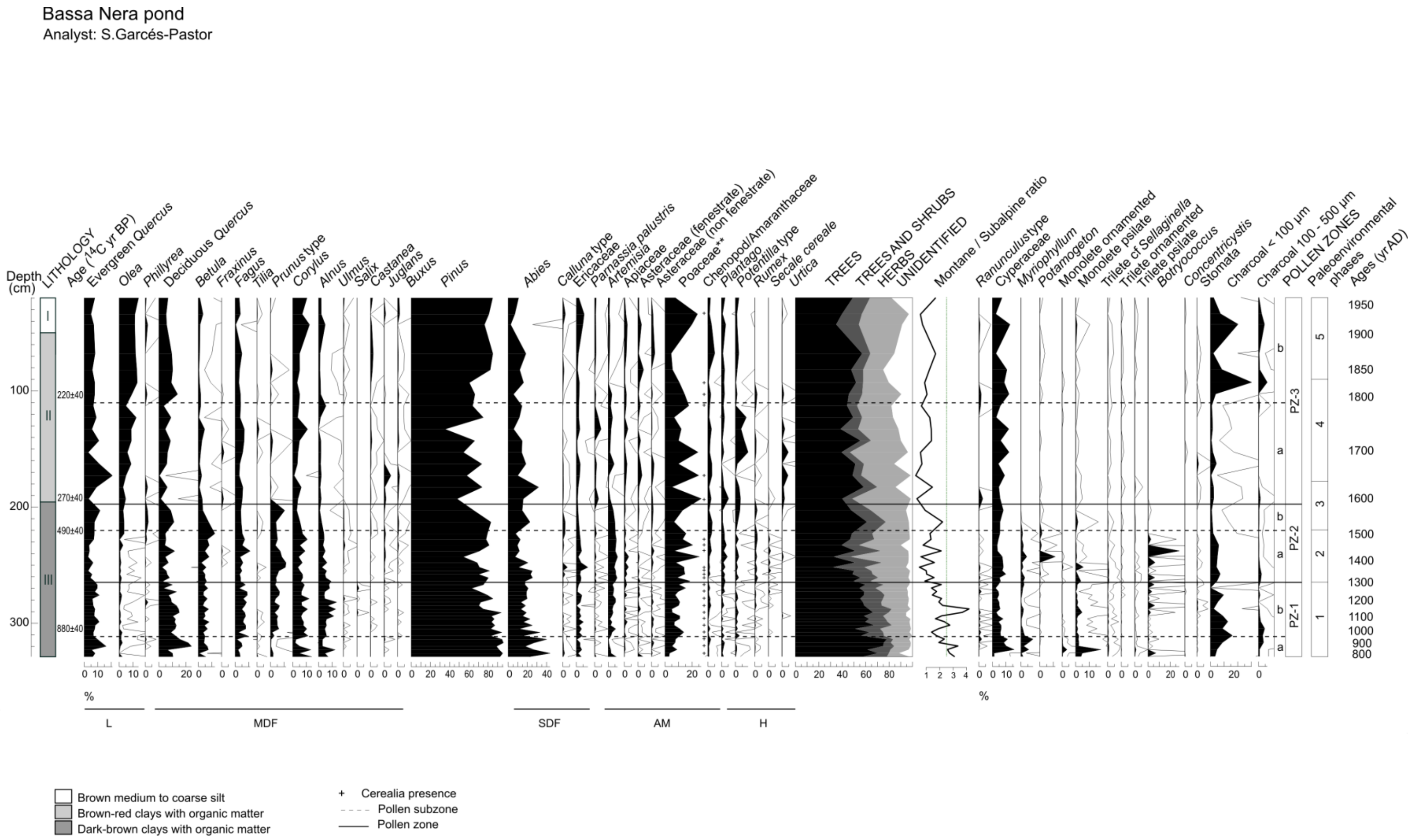


Figure 2.3. Percentage diagram of sporomorph, including the total pollen (relative abundance >1%), charcoal percentages and M/A ratio. *Pinus* pollen, wetland plants and fern spores were excluded from the pollen sum (ΣP). *Pinus* percentage was calculated with the pollen sum plus *Pinus* pollen vegetal associations: lowland (L), montane deciduous forest (MDF), subalpine deciduous forest (SDF), alpine meadows (AM), and human related taxa (H). The continuous horizontal lines correspond to statistically significant zones (Bennett, 1996) and the dotted lines correspond to subzones. Percentages of the elements out of the pollen sum were calculated by dividing them by the pollen sum. Palaeonvironmental phases (see the 'Discussion' section) are indicated at the right side of the diagram. Poaceae** shows the combined frequencies of Poaceae and Cerealia-t pollen.

2.3.2 Pollen and charcoal record

The pollen diagram (Figure 2.3) is dominated by arboreal taxa, mainly *Pinus* and *Abies*, with a notable abundance of deciduous taxa. Several anthropogenic taxa and various Mediterranean species are present along the whole sequence, increasing from 180 cm to the top. Three pollen zones (PZ-1–PZ-3) have been identified.

PZ-1 (330–265 cm, 22 samples). The PZ-1 zone is characterized by the maximum values of coniferous taxa (*Pinus* and *Abies*), with notable abundances of some deciduous taxa (deciduous *Quercus*, *Alnus*, *Betula* and *Fagus*). Shrub elements such as *Corylus*, *Prunus* and Ericaceae are well represented, while herbs show low percentages. This zone shows the highest montane pollen ratio of the studied sequence, with values of approximately 2.75. Charcoals are particularly abundant. Two subzones can be distinguished. The subzone PZ-1a has a clear *Abies* dominance (45%), with a high amount of deciduous Quercus and large percentages of Cyperaceae, *Myriophyllum* and ferns. In contrast, subzone PZ-1b is characterized by a decrease in conifers and deciduous *Quercus* and a marked rise of *Prunus*, *Artemisia* and Poaceae. Cyperaceae sharply decrease from 10% to 2%, while Myriophyllum and ferns are significantly reduced. In addition, *Botryococcus* rises by the middle of the subzone, coinciding with the appearance of conifer stomata and the decline of charcoal.

PZ-2 (265–197.5 cm, 13 samples). This zone is distinguished by a drop of arboreal pollen from 60% to 35%, mainly caused by the decrease in conifers (*Abies*, *Pinus*). Deciduous taxa decline slightly, but a minor increase in some shrub and herbaceous elements (*Prunus*, Poaceae, *Rumex*, *Artemisia*, Apiaceae and *Plantago*) is observed. *Myriophyllum* dominates the aquatic taxa. *Potamogeton* shows a remarkable increase; this increase is followed by a *Botryococcus* peak. The montane ratio displays short periodic fluctuations ranging from 0.5 to 2.2. Charcoal notably diminishes by the top of this zone, coinciding with the disappearance of stomata. Two subzones can be distinguished according to cereal presence and changes in the wetland and aquatic communities. Subzone PZ-2a is characterized by high abundance of *Secale cereale*, Cerealia-t and wetland and aquatic plants (Cyperaceae, Ranunculaceae, *Myriophyllum* and *Potamogeton*). *Botryococcus* has the highest values of the sequence. The onset of subzone PZ-2b is marked by the disappearance of

Cerealia-t and *S. cereale* as well as by a progressive decrease in Artemisia and montane taxa (*Alnus*, *Corylus* and *Betula*). In contrast, *Olea* increases markedly. Cyperaceae remain abundant, but the other aquatic plants virtually disappear. *Botryococcus* also declines. Charcoal decreases and stomata are absent.

PZ-3 (197.5–0 cm, 16 samples). The PZ-3 zone shows a notable decrease in *Alnus*, *Betula* and *Prunus*. Although *Artemisia* diminishes, the herbaceous taxa spread. *Olea* reaches its maximum values, and there is a rise in broken and damaged pollen by the middle of the zone. Cyperaceae have the same low frequencies as in PZ-2b. *Potamogeton* becomes the only aquatic macrophyte, with very low abundances, while *Botryococcus* disappears. The montane ratio continues with oscillations ranging from 0.2 to 1.7 and charcoal increase by the middle of this zone. Two subzones can be differentiated. The PZ-3a subzone is characterized by an increase in human-related taxa (*Urtica* and *Potentilla*) and *Parnassia*. In contrast, *Abies* shows a slight decreasing trend. Poaceae follow an oscillating pattern ranging from 25% to 5%. The evergreen *Quercus* peaks, and *Olea* spreads. Additionally, charcoal is scarce. The PZ-3b subzone is marked by an abrupt decrease in *Potentilla*, *Urtica* and *Parnassia*, while *Olea* reaches its maximum values. Cyperaceae and charcoal increase markedly, with two charcoal peaks at 50 and 90 cm. At the top of the subzone, Ericaceae and *Corylus* increase.

2.3.3 Diatom record

Diatom assemblages are composed of 240 taxa distributed among 52 genera. A substantial portion of these taxa are benthic (Figure 2.4). The most significant features in the diatom stratigraphy are the high percentages of small Fragilarioid taxa, with only five tychoplanktonic species showing abundances >10% at any given time along the sequence (*Achnanthidium minutissimum* (Kützing) Czarnecki, Staurosirella pinnata (Ehrenberg) Williams and Round, *Staurosira construens* var. *venter* (Ehrenberg) Hamilton, *Pseudostaurosira alvareziae* (Cejudo-Figueiras, Morales and Ector) and *Stauroforma exiguiformis* (Lange-Bertalot) Flower, Jones and Round). According to changes in the diatom assemblage, three diatom zones are differentiated for the last millennium, which can be divided into subzones.

DZ-1 (330–207.5 cm, 18 samples). In this zone, diatoms reach their maximum concentrations in the record, showing periodic fluctuations from 1.61 to 2.54 × 109 valves g sed-1. The Ce/Pe and P/F ratios also present their highest values in the record. H′ ranges from 2.8 to 3.7, and the DDI indicates that diatom frustules are well preserved. Assemblages are dominated by the periphytic *S. construens* var. *venter*, *A. minutissimum* and *S. pinnata*, while the abundances of *Encyonopsis subminuta* Krammer and Reichardt, *Nitzschia fonticola* Grunow in Cleve and Möller and Brachysira procera Lange-Bertalot and Moser fluctuate. Minor diatom assemblages formed by *Kobayasiella* sp., *Psammothidium subatomoides* (Hustedt) Bukhtiyarova and Round and *Sellaphora* cf. *radiosa* are found exclusively in this zone. *Aulacoseira nivaloides* (Camburn) English and *Potapova* and *Aulacoseira valida* (Grunow in Van Heurck) Krammer are abundant in the planktonic assemblage. Two subzones can be differentiated. The DZ-1a subzone is dominated by *S. pinnata*, *A. minutissimum* and *S. venter*. In the DZ-1b subzone, *S. pinnata* decreases and *S. venter* shows a dramatic drop at 252 cm. However, *P. alvareziae* and *Staurosirella oldenburgiana* (Hustedt) E. Morales show an increasing trend towards the top of this subzone. The species that characterize this zone prefer habitats with low nutrient and alkaline water bodies (Van Dam et al., 1994). The Cr/Di ratio is low. The presence of spicules of the sponge *Ephydatia muelleri* (Lieberkühn) (Økland and Økland, 1996) is noteworthy except between 222.5 and 152.5 cm.

DZ-2 (207.5–177.5 cm, three samples). At the transition between the DZ-1 and DZ-2 zones, the diatom concentration decreases drastically to 9.43 × 107 valves g sed-1 and recovers shortly thereafter. The Ce/Pe and P/F ratios decrease, and H′ decreases markedly at 192.5 cm. This zone is characterized by the prominent peak of the freshwater species *P. alvareziae*, which becomes the dominant species, reaching 70% abundance at 192.5 cm. The previously dominant species *S. pinnata* declines to 5%. Furthermore, S. venter and the epiphytic *A. minutissimum* and *E. subminuta* virtually disappear for the first time in the entire record while *S. oldenburgiana* remains constant. In contrast, *Eunotia arcus* Ehrenberg, *S. exiguiformis* and the acidophilus *Tabellaria flocculosa* (Roth) Kützing increase slightly, peaking at approximately 203 cm. In the planktonic assemblage, *A. nivaloides* and *A. valida* are replaced by the acidophilus *Aulacoseira tethera* Haworth (10%). This latter taxon has

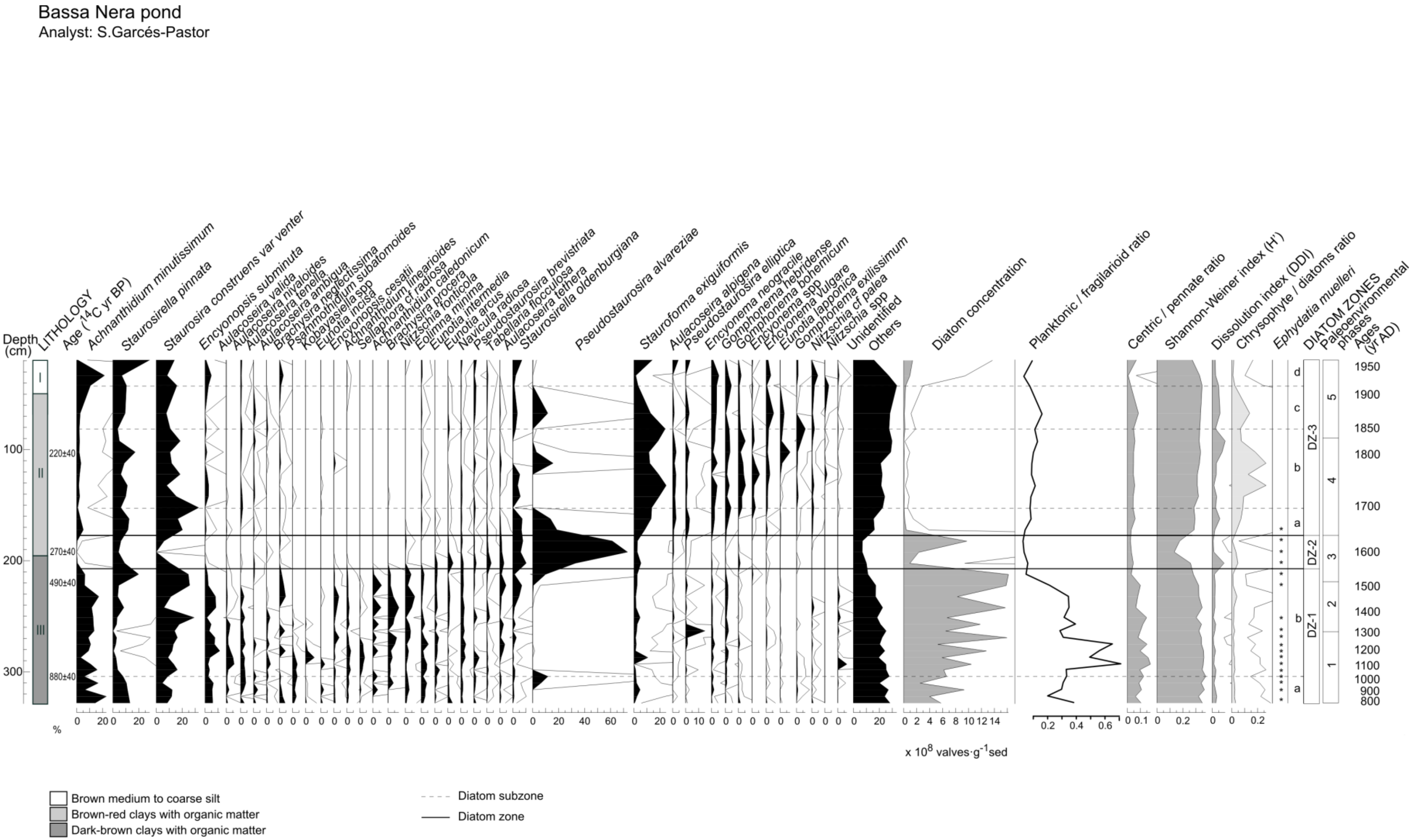


Figure 2.4. Relative abundances (>3%) of diatom taxa throughout the Bassa Nera record. The continuous horizontal lines delimitate three statistically significant zones (Bennett, 1996) and the dotted lines arbitrarily defined subzones. Diatoms are arranged in order of appearance.

been identified according to Krammer and Lange-Bertalot (2004a) and Bey and Ector (2013). The density and width of striae together with the rows of areolae and the dimensions of the cells seem to indicate that the observed species is indeed *Aulacoseira*, although it could be close to the morphotype described in Buczkó et al. (2010). The diatom assemblages of the DZ-2 zone are commonly present in habitats with a low-to-medium electrolyte content, circumneutral waters and moist places (Van Dam et al., 1994). Even the Cr/Di ratio remains low, reaching 0.048 at 202.5 cm. Meanwhile, *E. muelleri* spicules are present throughout the entire zone.

DZ-3 (177.5–0 cm, 14 samples). This zone is marked by the abrupt drop of the diatom concentration to 1.81 · 106 valves g·sed–1. The Ce/Pe value slightly increases, while H′ oscillates approximately 3.2 bits and decreases towards the top of the zone. The DDI reaches its highest values found in the record by the middle of the zone, and the P/F ratio remains low. *P. alvareziae* decreases significantly and is substituted by *S. exiguiformis* (25%), which peaks at 81.5 and 152.5 cm. *S. pinnata* and *S. venter* recover to their former abundances (DZ-1 zone). Indeed, *S. venter* has a prominent peak at 152.5 cm, reaching 30%. Further minor diatom assemblages formed by *Gomphonema bohemicum* Hustedt, *Gomphonema exilissimum* (Grunow) Lange-Bertalot and E. Reichardt, *Encyonema vulgare* Krammer, *Encyonema neogracile* Krammer and *Nitzschia* sp. show slight peaks. Four subzones can be identified. The DZ-3a subzone is characterized by a decrease in *P. alvareziae* and *S. oldenburgiana* and an increase in *S. venter*. Planktonic *A. tethera* decreases, to be replaced by *Aulacoseira alpigena* (Grunow) Krammer. The DZ-3b subzone is marked by two peaks of *S. exiguiformis*. At 122.5 cm, *P. alvareziae* reappears, with a peak that coincides with decreases in *S. exiguiformis* and *S. venter*. The DZ-3c subzone is distinguished by a decreasing trend of *S. exiguiformis* and a peak of *P. alvareziae, A. minutissimum, S. pinnata* and *S. venter*, while *S. oldenburgiana* shows an increasing tendency. The DZ-3d subzone is characterized by a peak of *A. minutissimum* at 33.5 cm, coinciding with a minimum of *S. pinnata*, *S. venter* and *S. exiguiformis*. At the end of this subzone, *S. pinnata, S. venter, S. oldenburgiana, S. exiguiformis, P. elliptica* and *A. alpigena* increase until the present. DZ-3 species are usually found in oligotrophic habitats with alkaline waters in freshwater bodies (Van Dam et al., 1994). *E. muelleri* disappears at 162.5 cm; however, chrysophytes increase, peaking at 132.5 and 112.5 cm.

2.4. Discussion

2.4.1 Vegetation and palaeoenvironmental reconstruction

Our reconstruction of the past environment and vegetation dynamics in the Bassa Nera catchment is based on pollen and diatom zonations (Figure 2.5). Our results suggest that aquatic habitats progressively shrank through time due to changes in hydrological conditions. In the record, temperature changes have led to shifts in vegetation ecotones. The study of the montane ratio in this highland ecosystem has helped to detect these replacements. The discussion will be structured into five main phases according to lacustrine, vegetation and montane ratio changes (Figures 2.3 and 2.4). Figure 6 shows the ecosystem dynamics and anthropogenic pressure on regional sequences located in the Central Pyrenees and pre-Pyrenees.

Phase I. 330–265 cm, AD 801–1297. The pollen record suggests that the surroundings of Bassa Nera were dominated by montane forest (deciduous *Quercus, Betula, Corylus* and *Prunus*) mixed with *Pinus*, *Abies* and Ericaceae. The conifer stomata demonstrate the close proximity of these taxa to the pond. The highest montane ratio values, which are close to the ones observed in the present montane belt samples, indicate the proximity of the montane vegetation. This upward shift of the montane boundary with respect to the current location suggests warmer conditions. In contrast, the increase in *Artemisia* and Poaceae after the charcoal peak points to forest clearance at approximately AD 1000. The *S. cereale* indicates the presence of local and regional crops by AD 1150. A change in water level occurred approximately AD 990, inferred from higher planktonic diatom percentages and the large decrease in *Myriophyllum* and Cyperaceae, usually associated with lake margins and shallow waters (0.4–4 m deep) (Grosjean et al., 2001). Some periphytic diatom species that live attached to this littoral vegetation, such as *S. pinnata*, also declined. The oscillations of diatom concentration, Ce/Pe and P/F might be related to periods of strong seasonality and hydric fluctuations. This environmental instability is also evidenced by the presence of tychoplanktonic and opportunistic small Fragilarioids (*S. pinnata, S. venter, P. alvareziae*) and *A. minutissimum*, which has been related to periods of increased mixing or turbidity (Axford et al., 2009; Corella et al., 2011; Scussolini et al., 2011). This phase is set in the context of the MCA (9th–14th centuries), characterized by an increment in temperatures and by relatively arid conditions in south-western Europe (Mann et al., 2009; Seager et al., 2007). The Iberian Peninsula (IP) experienced generally drier conditions during this period (Moreno et al., 2012). Indeed, some lakes of the Pyrenees such as Basa de la Mora and Burg (Figure 2.6) lowered their lake levels, with strong seasonality marked by higher summer/autumn temperatures (Catalan et al., 2009) and lower winter/spring temperatures (Pla and Catalan, 2005). Other nearby pre-Pyrenean lakes also showed a negative hydric balance (Catalan et al., 2009; Morellón et al., 2011a).

Phase II. 265–220 cm, AD 1297–1493. Between AD 1297 and 1493, the decrease in montane ratio suggests a downward shift of the montane vegetation belt. In regard to this finding, the pollen diagram shows a decrease in deciduous *Quercus* and *Alnus*. However, the high percentages of *Betula*, *Corylus* and *Prunus* indicate relatively close montane vegetation or mixed forest. This montane downward shift and the landscape opening, prompted by the increase in Poaceae and other herbs, suggest colder conditions during this period, which correspond to the MCA–LIA transition. In contrast, the maximum of *Artemisia*, together with an increase in some human-related taxa (Cerealia-t, *Plantago*, *Potentilla* and *S. cereale*) and small charcoal, evidence the use of regional fires for crops and grazing, as has been observed in nearby regions (Ejarque et al., 2010; Pèlachs et al., 2009). The small decrease in the Ce/Pe ratio jointly with the onset of *Potamogeton* and higher values of *Myriophyllum* suggests that the pond had relatively shallower water and poor nutrient status (Bornette and Puijalon, 2011). The decrease in P/F (Lotter and Bigler, 2000) and the increase in *S. oldenburgiana* might also be indicative of lower temperatures (Finkelstein and Gajewski, 2008). The decrease in *S. venter* and *A. minutissimum* could imply a substantial period of ice cover (Smol, 1988). Thus, the extreme fluctuations of diatom concentrations and the decrease in planktonic frequencies might be associated with hydrological fluctuations, which have also been observed in karstic Montcortès (1027 m a.s.l.) and Estanya Lakes (670 m a.s.l.) (Figure 2.1), between AD 1400 and 1460 (Morellón et al., 2009; Scussolini et al., 2011). In contrast, Bassa de la Mora lake presented higher lake levels (Pérez-Sanz et al., 2013) (Figure 2.6). Thus, unstable, cold and humid conditions were inferred for the MCA–LIA transition. In Europe, this period was humid with cold conditions (Pfister et al., 1998), whereas in the IP and the

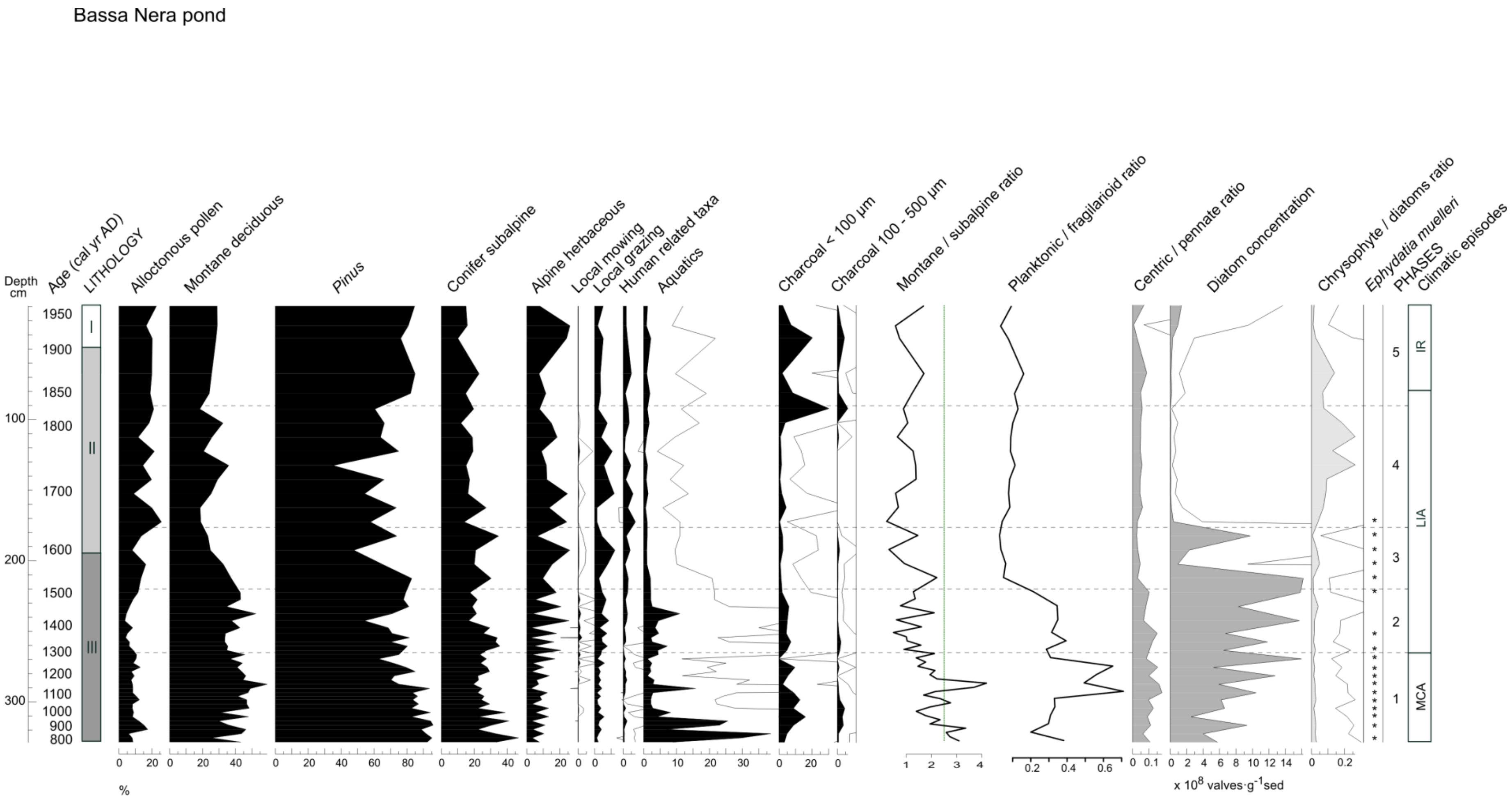
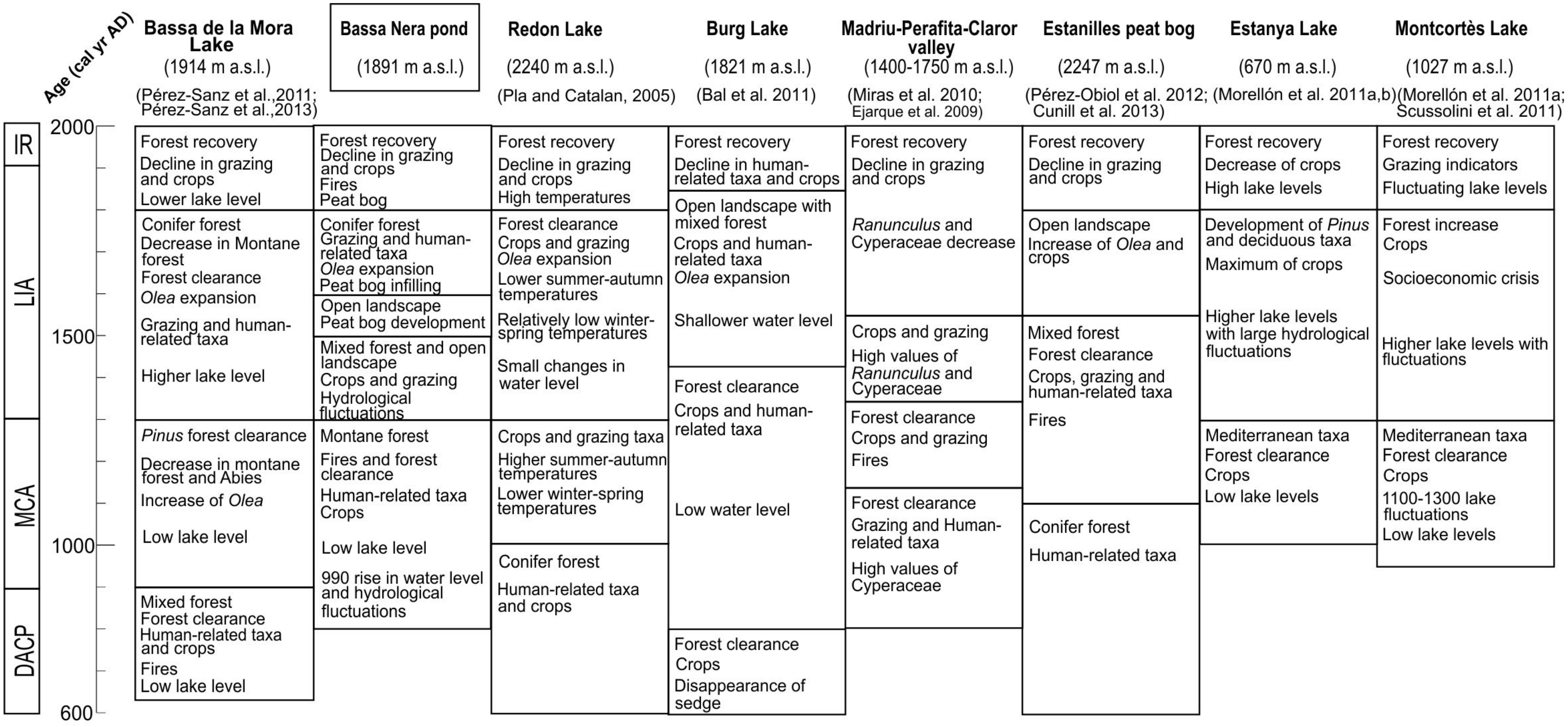


Figure 2.5. Summary diagram grouping pollen according to vegetal associations, with additional information about aquatics, charcoal, diatom concentration and ratios. Medieval Climate Anomaly (MCA), 'Little Ice Age (LIA)' and Industrial Revolution (IR) periods are indicated on the right. 'Local mowing' includes the following taxa: *Centaurea* spp, Cerealia-t, *Sanguisorba* spp and *Secale cereale*; 'Local grazing': *Galium*-t, *Juniperus* sp, *Plantago*-t, *Potentilla*-t, *Ranunculus*-t, *Rumex* sp and *Stellaria* sp and 'Human-related taxa': *Urtica* sp, *Asphodelus*-t and Chenopodiaceae/ Amaranthaceae.

Pyrenees, it was distinguished by fluctuating moist conditions and cold temperatures (Morellón et al., 2011a).

Phase III. 220–177.5 cm, AD 1493–1618. The increase in *Abies* coinciding with lower montane ratio values, evidenced by the decline of *Betula* and *Alnus*, points to a maintained downward shift in the vegetation communities and a temperature decrease. A conspicuous increase in Poaceae suggests a continuity in the openness of the vegetation. The expansion of *Olea* and *Quercus ilex* could reflect a high upward flow of regional pollen (Cañellas-Boltà et al., 2009), most likely due to the increasing development of agricultural practices in the lowlands and favoured by the landscape opening (Pérez-Sanz et al., 2013). The drastic decrease in aquatic taxa, the Ce/Pe decrease and the change from brown-dark to brown-red fibrous peat moss sediment suggest a lower rate of decay of material and the presence of the peat bog at the sampling site (Clymo, 1984). The decrease in submerged vegetation restricted the amount of habitat suitable for benthic and epiphytic diatoms (*A. minutissimum, A. valida* and *E. subminuta* (Rivera Rondón, 2013)) and caused a decrease in H'. These conditions lead to the replacement of *A. minutissimum* by a large amount of *P. alvareziae*, together with *S. oldenburgiana* and other Fragilarioids, suggesting colder conditions and, possibly, longer periods with ice cover (Lotter and Bigler, 2000). Indeed, a sequence extracted close to PATAM12 coring point also evidenced peat bog presence for the last millennium (Pèlachs et al., 2015). The presence of *Aulacoseira*, the main planktonic genus found in this study, could also indicate longer ice cover, given its low light requirements and its opportunistic nature (Rühland et al., 2008; Willén, 1991). Redon Lake also evidenced long-lasting ice cover during this period (Catalan et al., 2009). With the thawing of the ice cover, littoral habitats become first available to benthic and periphytic diatoms such as small Fragilarioid species, adapted to cold waters, short growing seasons and prolonged ice cover (Schmidt et al., 2004). This period represents the end of the MCA–LIA transition (AD 1300–1600)characterized by fluctuating, moist conditions and relatively cold temperatures in the IP and Southern Pyrenees (Morellón et al., 2011a). Some lakes, such as Basa de la Mora Lake, increased their water levels (Pérez-Sanz et al., 2013), whereas Redon Lake did not show remarkable shifts in its planktonic percentages, suggesting small changes in water depth (Catalan et al., 2009) (Figure 2.6). However, Burg Lake increased its Cyperaceae frequencies, while *Sparganium* decreased, implying shallower waters (Bal et al., 2011; Gacia et al., 2008). Estanya Lake also registered aridity and fluctuating water levels (Riera et al., 2004). Bassa Nera does not show evidence of moist conditions; this could be related to the possibility that local factors obscured any plausible regional relationship between climate and peat bog development (Mäkilä, 1997).

Phase IV. 177.5–90 cm, AD 1618–1823. During this phase, the vegetation was dominated by coniferous forest with some deciduous *Quercus* and *Corylus*. The low montane ratio indicates that the montane boundary remained below the altitude of the lake and suggests low temperatures during this period, matching with the second phase of the LIA (AD 1600–1850) (Morellón et al., 2011a). A notable increase in *Potentilla*, *Urtica* and Chenopodiaceae/Amaranthaceae suggests an intensification of human disturbance through grazing (Ejarque et al., 2010). The maximum values of *Olea* and evergreen *Quercus* imply intensified agricultural practices in the lowlands and an expansion of meadows (Cañellas-Boltà et al., 2009). Nearby lakes (Bal et al., 2011; Pérez-Sanz et al., 2013) and other parts of the Pyrenees also recorded high proportions of *Olea* at approximately the same time (Reille and Lowe, 1993) (Figure 2.6). The low macrophyte diversity, combined with higher frequencies of damaged pollen, might indicate periods of aerial exposure. Low diatom concentrations and less pelagic assemblages seem to indicate shallower waters because of the infilling process and the development of the peat bog in the pond edges. In this regard, it is important to note that the study of a single record adds some uncertainty to the evaluation of a general circumstance of the pond in front of a particular transition on the coring site because the rates of peat growth vary in different parts of the bog according to hydrological, topographical and edaphic factors (Mäkilä, 1997). The high values of Cr/Di suggest poor nutrient conditions (Smol, 1985). Some authors found similarly high proportions of cysts in littoral semi-aquatic mosses, where epiphytic diatoms are restricted (e.g. Duff et al., 1995). However, the stable frequencies of monoletes and triletes do not suggest a significant increase in mosses and ferns. The disappearance of the sponge *Ephydatia* could be due to a decrease in water temperature below 15°C (Økland and Økland, 1996) or limitations in food availability. The absence of wetland plants and the influx of humic acids from degrading peatbanks enclosing the lake



Vegetal associations: Mixed forest: *Pinus, Abies, Betula, Corylus,* deciduous *Quercus.* **Conifer forest:** *Pinus, Abies.* **Montane forest**: *Betula, Corylus*, deciduous *Quercus*

Human pressure: Crops: Cerealia-t and *Secale cereale.* **Grazing:** *Rumex,* Chenopodiaceae, *Urtica, Potentilla.* **Human-related taxa:** *Plantago,* Asteraceae, *Artemisia.*

Chronological stages (Morellón et al., 2011a): Dark Ages Cold Period (DACP), Medieval Climate Anomaly (MCA), Little Ice Age (LIA), Industrial Revolution (IR)

Figure 2.6. Overview of sediment results (diatom, pollen, algae, macrophyte remains) and resultant climate and environmental inferences of Bassa Nera and other lacustrine and peat bog sequences from the Central Pyrenees and pre-Pyrenees recording the last 1000 years. Dark Ages Cold Period (DACP), Medieval Climate Anomaly (MCA), 'Little Ice Age (LIA)' and Industrial Revolution (IR). Vegetal associations – Mixed forest: *Pinus, Abies, Betula, Corylus* and deciduous *Quercus*; Conifer forest: *Pinus* and *Abies*; Montane forest: *Corylus* and deciduous *Quercus*. Human pressure Crops: Cerealia-t and *Secale cereale*; Grazing: *Rumex*, Chenopodiaceae, *Urtica* and *Potentilla*; Human-related taxa: *Plantago*, Asteraceae and *Artemisia*.

(Pérez-Haase and Ninot Sugrañes, 2006) would have changed the biochemical conditions of the pond, colouring the clay sediment to brown-red and perhaps favouring a decrease in Botryococcus (Demetrescu, 1998). This would have prompted the replacement of planktonic diatoms by *S. exiguiformis*, *A. alpigena* and *T. flocculosa*, which are periphytic forms that can attach to mosses (Krammer and Lange-Bertalot, 2004b). The second major phase of the LIA was remarkably cold in Europe (Mann et al., 2009), while in the Southern Pyrenees it was characterized by colder temperatures, higher humidity and maximum glacier advances (González Trueba et al., 2008; Morellón et al., 2011a). The Bassa Nera record does not evidence increasing moisture, as also occurred in nearby basins as Basa de la Mora Lake and Perafita Valley (Bal et al., 2011; Miras et al., 2010). However, other lakes in the pre-Pyrenees such as Estanya show periods of large hydrological fluctuations (Morellón et al., 2011b; Riera et al., 2004) (Figure 2.6). On the other hand, the peat accumulation in Bassa Nera could have been favoured by the cold conditions (Martinez-Cortizas et al., 1999).

Phase V. 90–0 cm, 1823 to present. At the onset of this phase, the montane ratio remains low, suggesting the downward continuity of the montane vegetation belt boundary and low temperatures until the past century, when it increases again. A slight decrease in *Abies* at approximately AD 1921 and the increase in deciduous elements suggest a recent dominance of mixed forest. The higher proportion of herbs during the past century indicates an open landscape around the catchment. These non-forested areas are prone to be eroded and may produce higher sediment input to the pond, explaining the switch from clay to sandy silt during this period. The charcoal abundance suggests two periods of frequent regional and local fires. The increase in Poaceae, *Pinus* and *Corylus* at approximately AD 1915 could be due to their ability to reappear after fires. The type and acidophilus character of diatoms as well as the significant presence of chrysophytes indicate the continuity of shallow waters and the expansion of periphytic habitats (Douglas and Smol, 1995). A minor shift in diatom assemblage during the past century, marked by an initial decline in *S. venter* and *P. alvareziae* with a strong peak in the periphytic *A. minutissimum* and *S. pinnata*, reflects an increase in subaquatic vegetation and greater nutrient-rich conditions (Van Dam et al., 1994). This phase includes the end of the LIA in the IP (Morellón et al., 2011a) and the onset of a warmer and more arid period, coinciding with the IR and the CGW (Seager et al., 2007).

2.4.2 Comparison with other peat bogs

The Bassa Nera record shows some peculiarities in aquatic and vegetal trends compared with those found by other studies in the nearby region (Bal et al., 2011; Cunill et al., 2013; Pérez-Obiol et al., 2012). Some of these unique features might be due to differences in ecosystem sensitivity to climate between peat bogs and lakes. Our results agree with those from other peat bogs that show analogous conditions and are more geographically distant. In the IP, the distribution of peatlands is mainly in the northern areas, within the Eurosiberian bioclimatic region (Hernández-Beloqui et al., 2015; López-Moreno et al., 2010; Martınez-Cortizas et al., 2001; Pérez-Díaz et al., 2016; Pérez-Díaz and López-Sáez, 2014). Some Northwestern peat bogs also recorded wet periods between AD 1110–1210 and AD 1345–1475 (Mighall et al., 2006) with several rapid and brief dry episodes between AD 1200–1300, AD 1400–1450 and AD 1600–1700 (Castro et al.,2015) and higher intra-annual fluctuations after the mid-16th century (Silva-Sánchez et al., 2016) that could match with the hydrological fluctuations of Bassa Nera. In the Alps, the Mauntschas mire showed a change in hydrological conditions from AD 1572, favouring peat bog development and decreasing the water level (Van der Knaap et al., 2011). Similarly, in northern Poland, the Kusowskie Bagno bog presented water table fluctuations from AD 1150, increasing abruptly at approximately AD 1240 and then decreasing by AD 1500 (Lamentowicz et al., 2015). Carpathian peatlands also showed a substantial increase in the water table after AD 1400 and a marked change to drier conditions after AD 1580 (Schnitchen et al., 2006). Most of these peat bogs responded to the MCA with water level fluctuations, and all of them recorded an abrupt change to drier conditions with the onset of the LIA. The ecological response and sensitivity revealed by these palaeoenvironmental records provide insights into the nature and timing of the response that we may expect the CGW to trigger. Note that peat bogs are known to impact the global water cycle because of their water-retention properties (Moore, 2002). This capacity might be important in buffering the effects of precipitation decrease due to global warming expected for the Mediterranean region (Giorgi and Lionello, 2008). Therefore, a surveillance network along latitudinal gradients of peatlands would act as a sentinel and help to apply the appropriate measures of conservation and management.

2.4.3 Lag between pollen and diatom responses

Figure 2.7 displays the trends of montane and P/F ratios representing 35 samples taken at the same core depth. For this purpose, we considered only local vegetation, since the presence of the genera used in montane ratio imply the nearby occurrence of the pollen source. Our results show a possible faster response of diatoms than the local vegetation to the global climatic signal in all the climatic periods studied in this work, with a lag of several years to decades, coinciding with Scussolini et al. (2011). These trends, however, must be interpreted with caution because of the relative large fluctuations of both ratios and the likely influence of anthropogenic disturbances. The most coincident trends occur during MCA and IR, suggesting more similar response times between the two proxies during warm conditions. However, during the IR, the vegetation seemed to respond more intensely, while diatoms had a weaker response, most likely explained by the impoverished tychoplanktonic assemblage resulting from peat bog infilling. During the LIA, the response of these proxies clearly differs. At the species level, the peak in *P. alvareziae* (Figure 2.4) is interpreted as a manifestation of LIA cooling. Since then, the aquatic organisms show a decreasing trend during the rest of the sequence. In contrast, Abies peaks shortly before the Fragilariaceae, while *Pinus* and *Prunus* decline (Figure 2.3), and the vegetation strongly fluctuates thereafter, showing range expansions and contractions.

2.4.4 Human impact

The presence of human-related species along the whole diagram indicates that the Aiguamòg Valley has been subjected to low intensity anthropogenic pressure throughout the studied period. The increase in *Artemisia* (AD 990) and Cerealia-t (AD 1144), with the high frequency of local and regional charcoal, suggests the use of fire to maintain open spaces for cultivation during the MCA (Bal et al., 2011; Pérez-Obiol et

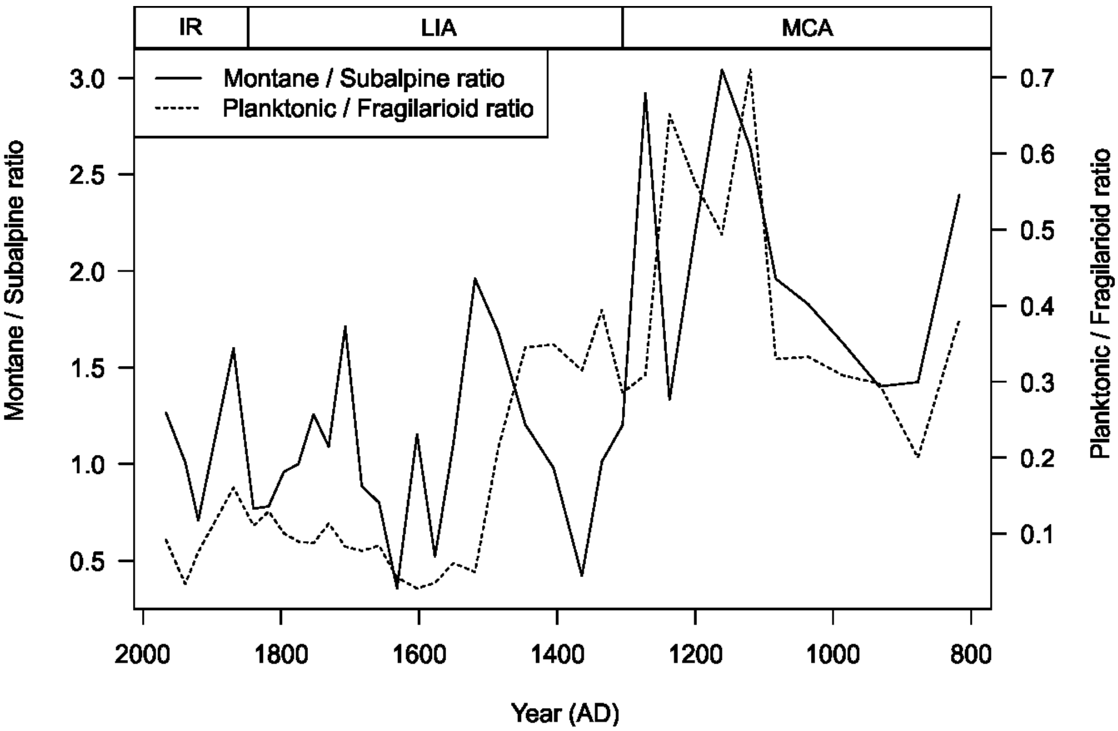


Figure 2.7. Lag between pollen and diatom responses to the same climatic pressure defined in the MCA, LIA and IR periods.

al., 2012) (Figure 2.6). Later, the increase in *S. cereale* and ruderals (*Plantago* and *Potentilla*) reflect an increase in farming activity and local grazing in the first stages of the MCA–LIA transition (AD 1300–1600), which might have increased lake turbidity. Other studies performed in adjacent regions have also recorded extensive *S. cereale* cultivation during this period (Cunill et al., 2013; Miras et al., 2010; Pérez-Obiol et al., 2012). However, these results contrast with nearby pre-Pyrenees regions, where wars and the devastating 'black death' epidemic (AD 1347-1353) prompted the abandonment of lands and crops (Rull et al., 2011). In contrast, Bassa Nera increased and shows diversified crops between AD 1300 and 1500. This finding could be due to the significant migration from towns to farmland after the large epidemic, together with the Querimonia (Gómez, 2007), a privilege document signed by King Jaume II in AD 1313 that granted the ownership of mountain lands to Aranese institutions and allowed their free use for grazing and farming. From a geographical point of view, the harsh winters and poor communication across the mountain passes of Viella and Bonaigua that surround the region favoured the geographic isolation of the Aran Valley (Boya-Busquet and Cerarols-Ramirez, 2015), affording protection against disease outbreaks and favouring regional activities. By the end of the MCA–LIA transition, the disappearance of *S. cereale* at AD 1500 suggests that crops suddenly ceased to occur, with only some grazing evidence remaining (*Potentilla* and *Urtica*), indicating that people abandoned the farming of the high ranges to develop farming in the lowlands; only the livestock persisted because of the increasingly colder conditions. The low charcoal values in our record between AD 1583 and 1736 indicate a low frequency of fires in the area. However, the short-term decreases in *Pinus* and the increase in *Artemisia* and *Potentilla* could indicate periods of forest clearance and increases in grazing lands at higher altitudes, as also observed in nearby Basa de la Mora Lake sequence (Pérez-Sanz et al., 2013). This pattern contrasts with nearby high-mountain valleys that experienced higher pastoral pressure and the use of fires for forest clearance or metallurgical activities (Catalan et al., 2013; Ejarque, 2009; Pèlachs et al., 2009), highlighting the differences in land use management between regions. By the mid-19th century, the frequent fires, forest clearance and the Poaceae peak might be the result of an increased need for supplies and raw materials during the IR (Ferrer i Alòs, 2012). The social and economic changes during the mid-20th century forced migration from the Pyrenees to cities. Therefore, the abandonment of rural lands and the establishment of Aiguestortes i Estany de Sant Maurici National Park in AD 1955 and the protection of its surroundings in AD 1990 favoured the expansion of arboreal taxa, such as *Pinus* (Améztegui et al., 2010; Bal et al., 2011). As in Bassa Nera, the abandonment of traditional land uses in the Pyrenees and many Mediterranean mountains has led to recolonization of deforested areas, shrub encroachment and densification of treelines (MacDonald et al., 2000). This afforestation reduced the grassland extensions and landscape diversity, increased water consumption and evapotranspiration and produced marked alterations in hydrological responses (Barrio et al., 2013; López-Moreno et al., 2010). These large amounts of accumulated deciduous biomass and the expected concomitant increase in temperatures and drought events (IPCC, 2007) might cause a shift to flammable material and trigger fire frequency and large-scale fire hazards, as occurred in the mid-Holocene (Gil-Romera et al., 2014; Lasheras-Alvarez et al., 2013). Applying strategies that minimize the impact of CGW to biodiversity may become essential, such as traditional grazing activities and associated management practices with ecological forest management (Ninot et al., 2008). Our palaeoecological results are in agreement with Pérez-Sanz et al. (2011), indicating that climate changes have not only influenced environmental evolution during recent times but might also have modulated the degree of human pressure in the high ranges.

2.4.5 Expected future mountain scenarios under CGW

In this study, the observed increase in montane vegetation by the mid-20th century points to an ongoing upward range extension in species distribution in the Aiguamòg region because of the CGW, which would possibly match with the rapid response of alpine Pyrenean treelines to climate registered by Camarero et al. (2015). Other studies have also demonstrated recent vegetation shifts in high-mountain ecosystems (Gottfried et al., 2012; Thuiller et al., 2005). From approximately 1940 to 1968, the montane ratio increased from 0.57 to 1.6. In this period, the mean temperature in the Pyrenees increased at an average rate of +0.3°C per decade (López-Moreno et al., 2010) and raised tree establishment and density within the treeline ecotone (Camarero and Gutiérrez, 2004). Considering that the predicted temperature increase for mountain regions by 2030 is 0.5–1.5°C (IPCC, 2013) and 1.5–2°C by 2021–2050 in the Pyrenees (IPCC, 2007), we could expect an acceleration of the upward shift of the montane belt in the Pyrenean region in a short period of time. This, together with a likely decrease in snow accumulation and reinforced Mediterranean summer droughts (IPCC, 2007; López-Moreno et al., 2009), could significantly reduce the available area for subalpine and alpine ecosystems in the Mediterranean mountains.

2.5. Conclusions

The multi-proxy approach used in this work has helped to produce a detailed and comprehensive picture of the main events that occurred during the last millennium in the surroundings of the Bassa Nera as an example of a temperate high-mountain environment. This study shows that the vegetation of the Bassa Nera catchment responded strongly to climate with altitudinal shifts and is most likely currently responding to the CGW. From the MCA to the MCA–LIA transition, the montane–subalpine ecotone reached the Bassa Nera catchment. We might expect that with the current temperature projections for 2100, this ecotone will eventually reach the Bassa Nera again. This scenario could be extrapolated to other high-mountain environments of the Mediterranean region.

To the best of our knowledge, this study is the first attempt to link a pollen ratio to past altitudinal shifts in the montane–subalpine ecotone. The montane ratio has proven the usefulness of good pollen indicators for revealing vegetation trends, providing a suitable tool for palaeoecological studies and for monitoring regional changes in natural communities in response to CGW. This pollen analysis is site-specific, and the application of this ratio for interpreting different biogeographic locations should be adapted by including local species with similar ecological characteristics. The use of the montane ratio in highland peat bog ecosystems would help with early detection of the replacement of vegetation predicted by IPCC (2007).

Additionally, human management of natural resources has changed over the past millennium. Through the MCA and MCA–LIA transition, the people of the region used fires to open the forests for cultivating and grazing. With the LIA cooling, grazing was the main form of resource exploitation. During the IR, some farming activities were still conducted until the authorities restricted resource exploitation by creating the National Park.

Aquatic taxa, diatom communities and sedimentary units allowed to describe the peat bog development at the coring site and its infilling at approximately AD 1565. During the past millennium, the small Fragilarioid species dominated the community. These opportunistic species had a particularly higher incidence during peat bog development in the LIA period, seizing an advantage when the diatom diversity diminished due to unfavourable and cold conditions.

Consistent shifts in vegetation, fire activity and aquatic communities throughout the sequence are clearly related to climatic signals such as the MCA and LIA phases. Although the studied lakes nearby such as Basa de la Mora, Burg and others located in the pre-Pyrenees (Pérez-Sanz et al., 2013; Riera et al., 2004; Scussolini et al., 2011) had shallow waters during the arid conditions of the MCA, the Bassa Nera might have maintained or increased its water levels. This finding underlines the contrasting responses of lakes and peat bogs to similar climatic pressures.

In this study, we have observed the past biotic responses to climate changes and considered possible future responses under the scenarios of the CGW. Because high spatial resolution has shown that the forecasted climatic changes will not be uniform throughout regional areas (Barrera-Escoda and Cunillera, 2011), the information provided by this study will help to better understand spatial variability in the impacts of climate change on high mountain ranges.

This study has been based on a single record recovered from the shore of Bassa Nera and mainly reflects the evolution of the sedimentological, ontogenic and palaeoecological processes occurred in the littoral zone in order to improve the obtained palaeoecological and palaeoenvironmental reconstructions and inferences, and it would be interesting to investigate other depositional environments like the pelagic zone of the pond and other parts of the peat bog and catchment.

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Chapter 3

Environmental history and vegetation dynamics in response to climate variations and human pressure during the Holocene in Bassa Nera, Central Pyrenees

Original publication (*Appendix 2 in the supplementary material*):

Garcés-Pastor, S., Cañellas-Boltà, N., Pèlachs, A., Soriano, J. M., Pérez-Obiol, R., Pérez Haase, A., Calero, M.M., Andreu, O., Escolà, N., and Vegas-Vilarrúbia, T. (2017). Environmental history and vegetation dynamics in response to climate variations and human pressure during the Holocene in Bassa Nera, Central Pyrenees. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *479*, 48-60.

Abstract

With the aims of investigating the causes of environmental changes in high mountain ecosystems during the Holocene in relation to climate forcings and identifying thresholds for vegetation community shifts, we performed a multi-proxy palaeoecological reconstruction based on two sediment cores from Bassa Nera, a lentic system located close to the montane–subalpine ecotone in the Central Pyrenees. Using pollen, plant macroremains, charcoal, chemical elements and loss-on-ignition at centennial to decadal resolution, we reconstructed the vegetation and lacustrine dynamics during the last 10,000 years. A montane pollen ratio was used as a palaeoecological indicator to track altitudinal shifts in high mountain vegetation, which was compared to the ice-rafted debris index (IRD) as a proxy for summarizing the climatic influence of the North Atlantic Circulation. Our results show upward shifts of deciduous forest and its presence in Bassa Nera from the onset of the Holocene until 4200 cal yr BP, when it was replaced by coniferous taxa. The montane ratio showed a link between vegetation and North Atlantic influence, while changes in *Sphagnum* macroremains and aquatic taxa allowed description of local ontogenic changes from the initial pond to the present peatland. The loss-on-ignition record showed some flood events at Bassa Nera between 4500 and 3900 cal yr BP. The studied proxies allowed inferences concerning anthropic pressure in the catchment through grazing activities by 7300 cal yr BP and the appearance of cereal agriculture around 5190 cal yr BP. The highest human pressure occurred in the late Bronze Age, Roman Period and Middle Ages.

Keywords: Montane–subalpine shifts, Pollen, Sphagnum peat, Charcoal, Pyrenees, Holocene

3.1 Introduction

The development of environmental assessment tools to predict how current climate change will affect natural ecosystems is essential to apply proper management measures. Palaeoenvironmental reconstructions are crucial to understand ecosystem sensitivity and past environmental shifts, as they help to distinguish between the effects of climatic and anthropogenic forcings (Last and Smol, 2001; Willis and Birks, 2006; Catalan et al., 2013). Mountain ecosystems are well-suited to study such changes, since their hard environmental conditions make them less prone to intensive human influence.

The onset of the Holocene, characterised by relatively warmer temperatures and an increase in humidity in Europe (Walker, 1995), prompted a rapid expansion of deciduous forests in southwest European mountains (Jalut et al., 2009; Pérez-Obiol et al., 2011), including the Pyrenees (Benito et al., 2008; Montserrat-Martí, 1992). By the Middle-Holocene, a southward shift of the North Atlantic westerly jet (Bond et al., 2001) led to a change in precipitation seasonality (Pla and Catalan, 2005), a drastic decline in deciduous taxa and a progressive consolidation of conifers in the Pyrenees (González-Sampériz et al., 2006; Pèlachs et al., 2011). Such changes in plant community composition suggest that the North Atlantic climatic variability had sufficient magnitude and duration to affect the Pyrenean ecosystems and force them to cross a threshold into a different state. However, the precise features in the response of deciduous and conifer species to climatic shift are not yet fully understood, and their study becomes even more complicated when anthropogenic influence is taken into account.

With the aim of investigating how the high mountain ecosystems of the Central Pyrenees responded to the Holocene climate and anthropogenic forcings, we performed a multi-proxy palaeoecological reconstruction of Bassa Nera (BSN), a pond with a ring of Sphagnum moss close to themontane-subalpine boundary of the Aiguamòg valley (Aran valley). There are several palaeoecological studies in the Central Pyrenees covering the Holocene that record a marked climate variability with well-defined arid and cold events (Pla and Catalan, 2005; González-Sampériz et al., 2006; Pérez-Sanz et al., 2013), vegetation responses through treeline shifts and biomassfire interactions (Cunill et al., 2013; Gil-Romera et al., 2014).

Regarding human influence, the Central Pyrenees have been occupied and exploited by prehistoric societies since at least the Neolithic (Ejarque et al., 2010; Gassiot et al., 2014) through fire and complex land use management (Bal et al., 2010; Pérez-Obiol et al., 2012). Phases of higher anthropogenic pressure in high mountain areas are known since the Early and Middle Neolithic, with an important threshold in the Early Bronze Age (Gassiot and Jiménez, 2006; Miras et al., 2007). Since then, it has been widely assumed that the configuration of high mountain landscapes has been influenced by humans (Ejarque et al., 2010; Bal et al., 2011; Cunill et al., 2013), or at least that humans have accentuated the effects of climatic constraints on vegetation during the late Holocene (Jalut et al., 2009) through mining activities, farming or cattle raising (Pèlachs et al., 2009a; Cunill et al., 2013; Garcés-Pastor et al., 2016). In this study, we perform a high-resolution reconstruction of vegetation in order to detect the onset of the anthropic pressure in Bassa Nera caused by grazing and agriculture farming.

Pèlachs et al. (2011) found a close coupling between regional climatic patterns using the ice-rafted debris index (IRD; Bond et al., 2001) from the North Atlantic and the accumulation of organic matter in a mountain wetland system in the Central Pyrenees (Pèlachs et al., 2011). However, it is advisable to check whether this coupling between climatic influence and organic matter deposition may be generalized to other lentic systems in order to test the applicability of the IRD to palaeoecological reconstructions. Hence, this paper studies the response of organic matter accumulation in Bassa Nera to North Atlantic regional climatic patterns and compares it with similar regional essays. In a previous study, Garcés-Pastor et al. (2016) introduced a montane pollen ratio that was useful for monitoring local upward migrations of the montane-subalpine boundary. The present work uses this montane ratio to track the response of high mountain vegetation to the Holocene climate variability and North Atlantic influence (IRD) and, if possible, to identify possible thresholds in vegetation communities. In this paper, we combine diverse proxies (pollen, charcoal, macroremains, organic matter, chemical elements and sedimentology) from two independent records of Bassa Nera spanning the Holocene with the following objectives: (1) To reconstruct the local vegetation dynamics of BSN; (2) to evaluate the ecosystem response of the area to climate forcings and North Atlantic influence, describing the main arboreal dynamics at the local level as well as identifying thresholds in vegetation communities and their possible causes; (3) to test the response of organic matter indicators such as LOI in front of North-Atlantic regional climatic patterns (IRD), comparing the results from BSN to those obtained from other Pyrenean systems; and (4) to assess the human influence on the BSN region, determining the point at which this influence became strong enough to be detectable and how it was affected by the climatic patterns.

3.2. Study area

3.2.1. Environmental and geographical settings

The Bassa Nera (42° 38′ 18.5″ N, 0° 55′ 27.6″ E, 1891 m a.s.l.) is a small lentic system from glacial origin located in the peripheral zone of “Aigüestortes i Estany de Sant Maurici” National Park (PNAESM) (Figure 3.1). Its surface area is 2.01 ha, with a maximum depth of 5 m, and it drains by a small outlet into the Aiguamòg River. This pond is surrounded by mixed peat bogs and it is currently in the final stages of infilling (Pérez-Haase and Ninot Sugrañes, 2006, 2017). The climate is subalpine with Atlantic influence and precipitation is well distributed along the seasons (annual average=1152mm)(Ninyerola et al., 2003). Mean annual temperature is 4.25 °C, being January the coldest month (−3 °C in average) and July the warmest (14 °C in average). The BSN basin lies on a granodiorite bedrock from the Maladeta batholith, which dates from the Carboniferous-Permic age (Roca i Adrover et al., 2010). The main peat communities are geogenous fens (*Scheuchzerio-Caricetea fuscae*) and ombrogenous bogs (*Oxycocco-Sphagnetea*) (Pérez-Haase et al., 2012). The BSN is surrounded by a mixed conifer forest of *Pinus mugo* subsp. *uncinata* (Ramond) Domin. and *Abies alba* Mill., with *Rhododendron ferrugineum* L. in the understory and Poaceae meadows. Cañellas-Boltà et al. (2009) described the montane and subalpine vegetation altitudinal belts where the catchment area lies. The montane belt (b1600 m) is composed by deciduous oak forests of *Quercus petraea* (Mattuschka) Liebl. with

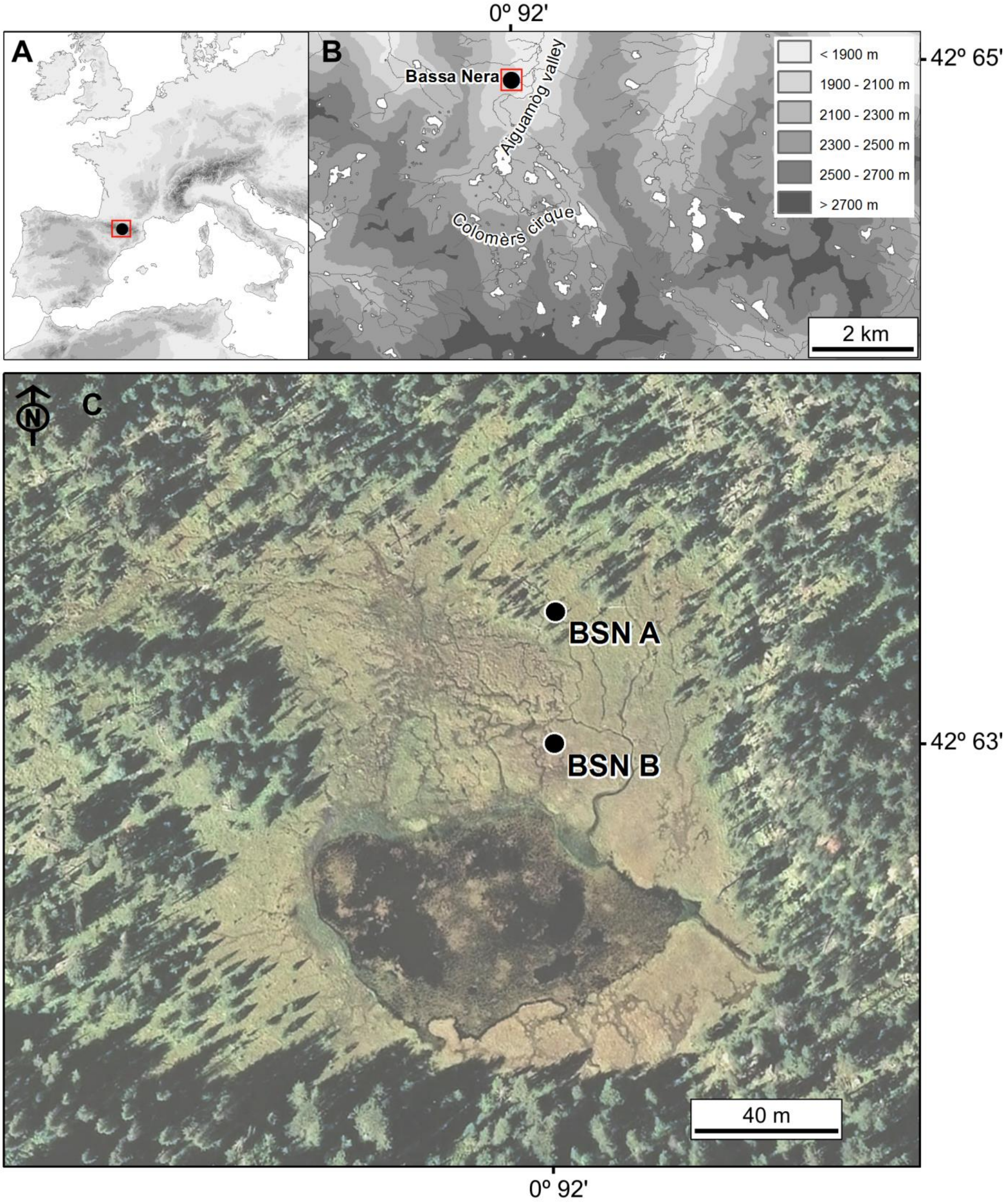

Figure 3.1. Location of the study area (A) Map indicating the relative location of Bassa Nera Pond in the Iberian Peninsula. (B) Topographic map of the terrain surrounding Bassa Nera. (C) Coring sites where the cores A and B were retrieved.

Betula pendula Roth.; riverine forests (dominated by *Alnus glutinosa* L., *Fraxinus excelsior* L., and *Salix* spp.); forests with *Tilia platyphyllos* Scop., *Prunus avium* L., and *Corylus avellana* L.; and mixed forests of *Betula pendula* Roth. and *Pinus sylvestris* L. The subalpine belt (1600–2250m) is dominated by coniferous forests of *Abies alba* and *Rhododendron ferrugineum* at the lowest parts and Pinus mugo subsp. uncinata with *R. ferrugineum* at the upper stages. Wetlands are mainly occupied by *Trichophorum cespitosum* subsp. *cespitosum* (L.) Hartm. communities, assemblages of *Juncus balticus* Willd, subsp. *pyrenaeus*, *Carex rostrata* Stokes beds., *Caltha palustris* L. flushes and *Sphagnum* peat bogs (carpets and hummocks) (Pérez-Haase and Ninot Sugrañes, 2006). This part of the valley has experienced low human pressure through pasturing and farming during the last millennium (Garcés-Pastor et al., 2016). Since the rural exodus of mid-20th century to the creation of the PNAESM in 1955, grazing, forest exploitation and hydroelectric electricity generation were the only activities. Afterwards, tourism has become an important activity in the national park.

3.3. Material and methods

3.3.1. Coring, sampling, dating and sedimentology

Two cores (PATAM-12 and BSN-6), separated by 47 m, were retrieved from the Sphagnum mire surrounding Bassa Nera (Figure 3.1). Core PATAM-12 provides a detailed record of the last seven millennia, but lacks the beginning of the Holocene. For this reason, we also studied core BSN-6, which covers the last ten thousand years and provides a wider environmental framework. The core BSN-6 (core A, 270 cm long) was collected in 2011 through the percussion and recover in one step of a 3 m PVC tube on a hummock composed by *Sphagnum magellanicum* and *S. capillifolium* (Figure 3.1). The core PATAM-12 (core B, 706 cm long) was obtained in 2007 with a "Russian" corer (Jowsey, 1966) on the *Sphagnum* quaking carpets (*Caricion lasiocarpae*) that surround the pond. Core A was sliced every 1 cm and core B every 3 to 5 cm. The chronological framework was based on AMS radiocarbon dates from peat and macroremains obtained at Beta Analytic Radiocarbon Dating laboratory (Miami, USA) and Keck Carbon Cycle (Irvine, USA), published in Pèlachs et al. (2016) and Garcés-Pastor et al. (2016). The radionuclide analysis (210Pb) for dating purposes of the uppermost 40 cm of core A was carried out at the Laboratory of Environmental

Radioactivity of the Universitat Autònoma de Barcelona (UAB, Spain). The supported 210Pb was found at 30 cm depth and 210Pb activities were determined by α-spectrometry through 210Po in equilibrium(Sanchez-Cabeza et al., 1998). Ages were calibrated with IntCal13.14C curve (Reimer et al., 2013) and the age–depth models (Figure 3.2) were performed with Clam 2.2. software using Smooth Spline function (Blaauw, 2010). The sedimentary facies of the cores were described following Schnurrenberger et al. (2003) (Figure 3.2).

3.3.2. Pollen, charcoal and macroremains

Pollen analyses were carried out in two different laboratories. A total of 62 samples along 270 cm (core A) and 114 samples in 706 cm (core B) were processed according to standard chemical methods (Moore et al., 1991) with KOH, HCl, HF digestions and mineral separation in heavy liquid (Thoulet solution; density 2.0 g/cm^3) at the Universitat Autònoma de Barcelona and the Catalan Institute of Human Paleoecology and Social Evolution. The pollen record from the first 330 cm from core B (51 samples) has been already studied in Garcés-Pastor et al. (2016). Pollen grains were identified according to Faegri and Iversen (1989) and Reille (1992), and counted until diversity saturation (Rull, 1987). Because most slides had *Pinus* superabundance that could hide vegetation dynamics, counts were increased in order to get a representative sample (minimum 200 pollen grains without *Pinus*). Stomata, non-pollen palynomorphs and algal remains were also counted. The palynological results are presented as percentage of the pollen sum excluding Pinus, spores and wetland plant pollen. Diagrams were plotted using Psimpoll 4.27 software (Bennett, 2002) and statistical significant pollen zones were calculated using the method of Optimal Splitting by Information Content (Bennet, 1996) on taxa showing abundances N1% (Figures 3.3 and 3.4). Some pollen taxa which appear in Figure 3.4 have been excluded from Figure 3.3 since they were less represented due to localisms. The montane pollen ratio used by Garcés-Pastor et al. (2016), based upon the modern pollen indicators of montane and subalpine/alpine stages identified by Cañellas-Boltà et al. (2009), was calculated to estimate altitudinal variations of the montane belt. This ratio was calculated using taxa that have a significant correlation between the abundance of pollen and local occurrence of parent taxa in montane and subalpine-alpine belts in this valley. Montane pollen types included *Alnus*, *Betula*, *Buxus*, *Corylus*, *Fraxinus*, deciduous *Quercus*, *Tilia* and *Salix*, while subalpine-alpine indicators included Asteraceae, *Calluna*, *Campanula*, Ericaceae, *Plantago* and Poaceae. The percentages of the montane pollen were summed and divided by the sum of the percentages of subalpine-alpine pollen (see more information in Garcés-Pastor et al., 2016). Values of 2.5 indicate the close presence of montane belt, while higher values imply the upward montane migration of the latter within Bassa Nera basin. It is important to highlight that this ratio has been inferred from only one altitudinal transect and has been useful for the palaeoenvironmental interpretation of BSN catchment (Garcés-Pastor et al., 2016), but it should be interpreted with caution if used in other areas or landscape mosaics.

To study the sedimentary charcoal, the Carcaillet protocol for lacustrine sediments was adjusted to peat bog (Carcaillet et al., 2001). Consequently, wet weight was used instead of volume in order to minimize the differences of density inside the peat bog. A total of 295 samples from core A were digested according to Carcaillet et al. (2001) and counted with a stereomicroscope at 40× magnification. Charcoal counts were combined and divided by sample weight to calculate charcoal concentration (mm^2/g) and divided by sedimentation rate to calculate the charcoal accumulation rate (CHAR, $mm^2/g/yr$). (Figure 3.3).

Plant macroremains at 64 depths from core A were analysed. Samples were processed according to Mauquoy et al. (2010) with KOH and sieved with a mesh of 150 µm (Birks and Birks, 1980). Taxonomic identification was done according to Brugués et al. (2007), Smith and Smith (2004) and Daniels et al. (1990) using a stereomicroscope and an episcopic microscope. Some Sphagnum macroremains were identified at species level while others could only be identified to their taxonomic section. In those cases, we could refine the identification within each section to a couple of taxa (*S. papillosum* or *palustre* and *S. denticulatum* or *subsecundum*). Results are expressed as presence/absence and *Sphagnum* percentage in Figure 3.3.

3.3.3. Loss-on-ignition and chemical elements

To estimate the organic matter content, 295 samples of core A were dried at 60 °C to determine the weight loss and burned at 550 °C for 4 h to oxidize organic matter, after absence of carbonates had been verified (Heiri et al., 2001; Luque, 2003). The loss-on-ignition results (LOI) are expressed as percentage of dry weight (Figure 3.3). In this work only Titanium (Ti) and Manganese (Mn) were considered as proxies of soil erosion and water oxygenation (Davies et al., 2015). For this purpose, 98 samples from core A were moulded and digested in a microwave oven (CEM, Marsmodel) using a solution of HNO3, HCl and HF with a parallel blank digestion. The extracted solutions were analysed with an inductively coupled plasma mass spectrometer (ICP-MS, Argilent model 7500 ce) at the Chemical Analytic Service of the UAB.

3.4. Results

3.4.1. Chronology and sedimentology

Figure 3.2 shows the age-depth models, the lithology and the correlation of the two cores. Seven radiocarbon dates and 31 210Pb dates were used to construct the core A age-depth model. It covers the last 10,211 cal yr BP in 270 cm with an average confidence interval error of ca. 220 yr and a sedimentation rate of 0.07 ± 0.21 cm yr^{-1}, ranging from 0.016 to 0.86 cm yr^{-1}. Date 104.1+/− 0.4 pMC was rejected because lack of consistency with the 210Pb dating at the same depth (30.2 cm). Core A shows four sedimentary units: Unit I-A (0–17 cm) is composed of living Sphagnum peat with abundant roots. Unit II-A (17–185 cm) is the largest of the record and is composed of a peat bog texture with light to dark-brown clayey silt and abundant organic matter. Two intercalated layers can be distinguished: one characterised by dark-brown fine silt with abundant organic matter (60–64 cm) and another with low organic sandy silt (115–117 cm). Unit III-A (185–263 cm) is composed of brown-black clay with imbedded pennate diatoms and organic matter and shows an intercalated layer of black fine silt with coarse-quartz sands and pennate diatoms (243–244 cm). Unit IV-A (264–270 cm) presents a transition from quartz pebble to coarse sands (till) and broken diatoms.

The age-depth model of core B was built with ten radiocarbon dates. One date (250±40 AMS 14C yr BP), obtained from a woody macroremain, was rejected as stratigraphically incongruent (see detailed information in Garcés-Pastor et al., 2016). The model spans ca. 7490 cal yr BP to the present, with an average confidence interval error of ca 150 yr and an average sedimentation rate of 0.2 ± 0.18 cm·yr^{-1}, ranging from 0.04 to 0.78 cm·yr$-^1$. It presents 3 sedimentary units: Unit I-B (0–50 cm) consists of massive brown sandy silt with scarce vegetal matter. Unit II-B (50–553 cm) is formed by brown-red clay with abundant vegetal matter and few pennate diatoms (50–195 cm), and dark brown massive clays with abundant organic matter (195–553 cm)with an intercalated layer of very fine silt with some broken diatoms and low organic matter (536–539 cm). Unit III-B (553–706 cm) is composed of clays with colours varying from grey to brown. This unit has little organic matter and scarce diatoms (553–586 cm) and brown-black clay with pennate diatoms and organic matter (586–706 cm), with an intercalated section of brown-red clay with abundant vegetal matter and few diatoms (603–677 cm).

The two cores were correlated according to their sedimentological features (Figure 3.2). One stratigraphic correlation was carried out between the sandy silt layer of Unit II-A and the very fine silt layer of Unit II-B. The second correlation was made between the base of Unit II-A and the brown-red clay and dark-brown clay layers located at 677 cm in Unit III-B. The lithological differences between the studied cores are likely due to the concave shape of the basin and the relative position of the sampling points. In summary, core A contains 10,211 years in 270 cm and core B 7490 years in 706 cm. Given the different temporal resolutions between the two records, core A will show the main palaeoenvironmental features of the Holocene, and core B the last 7000 years at a higher resolution.

3.4.2. Loss-on-ignition and metals

The LOI values come fromthree different sedimentary environments (Figure 3.3): (I) peat bog (0–184 cm), with N70% of organic material, showing an abrupt drop to 14% at 105–120 cm; (II) clays (185–263 cm), with an organic content between 30 and 94% and a sharp decrease below 194 cm; and (III) sands with b19% organic matter content (264–270 cm). Ti shows an increasing trend with an important drop at 60–64 cm (Figure 3.3). Since then, it displays relatively stable values except for a prominent peak at 112–116 cm and a smaller peak at 215 cm. On the other side, Mn shows low variability (3–46 µg/g), with two small peaks at 10 cm and 215 cm and one large peak at 112–116 cm (311–346 µg/g).

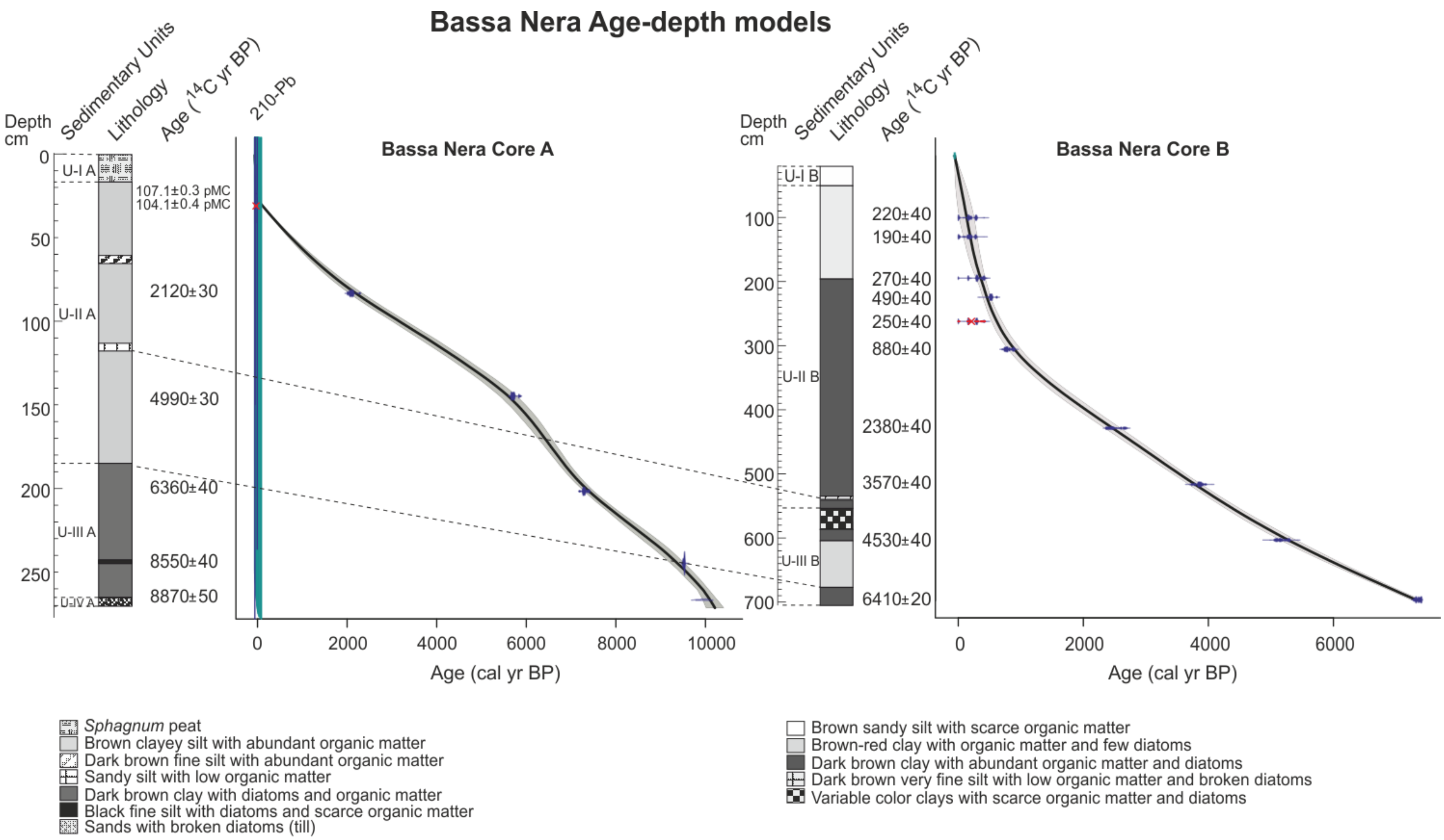

Fig.2: Age-depth models of Bassa Nera cores performed with Clam 2.2 software using Smooth Spline function (Blaauw, 2010) and correlation between their sedimentary facies. The core A presents the 210-Pb dates (marked in blue). In the cores A and B, one radiocarbon date was rejected as stratigraphically incongruent because the roots might have dragged down the wood macrorest.

Figure 3.2. Age-depth models of Bassa Nera cores based on radiocarbon dating, performed with Clam2.2 software using Smooth Spline function (Blaauw, 2010) and correlation between their sedimentary facies. Core A presents the 210Pb dates (marked in blue). In cores A and B, one radiocarbon date was rejected as stratigraphically incongruent, likely because roots might have dragged down the wood macroremain.

3.4.3. Palynological interpretation

The diverse sedimentation rates of the cores and their different locations within the lake basin make them suitable to reconstruct the palaeoenvironmental conditions in different ways. The information provided by the pollen, charcoal and macroremains of core A is used to show the main palaeoenvironmental events and vegetal dynamics that occurred in the BSN catchment during the Holocene, while the pollen from core B details
the most relevant landscape dynamics (Figures 3.3 and 3.4). The entire record is mainly dominated by arboreal pollen, where the deciduous species that abound at the base are replaced by coniferous taxa at around 4000 cal yr BP. Shrubs and herbs increase along the record. Mediterranean taxa are present during the entire sequence, while anthropogenic indicators and charcoal rise by the middle of the sequence. Seven phases are described and summarized according to statistically significant pollen zones.

3.4.3.1. Phase I

This phase is dominated by herbs, mainly Poaceae and *Artemisia*, suggesting that the pond was surrounded by steppe-like grasslands (10211–10,070 cal yr BP, core A: 270–264 cm, 2 samples). The lowest montane ratio values and high proportions of *Betula* point to a nearby birch forest. This agrees with studies that indicate an early phase of birch colonization in the Pyrenees during the Late Glacial-Holocene transitional period (Reille and Lowe, 1993; Cunill et al., 2013; Gil-Romera et al., 2014). Therefore, this phase could be a transition from the steppe taxa that abounded during the colder and more arid Younger Dryas to a deciduous forest, characteristic of the start of the Holocene (González-Sampériz et al., 2006; Reille and Lowe, 1993; Jalut et al., 1992).

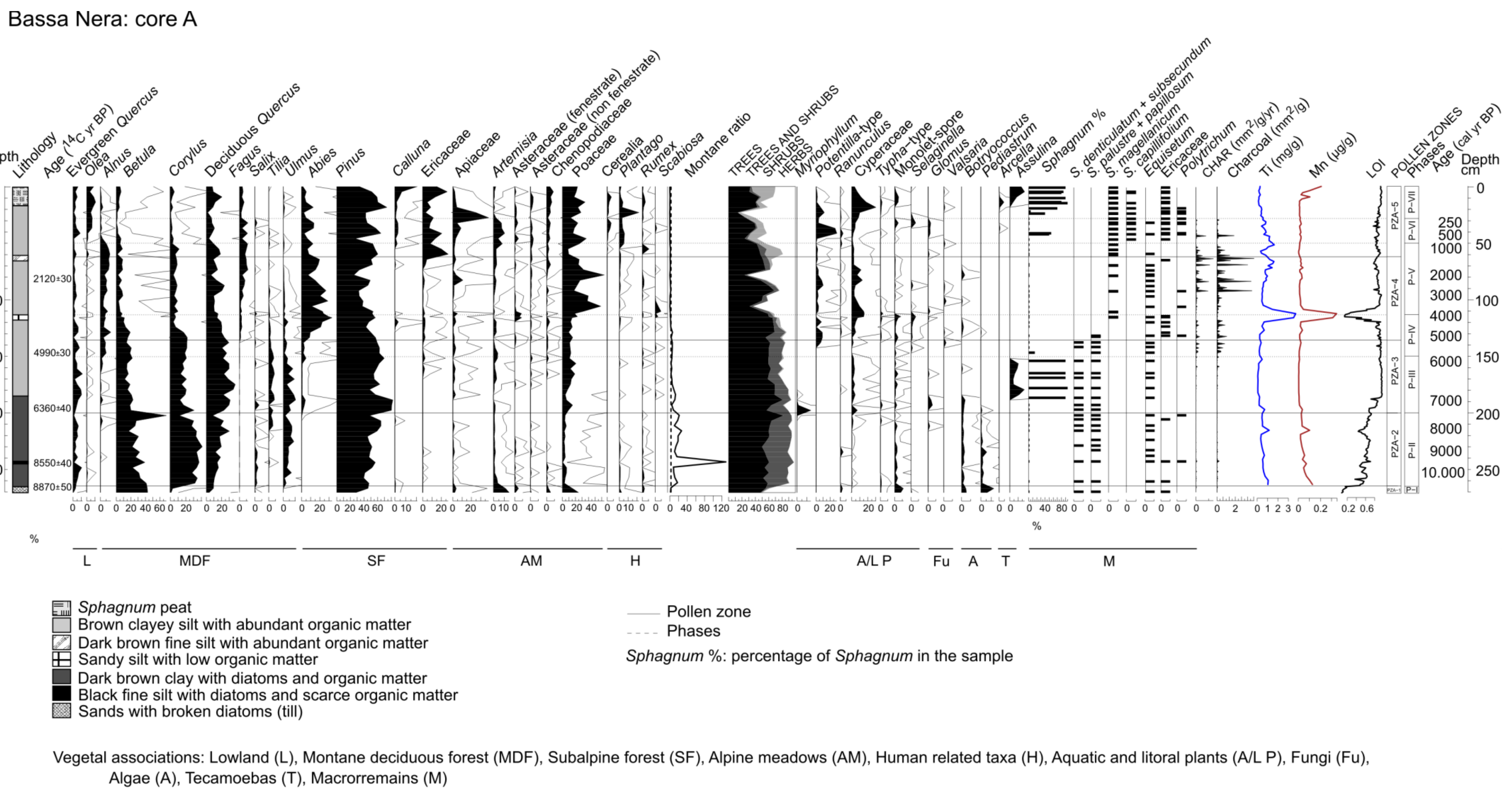

Figure 3.3. Percentage diagram of sporomorphs of core A including: the total pollen (relative abundance ≥1%), montane ratio, aquatic elements and plant macroremains, charcoal concentration (mm²/g) and charcoal accumulation rate (CHAR,mm²/g/yr), chemical elements and Loss on Ignition. *Pinus* pollen, wetland plants and fern spores were excluded from the pollen sum (ΣP). *Pinus* percentage was calculated with the pollen sum plus *Pinus* pollen. *Sphagnum palustre* or *papilosum* and *Sphagnum denticulatum* or *subsecundum* means that the two species could not be distinguished. The dotted line in montane ratio corresponds to the threshold value of 2.5, which indicates the close presence of montane-subalpine ecotone. Higher values imply the occurrence of upward montane vegetation in Bassa Nera. Vegetal associations: Lowland (L), Montane deciduous forest (MDF), Subalpine deciduous forest (SDF), Alpine meadows (AM), Human related taxa (H). The continuous horizontal lines correspond to statistically significant zones (Bennett, 1996).

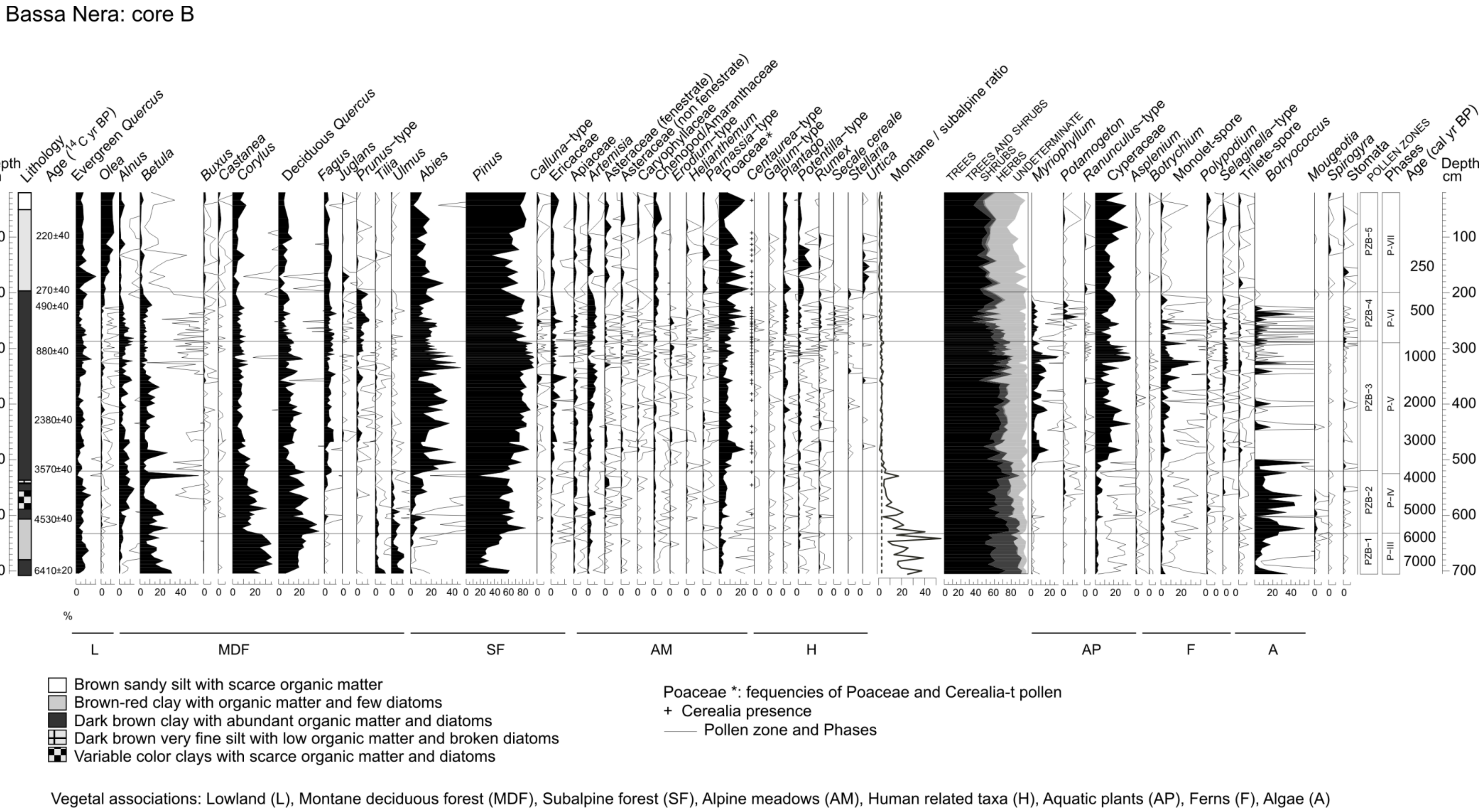

Figure 3.4. Percentage diagram of sporomorphs of core B including: the total pollen (relative abundance ≥1%),montane ratio and aquatic elements. *Pinus* pollen, wetland plants and fern spores were excluded from the pollen sum (ΣP) *Pinus* percentage was calculated with the pollen sum plus Pinus pollen. Due to the low values of Cerealia-t, its presence has been represented by a cross in the Poaceae plot. The dotted line in montane ratio corresponds to the threshold value of 2.5, which indicates the close presence of montane-subalpine ecotone. Higher values imply the occurrence of upward montane vegetation in Bassa Nera.

The maximum values of planktonic algae (*Pediastrum*, *Botryococcus*) might indicate an increase of primary production (Jankovská and Komárek, 2000) as a result of the relative increase of temperatures in a cold freshwater environment. The presence of *Sphagnum denticulatum* or *Sphagnum subsecundum* macroremains is characteristic of semi-submerged or flooded areas. The occurrence of *S. palustre* or *S. papillosum* also points to the early presence of *Sphagnum* lawns or low hummocks. The sediment granulometry and the lowest LOI values also suggest an open water phase with scarce vegetation. The presence of Ranunculus and ferns implies high moisture and an increase in the lake primary production, while the sands with broken pennate diatoms (sedimentary unit IV-A) suggest intense hydrodynamic material input events.

3.4.3.2. Phase II

A drop in herbaceous taxa together with the highest frequencies of deciduous taxa characterise this phase (10070–7343 cal yr BP, core A:264–199 cm, 14 samples). Even though that there is no noticeable increase of montane taxa, the decrease in subalpine-alpine taxa prompted by a drop in Poaceae and Asteraceae led to an increase in the resulting montane ratio. According to modern analogues, the high montane ratio values correspond to the presence of the lower montane altitudinal belt (Garcés-Pastor et al., 2016). In this period, the forest shifted froma *Betula* woodland to a mixed *Corylus* and *Betula* forest with deciduous *Quercus*, *Ulmus* and some *Pinus* (Figure 3.3). Meadows became scarce. The replacement of the sun-tolerant *Betula* by *Corylus* matches the natural succession of secondary species. The higher frequencies of *Corylus* compared to deciduous *Quercus* during the entire phase emphasize the colonizing capacity of the former and suggest stronger oceanic influence (González-Sampériz et al., 2006; Montserrat-Martí, 1992). This coincides with studies that reported the warmest summer temperatures during the early Holocene in the Northern Hemisphere due to maximum summer radiation and minimum winter radiation (Heiri et al., 2003; Anderson et al., 1988; Cacho et al., 2010).

In the aquatic system, the decrease in freshwater algae (Botryococcus, Pediastrum) could indicate shallower or more-turbid waters (Jankovská and Komárek, 2000), which is consistent with the presence of pennate diatoms in sedimentary unit III-A. On the other hand, the co-occurrence of species with affinity for moist

(*Sphagnum denticulatum* or *subsecundum*) and dry conditions (*Sphagnum palustre* or *papillosum*, *Polytrichum*, Ericaceae) evidences a complex *Sphagnum* landscape. The decrease in Ti and Mn, with a change from sands to clays, suggests decreased sedimentary input (Nesje and Dahl, 2001). This coincides with the establishment of forests surrounding BSN, which would have promoted the rise of LOI, stabilized the soils and limited the erosion input into the lake (Figure 3.3). The intercalated layer of fine silt between 9397 and 9358 cal yr BP coincides with an important montane ratio peak (243 cm), suggesting a punctual event of higher moisture that might have favoured the growth of deciduous forest. This could be related to the meltwater events of 9300 cal yr BP described in the Pyrenees and Mediterranean regions, when increased snow accumulation in winter and large snowpack melt during warmer summers led to higher run-off (Pérez-Sanz et al., 2013). Shortly later, between 9195 and 8789 cal yr BP, the drastic decrease in montane values with the rise of meadows (Apiaceae, *Artemisia* and Asteraceae) would point to colder and drier conditions. This period coincides with an episode of forest decline at around 9200 cal yr BP in the western Mediterranean (Fletcher et al., 2013) and a cold and arid event registered at 8800 cal yr BP in Basa de la Mora Lake and the rest of the Iberian Peninsula (Pérez-Sanz et al., 2013).

By 8164 cal yr BP, the occurrence of Ti and Mn peak and a drop in LOI shortly followed by the presence of chlamydospores of the mycorrhizic fungus *Glomus* suggest punctual runoff events (López-Vila et al., 2014). The posterior decline of the montane ratio reflects a downward shift of montane forest led by a *Betula* decrease which can be also found in Bassa de la Mora lake and the Portalet peat bog, which could point to drier summer conditions (Pérez-Sanz et al., 2013; González-Sampériz et al., 2006). The shallower waters, inferred by decrease of algae and rise of Cyperaceae and *Typha*-t pollen, could have prompted the increase of emerged littoral areas. The drier conditions, the peaks of Ti and Mn and the drop in LOI could be related to the 8200 cal yr BP cold event that brought generally cold and dry conditions to the Northern Hemisphere (Alley and Ágústsdóttir, 2005; Rohling and Pälike, 2005) and the Central Pyrenees (González-Sampériz et al., 2006).

3.4.3.3. Phase III

This phase is characterised by a sharp decrease in *Corylus* and *Betula*, as the forest succession evolves (7343–5832 cal yr BP, 11 samples core A: 199–150 cm; 13 samples core B: 706–632 cm). The montane ratio decreases and fluctuates around a value of 20, which suggests punctual episodes of montane forest downward migration. Even so, the lower montane belt was still present at the site, composed of a mixed *Corylus* and deciduous *Quercus* woodland with some *Betula*, *Tilia* and *Ulmus*. Meadows increase, indicated by the rise of Poaceae and *Artemisia*. *Salix* slightly drops and *Ulmus* shows a decreasing trend, while *Alnus* rises around the end of the phase. *Abies* appears in the beginning of this phase and rises by 6356 cal yr BP This is in line with the east-to-west colonization of the Central Pyrenees by firs (Pèlachs et al., 2009b; Matías et al., 2016). The marked reduction in *Pinus* around 7000 cal yr BP observed in core B could point to an increase in temperature and precipitation, as reported across the Iberian Peninsula (Pérez-Díaz et al., 2016) (Figure 3.4). On the other hand, a higher fire frequency is inferred from the noteworthy increment of charcoal by 6200 cal yr BP (150 cm). The rise of Cyperaceae points to shallower waters and higher extent of littoral areas and, which is corroborated by the differences between both records due to small-scale spatial variability.

Among the aquatic and littoral elements, the spatial differences illustrate two scenarios. Core A shows the peatland development at the shore of the lacustrine system by 6866 cal yr BP, likely forming lawns. This is inferred from the increase in Sphagnum percentages dominated by *S. palustre* or *S. papillosum* with the occurrence of the cercozoan *Assulina* (185–150 cm), characteristic of non-water-saturated topsoils (Charman et al., 2000). The rise in LOI and Cyperaceae together with a decrease in planktonic algae and the change from clay with abundant diatoms to peat also supports this interpretation. In core B, the dominance of *Botryococcus*, fewer sedimentary diatoms (unit III-B) around 6822 cal yr BP and low Cyperaceae frequencies points to some water level. The change from brown-dark to brown-red clay with organic matter suggests periods of subaerial exposure or hydric fluctuations (Figure 3.2). These changes in lake level might have increased water turbidity in shore environments, perhaps affecting algae development and favouring the disappearance of *Pediastrum* in core A (Jankovská and Komárek, 2000), while *Botryoccoccus* and

Mougeotia rise in the shallow waters of core B. Nearby localities such as Portalet and Estanilles peat bogs and Burg Lake also recorded a transition from a lake to a peat system at similar times (González-Sampériz et al., 2006; Pérez-Obiol et al., 2012; Pèlachs et al., 2011).

3.4.3.4. Phase IV

A transition from deciduous to coniferous taxa marks this phase. The decrease in deciduous *Quercus* and the drop in *Betula* and *Corylus* indicate a downward shift of deciduous forest (5832–3912 cal yr BP, 10 samples core A: 150–112 cm; 19 samples core B: 632–525 cm). However, montane ratio values around 5 indicate the occurrence of upper montane forest on the site. On the other hand, *Abies* expanded and *Fagus* appeared by 4492 cal yr BP. This vegetation shift could point to rainy and warm summers, where *Abies* rose in altitude to avoid the warming, and a change to greater precipitation might have promoted a downward displacement of optimal deciduous habitats (Alba-Sánchez et al., 2010; Pèlachs et al., 2011). The replacement of *Ulmus* by *Alnus* in 5286–4054 cal yr BP is coherent with the decline of elm in the rest of the Pyrenees (Montserrat-Martí, 1992; Reille and Lowe, 1993). The establishment of emerged lands and lakeshore environments might have favoured colonization by *Alnus* (Pérez-Obiol et al., 2016; Revelles et al., 2015). The alpine meadows continued their rising trend, dominated by Poaceae, *Artemisia* and some Apiaceae. This coincides with an intensification of anthropic pressure, as inferred by the increase in fires and agropastoral pollen indicators (*Centaurea*, Cerealia-t, *Potentilla*).

In the wetland plant communities, littoral site A has a peaty marsh environment, as inferred by the occurrence of aquatic plants (*Ranunculus*-t and *Typha*-t) and *Selaginella*-t since 5526 cal yr BP and a drastic decrease in Sphagnum percentages. Around 5043 cal yr BP, both *S. palustre* or *papillosum* and *S. denticulatum* or *subsecundum* disappeared in conjunction with a rise in Ericaceae and some *S. magellanicum*. The ombrotrophic affinity of these taxa points to drier peaty habitats. On the other hand, the water level increased at site B, as indicated by the occurrence of *Myriophyllum* and planktonic algae (*Botryococcus* and *Mougeotia*) (Grosjean et al., 2001; Van Geel, 2001). Subsequently (590 cm), the watershed shrank, giving rise to marshy conditions with the increase of *Potamogeton* and Cyperaceae and the reduction of diatoms in clay sediments, leading to peat formation by 4359 cal yr BP with a change to dark-brown clay.

A remarkable increase in erosion, likely due to a rise in the recurrence of flood events, occurred around 4049 cal yr BP, as evidenced by a sharp decrease in LOI, peaks in Ti and Mn and the presence of an inorganic silt layer in both cores. This event might have damaged the forest that surrounded the pond, with a decrease in deciduous *Quercus*, *Abies* and *Pinus* and may have prompted the rise of the montane ratio, possibly explained by the colonization of the degraded terrain by *Betula*. The presence of *Equisetum* and the absence of Ericaceae at site A, characteristic of pond margins, also suggest an increase in moisture around 3992 cal yr BP (Pérez-Haase and Ninot Sugrañes, 2006). These flood events that appear locally exaggerated in BSN are in accordance with a period of wetter conditions registered between 4500 and 3900 cal yr BP at nearby study sites such as Basa de la Mora Lake and the rest of the Iberian Peninsula, such as Sanabria and Enol Lakes (Pérez-Sanz et al., 2013; Jambrina-Enríquez et al., 2014; Moreno et al., 2011). Indeed, lakes from France and Italy have also registered phases with increasing humidity in this period (Magny et al., 2013; Simonneau et al., 2013), a dynamics attributed to positive synergies between cold climatic oscillations and human-induced soil destabilization and erosion.

3.4.3.5. Phase V

The highest abundances of coniferous taxa suggest that the vegetation surrounding the pond was dominated by *Abies* and *Pinus* with some mixed montane forest (deciduous *Quercus*, *Betula* and *Fagus*) (3912–792 cal yr BP, 14 samples core A: 112–50 cm; 45 samples core B: 525–290 cm). Although the montane ratio continued to decline, the values around 2.75 indicate the proximity of the montane upper limit to BSN; *Alnus* still dominated the riverine forest. On the other hand, a higher anthropic pressure (3000 cal yr BP) through forest clearance is evidenced by the intensification of fires, the noteworthy rise of shrubs (Ericaceae) and the increase of agropastoral indicators (Cerealia-t, *Potentilla*).

Regarding the aquatic system, site A continues showing a pond margin scenario at 4049–1455 cal yr BP with the continuity of *Equisetum* and the absence of Ericaceae, while site B still has some water level, as inferred by the rise of

Myriophyllum and the presence of diatoms in the sediment (Grosjean et al., 2001). However, their decrease at 3169–2303 cal yr BP points to shallower waters and matches a period of intensified aridity in the Pyrenees and across the Mediterranean (Pérez-Sanz et al., 2013; Jalut et al., 2000). The chemical elements remain stable along the phase until 1369–1231 cal yr BP, when an important drop in Ti coincides with an intercalated layer of fine silt (unit II-A) and Ericaceae macroremains at site A, pointing to a period of dried substrate and lower sedimentary input.

3.4.3.6. Phase VI

This phase is marked by the highest values of *Prunus*-t and a notable rise in herbs (Poaceae, *Artemisia*, Chenopodiaceae/Amaranthaceae) (792–386 cal yr BP, 3 samples core A: 50–38 cm; 21 samples core B: 290–200 cm). *Abies* and *Pinus* forest remained in detriment of deciduous vegetation (deciduous *Quercus*, *Fagus* and *Betula*), which experienced a downward shift, supported by montane values below 2.75. The increase of meadows and the co-occurrence of charcoal and anthropogenic pollen indicators (*Secale cereale*, Cerealia-t) indicate the continuity of forest clearances for agropastoral purposes.

In the wetland community, Sphagnum values > 40% in site A show the development of drier *S.magellanicum* and *S. capillifolium* hummocks, with Ericaceae and *Polytrichum* (Pérez-Haase and Ninot Sugrañes, 2006) between 499 and 465 cal yr BP. At site B, the transition from *Myriophyllum* to *Potamogeton* by 544 cal yr BP points to a lower lake level. Moreover, a paleoecological study of the last 1000 years in BSN (Garcés-Pastor et al., 2016) reported extreme fluctuations of diatom concentrations and a decrease of planktonic frequencies, which were interpreted as responses to periods of strong seasonality and hydric fluctuations (Figure 3.4).

3.4.3.7. Phase VII

The highest values of Poaceae and lowland pollen (evergreen *Quercus*, *Olea*) characterise this phase (386 cal yr BP–present, 8 samples core A: 38–0 cm; 16 samples core B: 200–0 cm). A conifer forest (*Abies*, *Pinus*) surrounded BSN, while the low montane ratio values show the continuity of the montane boundary at lower altitudes. The understorey (Ericaceae) and alpine meadows grew. The decrease in fires and agropastoral indicators (*Prunus*-t, Cerealia-t), with the highest values of *Potentilla* between 160 and 110 cm, suggest a complex agroforestal system with a dominance of pastoral activities (Garcés-Pastor et al., 2016).

On the aquatic system, the dominance of Cyperaceae points to the continuity of a thin water layer. The establishment of the peat bog at site B at around 370 cal yr BP was marked by a change from dark-brown to red-brownish fibrous peat moss. At the littoral site A, the disappearance of *Equisetum* by 171 cal yr BP suggests less moisture. Finally, the higher percentages of *Sphagnum* (*S. capillifolium* and *S. magellanicum*) and Ericaceae point to the establishment of an ombrotrophic hummock in recent times.

3.5. Discussion

3.5.1. Vegetation response to the North Atlantic influence: montane ratio and IRD

The ice rafted debris (IRD) index from the North Atlantic (Bond et al., 2001) has been used as an indicator of general climatic patterns during the Holocene in regions influenced by North Atlantic Ocean circulation (Battarbee et al., 2004). High IRD values have been correlated with southward displacements of the Atlantic westerly jet, prompting increased precipitation and higher water levels in mid-European lakes (Magny et al., 2001; Magny, 2004). Likewise, in the Pyrenees, González-Sampériz et al. (2006) reported a translation of climate variability from the North Atlantic to the mid-latitudes, and Pèlachs et al. (2011) found a correlation between IRD and deciduous tree pollen percentages in Burg Lake, where high IRD corresponded to wetter and occasionally colder conditions.

With the aim of evaluating the North Atlantic influence in BSN vegetation shifts, we compared the montane ratio with the IRD index (Figure 3.5). Cores A and B present different behaviour because of their varying sedimentation rates. Core A shows a smoothed curve along the record since it has a lower sedimentation rate. On the other hand, core B offers a greater detail of the altitudinal shifts due to its higher sedimentation rate and resolution. Similar montane ratio and IRD trends are apparent during the first half of our records (between 10,200 and 5300 cal yr BP), when high IRD values (the main Bond events 6, 5 and 4 and other secondary peaks) coincide with increases in the montane ratio (10,000; 9400; 8100; 7300; 6800; 5750; 5500; 5300 cal yr BP). The remarkable montane peak at around 9400 cal yr BP (core A), which could be related to a punctual high moisture event, has also been registered in Burg and Redon Lakes as a phase of low LOI and colder winter/spring temperatures (Pla and Catalan, 2005; Pèlachs et al., 2011). However, by the end of Bond event 4, the montane ratio starts to decouple from IRD, and fully decoupled trends can be seen henceforth during the most recent half of our records, when the montane ratio maintains stably low values, despite the occurrence of several Bond events with high IRD values (Figure 3.5). Therefore, montane ratio peaks during the first half (10200–5300 cal yr BP) may be related to the growth of deciduous taxa in response to a regional rise of moisture in the Northern Hemisphere, in agreement with Pèlachs et al. (2011). However, the increasing mismatch between both trends suggests that other pressures besides the North Atlantic climate (probably at a more local level) have ultimately influenced BSN vegetation. Regarding those pressures, charcoal notably increased around 6700 cal yr BP, indicating that fires in the area might have affected the montane forest and prompted the early decrease of the montane ratio by 6650 cal yr BP. Shortly later, in 6400–6100 cal yr BP, charcoal becomes recurrently abundant, coinciding with a notable decrease of *Betula* and the montane ratio. Indeed, *Betula* and the montane ratio fall to even lower values around 6140 cal yr BP, when a notable charcoal peak occurs (Figure 3.5). During that period, frequent fires might have interfered with the response of montane vegetation to the regional moisture increase inferred from higher IRD. By 5700 cal yr BP, charcoal notably increased, suggesting higher fire frequency. This coincides with a drop of *Corylus*, suggesting that fires may have burnt the montane forest and favoured resprouting species such as Ericaceae. Even though the fire frequency was reduced around 5400 cal yr BP, the montane ratio did not respond to the rise of IRD by 5250 cal yr BP. However, a period with less fires could have prompted the recovery of the montane ratio by 5100 cal yr BP, which responded to IRD with a slight delay until 4750 cal yr BP.

Thus, the montane vegetation responded to climatic forcing from 10,200 to 6700 cal yr BP, followed by a transition period (5700–5250 cal yr BP) when fires could have affected the resilience of deciduous vegetation, hampering its response to

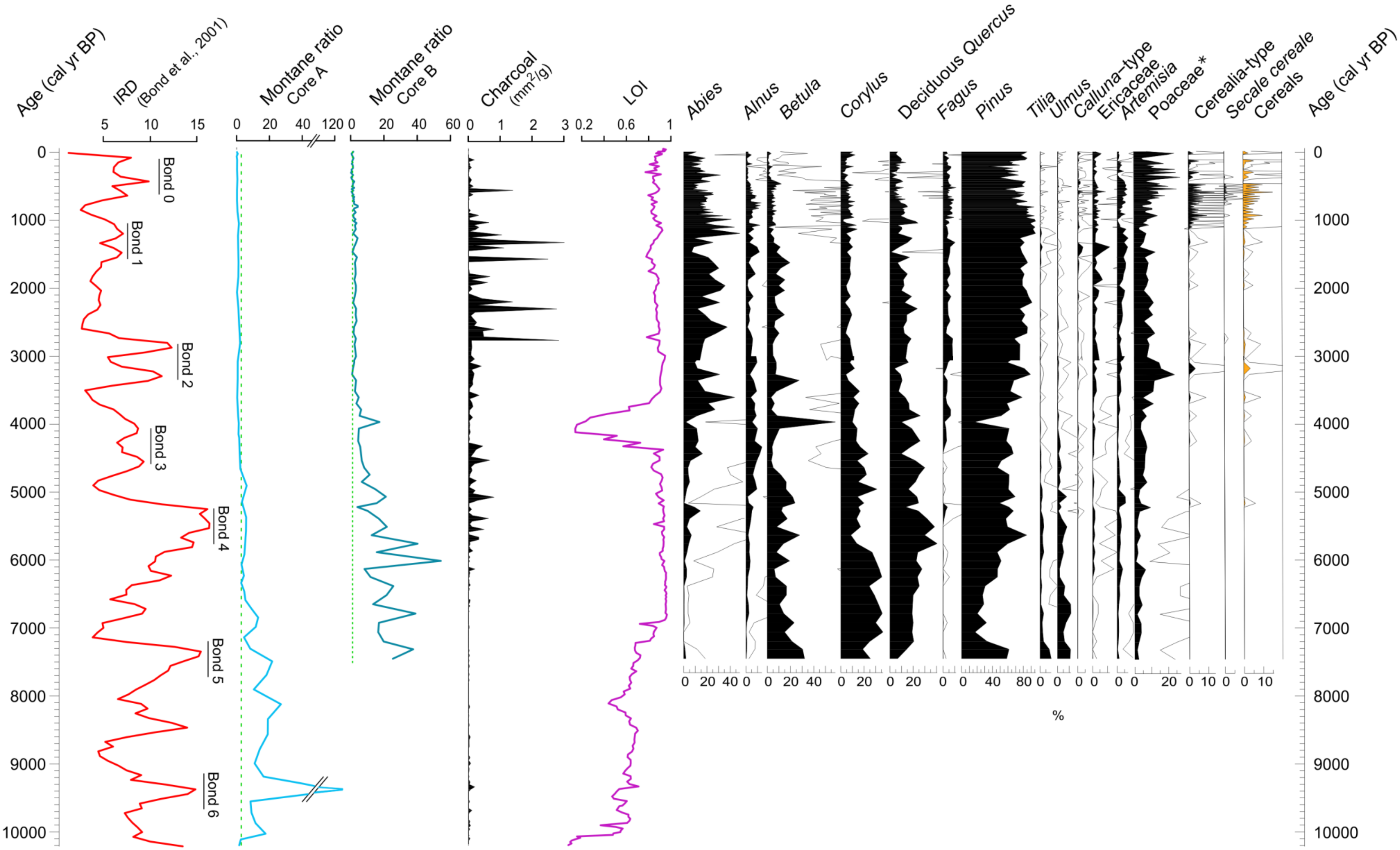

Figure 3.5. Summary diagram grouping IRD (Bond et al., 2001), Bond events, montane ratio of cores A and B, Charcoal concentration (mm²/g), LOI and relevant selected pollen taxa of core B.

climate. These results agree with those found in the nearby Basa de la Mora Lake, which also presented a retreat in deciduous taxa from 5700 cal yr BP, when frequent fires and climatic forcings overcame the regeneration capacity of the vegetation (Lasheras-Álvarez et al., 2013). Although Portalet peat bog shows a hiatus by this transition period, it also recorded a contraction of deciduous forest from 7700 cal yr BP, attributed to a threshold response of the ecosystem to high fire frequencies (Gil-Romera et al., 2014). Burjachs and Expósito (2015) also noted that vegetation in the Mediterranean area of the Iberian Peninsula was more resilient during the first half of the Holocene than in the recent Holocene. By 5250 cal yr BP, climate and fires could have weakened the resilience of deciduous vegetation beyond a threshold that prompted the progressive downward shift of montane vegetation between 5300 and 4200 cal yr BP in BSN. Indeed, Pla and Catalan (2005) showed that the climate was more continental before 4000 cal yr BP, whereas Jalut et al. (2000) and Pérez-Sanz et al. (2013) reported a change in precipitation seasonality towards more frequent summer droughts, namely, a transition from a significant Atlantic influence to a typical Mediterranean climate that affected the deciduous forest composition and resilience while promoting fires.

3.5.2. Local differences in the response of organicmatter accumulation to the North Atlantic influence: LOI and IRD

The close correlation found between Bond IRD oscillations and the accumulation of organic matter reported in Burg Lake (1821 m a.s.l.) by Pèlachs et al. (2011) suggests a direct North Atlantic influence on climate fluctuations in the Central Pyrenees. Of the Pyrenean studies that have used loss on ignition as an organic matter indicator (Pla and Catalan, 2005; González-Sampériz et al., 2006; Pèlachs et al., 2011), Burg is the only lentic system with a continuous record that can be compared to our results. The proximity between BSN and Burg (34.5 km) makes them suitable places to study the response of subalpine ecosystems at the regional level. However, the LOI trend at BSN differs from the oscillating record at Burg. In BSN, the LOI values approach 90% by 6900 cal yr BP (Figures 3.3 and 3.5) and stay rather constant except for a remarkable decrease around 4049 cal yr BP. Conversely, Burg has stronger LOI oscillations, reaching 90% values by 5100 cal yr BP and fluctuating thereafter following the IRD trend (Pèlachs et al., 2011). Although both records have a different response amplitude, they present some similarities in 5500–3800 cal yr BP, and we hypothesise that this corresponds to a wetland phase, according to a sediment change from organic silt to peat and higher LOI values at both sites. In this general trend, several aridity phases have been identified at different moments on the Iberian Peninsula during the Middle Holocene, for example, between 6200 and 5600 and 4600–4300 cal yr BP (Pérez-Sanz et al., 2013; Pèlachs et al., 2007; Azuara et al., 2015). The LOI at BSN is less sensitive to global climate changes than at Burg and thus shows a high influence of local factors, which might have hidden any direct relationship between IRD oscillations and BSN peat bog development (Mäukiläu, 1997). The accumulation of organic matter in a wetland is promoted by temperature and/or water inundation (Crawford et al., 2003), the latter being mainly controlled by precipitation (Charman et al., 2009). Therefore, we can attribute differences in the LOI trends between both sites to contrasting water inundation patterns. The higher and more constant values of LOI in BSN suggest less water table variations than Burg. These differences could have been influenced by the orographic conditions of both sites, which would have determined the precipitation regime. Although significant environmental changes occurred in BSN during the entire record, these shifts do not seem to have influenced the LOI values, highlighting that the local influence is too important to allow climatic inferences from this variable. On the other side, Burg could have been more dependent on precipitation that would have prompted an increase of paludification and LOI fluctuations. On the other hand, the Portalet peat bog (1802 m a.s.l.) was established after 6400 cal yr BP, reached LOI values around 40% and fluctuated thereafter due to alternate periods of peat and shallow clastic lake deposition, until a hiatus in 5300 cal yr BP (González-Sampériz et al., 2006). Finally, the Molina peat bog, located in north-west Spain (Cantabrian region) was established around 6000 cal yr BP and reached LOI values close to 90% and then remained stable until the last millennium (Pérez-Obiol et al., 2016). These different scenarios show how local influence could limit the suitability of LOI as a paleoclimate indicator in the case of peat bog systems. However, it may be more useful in environments with higher Mediterranean influence where climatic oscillations are more pronounced (Azuara et al., 2015).

3.5.3. Anthropic influence: fire and crops

The low fire frequency and the absence of anthropic indicators between 9968 and 8548 cal yr BP in Bassa Nera could be attributed to natural fires prompted by the large amounts of deciduous biomass, as occurred in the nearby Portalet peat bog and Basa de la Mora Lake in the same period (Gil-Romera et al., 2014; Lasheras-Álvarez et al., 2013).

The BSN catchment is surrounded by many archaeological sites that evidence human occupation and exploitation of the high mountains of the Pyrenees since the Neolithic (from 9000 to 8571 cal BP) (Gassiot et al., 2014). The first anthropic evidence in the PNAESM area is some hunting artefacts from7650 to 7325 cal yr BP found in Sardo cave, located 9 km from BSN (Gassiot et al., 2012). Shortly after, the rise in agropastoral indicators in BSN (*Artemisia*, Asteraceae, *Potentilla*, *Galium*, *Centaurea*, *Rumex*) and charcoal particles indicate grazing activities between 7343 and 5832 cal yr BP. This is coherent with agropastoral activities recorded in the nearby records of Sardo cave (Gassiot et al., 2012), Bosc dels Estanyons (Miras et al., 2007) and the Estanilles peat bogs (Cunill et al., 2013). The intermittent presence of Cerealia-t pollen in our records between 5190 cal yr BP and 2000 cal yr BP, together with agropastoral indicators and charcoal, might be evidence of some cereal-based agriculture near BSN. Although the first record is a punctual event (5190 cal yr BP), it fits with the development of cereal-based subsistence in the area, coinciding with cereal seeds found in Sardo cave and Cerealia-t pollen recorded in the Burg lake and Madriu valley records (Pèlachs et al., 2007; Miras et al., 2007; Gassiot et al., 2014). A higher fire frequency in 5190–4300 cal yr BP and the rise in pastoral indicators (*Potentilla*, *Rumex*) suggest an increase in grazing activities in BSN, coinciding with an intensification of archaeological settlements found in PNAESM (Sardo cave, Estany de la Coveta I, Obagues de Ratera and Saboredo). These lines of evidence of high mountain exploitation in the Central Pyrenees (Gassiot et al.,2014;Pèlachs et al.,2007; Jalut et al., 2000) were probably prompted by the increased frequency of dry summers around 4600–4300 cal yr BP in the Mediterranean area (Jalut et al., 2000; Azuara et al., 2015). The rise of fires during the Bronze Age and the scattered presence of Cerealia-t (4230–3500 cal yr BP) point to forest clearance and occasional crops. The spread of *Fagus*, which occurred in the same period (Figure 3.4), could have been favoured by the resulting open spaces from anthropogenic disturbance followed by a change to higher precipitation (Miras et al., 2007; Pèlachs et al., 2009b). The rise of *Fagus* in BSN fits with its expansion in the Pyrenees and Cantabrian mountains (Pérez-Sanz et al., 2013; Montserrat-Martí, 1992; Magri, 2008). During the Late Bronze Age (3150–2650 cal yr BP), agricultural landscapes were established in BSN with the rise of Cerealia-t and agropastoral indicators (*Centaurea*, *Potentilla*, *Artemisia*, *Asphodelus*). This agrees with the record of higher anthropic pressure through farming and pasturing activities found in the nearby Burg, Estanilles and Bosc dels Estanyons peat bogs (Bal et al., 2011; Pérez-Obiol et al., 2012; Miras et al., 2007). Fires increased by 2800 cal yr BP, and Cerealia-t reappeared with the Roman Period (2000 cal yr BP). By the Middle Ages (1100 cal yr BP), an intensification of agriculture and livestock is indicated by the rise of Cerealia-t, grazing indicators (*Galium*, *Potentilla*) and fires, coinciding with an increase of human settlements in PNAESM and the Central Pyrenees (Catalan et al., 2013). At 800 cal yr BP, the appearance of *Secale cereale* together with the rise of Poaceae, agropastoral and arboriculture indicators (*Rumex*, *Artemisia*, *Castanea*, *Juglans*) point to crop diversification and an increase of pastures (Garcés-Pastor et al., 2016). The rise of *Prunus*-t in this period could be related to cultivated species such as *P. domestica* or *P. avium*. From 450 cal yr BP, the scattered cereal presence and the higher amounts of Poaceae and pastoral pollen (*Potentilla*, *Urtica*) suggest the abandonment of farming, the spread of meadows and the highest grazing exploitation period of the sequence. The resulting opening of the landscape would have led to the higher upward flow of *Olea* and evergreen *Quercus* from an increase of agricultural practices in the lowlands (Cañellas-Boltà et al., 2009). Since 120 cal yr BP, the forest clearance and a peak in Poaceae might be the result of an increased need for supplies and raw materials during the Industrial Revolution (Garcés-Pastor et al., 2016). Later, the disappearance of cereals and the reduction of agropastoral pollen indicate crop abandonment. However, the presence of *Potentilla* suggests some grazing activity until the establishment of the PNAESM in 1955 cal AD and the protection of its surroundings in 1990 cal AD.

Agriculture in BSN has passed through short exploitation phases interspersed with periods of land abandonment and grazing. Some authors suggest that these human occupation phases could be a result of the synergic effects between climate changes and human activity (Gassiot et al., 2012; Jalut et al., 2009). To check whether the occupation phases in BSN could have been prompted by climate, we compared IRD with the Cerealia-t pollen frequencies (Fig. 5). Our results suggest that cereals rise when IRD presents low values (c. 5200, 4250, 3600, 3180, 1950, 1300 cal yr BP), in agreement with Pèlachs et al. (2011) in Burg lake. The only occurrence of cereals with high IRD values occurs with *Secale cereale* in 750–500 cal yr BP, known for its resistance to cold environments and which is also cultivated in the Estanilles peat bog (Cunill et al., 2013; Pérez-Obiol et al., 2012). In agreement with the appearance of cereals in other studies of the Central Pyrenees and coinciding with Magny (2004) the development of cereal-based subsistence in BSN could have been prompted by the rise of the regional population and by dry conditions. The farming activities in BSN and their influence in the environment became evident during the Bronze Age and intensified in the Roman period and Middle Ages.

3.6. Conclusions

In this multi-proxy study we reconstructed the palaeoecological and ontogenic events recorded in Bassa Nera during the last 10,200 cal yr BP. Changes in aquatic taxa, macroremains and sedimentary units show a non-linear development of the peat bog over the larger previous lake. The study of two separated cores allowed us to compare some remarkable spatial differences that took place within the same catchment. The pollen and the montane ratio were useful to infer structural and altitudinal changes in montane forest through the last 10,200 cal yr BP. Vegetation strongly responded to climate during the first half of the Holocene (10200–6700 cal yr BP) with punctual episodes of downward shift in the montane forest. *Abies* appeared by 6356 cal yr BP and expanded. Then, a transition period took place (5700–5250 cal yr BP) when climate and fires prompted a progressive downward shift of montane vegetation and its replacement by coniferous taxa, which has dominated the catchment with some mixed montane forest since 3912 cal yr BP. The montane ratio was a useful tool for summarizing palaeopalinological data, enabling the assessment of the potential correlations between changes in vegetal communities and the climatic forcing indicated by the IRD, and highlighting the different responses of the vegetation to the North Atlantic influence in Bassa Nera during the Holocene. The study of LOI and sedimentary units allowed us to infer important flood events between 4500 and 3900 cal yr BP. From 7300 cal yr BP onwards, charcoal and pollen indicators evidence human disturbance through grazing, pointing to the use of fire as a tool for forest clearance or maintaining open spaces. The first cereal crops in Bassa Nera occurred around 5190 cal yr BP and coincided with dry climate conditions until the cultivation of cold resistant species like *Secale cereale*. The second half of the Holocene features phases with increased occurrence of agricultural practices alternating with land abandonment and grazing. Notable periods of anthropic pressure include the Late Bronze Age, with the establishment of agricultural landscapes, followed by the Roman Period and Middle Ages. The results from this study highlight the sensitivity of the high-mountain vegetation of the Central Pyrenees to climate changes and anthropic pressures.

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Chapter 4

Subalpine forest dynamics reconstructed throughout the last 700 years in the Central Pyrenees by means of pollen and tree rings

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Garcés-Pastor, S., Gutiérrez, E., Martínez-Sancho, E., Dorado-Liñán, I., Camarero, J.J., Cañellas-Boltà, N., Vegas-Vilarrúbia, T. Subalpine forest dynamics reconstructed throughout the last 700 years in the Central Pyrenees by means of pollen and tree rings. *The Holocene*

Abstract

To predict the influence of the ongoing climate warming on growth and composition of Pyrenean forests and to improve our knowledge of the vegetation-climate-site complex, it is necessary to understand how climate has modulated them in the past.. The present work assesses the past dynamics of subalpine forests of Central Pyrenees during the last 700 years by studying the relationships between sedimentary pollen and tree-ring records, and their link with climatic drivers. With these aims, we compared the pollen record and calculated the montane-subalpine pollen ratio, an index obtained from sedimentary pollen that allows inferring past altitudinal variations in the montane-subalpine ecotone, with tree-ring width data from old *Pinus uncinata* subalpine forests located in the “Aigüestortes i Estany de Sant Maurici" National Park. To assess climate-growth associations, we related the dendrochronological data with instrumental meteorological records of the 1901-2010 period and with temperature reconstructions for the Pyrenees and Northern Hemisphere for the last 700 years. Few robust associations were found between any specific arboreal pollen taxa and the tree-ring width series of the surrounding forests. However, a significant correlation was found between the integrative montane-subalpine pollen ratio and the pines growth of nearby (less than 10 km apart) subalpine forests. This relationship could be potentially useful to reconstruct long-term forest productivity and growth changes at decadal to centennial scales using the montane-subalpine pollen ratio. On the other hand, our results suggest that the sensitivity of pines growth to climate has varied over the last 700 years. Similar results have been obtained for the last century as tree-growth variability at high elevations is more constrained by low than by high temperatures, although we also noted a relaxation of this constrain in recent decades.

keywords: dendroecology, tree rings, pollen, subalpine forests, Mountain pine, Pyrenees

4.1. Introduction

Climate and land-use changes are rapidly transforming mountain forests ecosystems at extraordinarily short-term scales (Améztegui et al., 2010). Unfortunately, the adaptability of those high-elevation ecosystems is limited in comparison to the rate of temperature change and land abandonment (Pauli et al., 2003). For instance, subalpine forests and alpine flora have shifted upwards in response to the current climate warming (Jump and Peñuelas, 2005) prompting changes in mountain biodiversity (Gottfried et al., 2012; Thuiller et al., 2005). Indeed, the IPCC (2013) estimations of future climate scenarios predicts large effects on the vegetation of some mountain ranges as the Pyrenees. For example, the Pyrenean forests showed an enhancement of tree recruitment and densification of the alpine ecotones over the 20th century in response to climate warming and land-use abandonment (Camarero and Gutiérrez 2004; Batllori and Gutiérrez 2008). However, the IPCC projections of climate-vegetation couplings are limited by the variability at multi-decadal and longer timescales (Dorado-Liñán et al., 2012), in part due to the short length of instrumental records and biotic proxies. Therefore, palaeoenvironmental studies are necessary to improve our comprehension of the long-term forest dynamics and better understand the actual forests composition as well as their future evolution, in order to apply appropriate management measures and to do more accurate projections of vegetation shifts and species distribution.

The present palaeoecological study focuses on pollen and tree-ring records, two well-studied proxies that encode different but complementary characteristics of forest ecosystems and tree performance. The sedimentary pollen allows to reconstruct the presence and abundance of vegetation taxa along thousands of years with multi-decadal resolution (Foster et al., 2006), whereas annually resolved tree-ring chronologies provide detailed information of environmental factors, mainly climate, that has affected tree growth (Fritts, 1976; Helama et al., 2010; Bosch and Gutiérrez, 1999). This study aims to combine the strengths of palynological and dendrochronological reconstructions to provide a temporal framework that will allow further studies to determine possible forest responses to future environmental conditions. In order to provide a solid knowledge about the rate, intensity and geographical extent of climate change, it is necessary to combine environmental reconstructions with instrumental climate data to have a proper assessment of the vegetation-climate-site complex. This paper will compare the palynological and dendrochronological data of subalpine forests of the Pyrenees with reconstructed temperature for the last 700 years and with the instrumental climate data of the 1901-2010 period.

In the Pyrenees as in other mountain ranges the coexistence and replacement of diverse tree species with different sensitivities to climate occurs along altitudinal gradients (Gutiérrez Merino et al., 1998; Tardif et al., 2003). High-elevation subalpine forests are especially sensitive to climatic change, since the species populations forming the uppermost distribution limit are growing under unfavourable conditions characterized by low temperatures and short growing seasons. In this regard, Batllori et al. (2012) have shown that sub-regional response patterns of growth and recruitment to temperature, which exerts a positive effect, are rather homogeneous across the Pyrenean altitudinal ecotone. These results could explain why natural forests limits appear at ca. 2300-2400 m a.s.l (Batllori and Gutiérrez 2008). There are evidences that during warmer periods in the past, the ecotone between the subalpine forest and montane forest vegetation belts reached higher altitudes in this range (Cunill et al., 2012; Garcés-Pastor et al., 2016). Nowadays, a higher tree recruitment near the treeline has been registered as a result of the recent increases of temperature (Camarero and Gutiérrez 2004; Batllori and Gutiérrez 2008), but forest ascent is not generalized across the Pyrenees. Our study takes place in the region of "Aigüestortes i Estany de Sant Maurici" National park (hereafter PNAESM) and nearby areas, in Central Pyrenees (Spain), a well-studied area in terms of palaeoecology and dendrochronology with many relatively undisturbed and old conifer forests (Pla and Catalán 2005; Garcés-Pastor et al., 2016; Camarero et al., 2015).

Forest-growth models predict that climate warming may enhance the productivity of mountain pine, which is mainly limited by low temperatures (Andreu et al., 2007; Camarero et al., 2015; Sánchez-Salguero et al., 2016). The referred studies have been carried out at large spatial scales (i.e. Pyrenees and Iberian Peninsula) to provide a general assessment of the vegetation response. But at the same time, they also found that some parameters of the projection models were influenced by the different local climatic conditions and thus highlighted the importance to perform local studies in areas that share similar environmental features. Assessing the relationships between radial growth and site conditions along ecological and altitudinal gradients will provide a proper background to improve the future climate tree growth models (Sánchez-Salguero et al., 2016).

In order to understand how the subalpine forests have changed in response to past climate forcings at local and centennial scales in the Central Pyrenees, we have used the palaeoecological information provided by the pollen data of a late Holocene lacustrine section from a high-elevation site (published in Garcés-Pastor et al., 2016) and the tree-ring width chronologies of mountain pine from 19 high-elevation sites (mostly forest limits) situated along the Central Pyrenees (published in Gutiérrez Merino et al., 1998; Tardif et al., 2003, Galván et al., 2014), providing a wide spatio-temporal information about mountain forests growth. We will also use the montane-subalpine pollen ratio, developed as an indicator of pollen types, to infer past altitudinal shifts of the montane-subalpine ecotone (Garcés-Pastor et al., 2016; 2017). This pollen ratio could also probably be used to monitor site-specific changes in natural communities in response to the current climate warming. The combination of this ratio with the mountain pine growth will provide information about the conditions at the subalpine stage and about both montane-subalpine and subalpine-alpine ecotones.

The direct comparison between pollen, montane-subalpine ratio and tree-ring width data series from the same area allows a broader estimation of subalpine vegetation responses to climate forcings at local scale. This paper analyses potential palynological and dendrochronological responses to climate, in order 1) to find the relationship between those proxies that better describes the dynamics of subalpine forests and montane-subalpine forest ecotones along the last 700 years, and 2) to assess how recent climate change can affect forests growth at local scales in the Central Pyrenees (PNAESM) by studying climate-tree growth relationships at different temporal scales. To fulfil these aims, first we analyse the relationships between mountain pine growth and climate reconstructions during the last 700 years, and second we repeat these analyses but using instrumental climate records for the last century at monthly and seasonal resolution.

4.2. Materials and methods

4.2.1 Study area

Among conifer species, the mountain pine (*Pinus uncinata* Ram) is known for its wide ecological tolerance and for being adapted to the harsh climate conditions and shallow soils depths, allowing it to dominate the high-elevation forests of the Pyrenees (from ca. 1800 to 2400 1750-2451 m a.s.l. (Ninot et al., 2007). Such uppermost subalpine forests are relatively little influenced by local human activities and trees record the regional climatic signal (Gutiérrez Merino et al., 1998; Tardif et al., 2003; Dorado-Liñán et al., 2012). Compared with other Pyrenean tree species, *P. uncinata* is the most long-lived species, with maximum ages of 800-1000 years (Galván et al., 2014). In comparison, silver fir (*Abies alba* Mill.) reaches maximum longevities of ca. 500 years (Macias et al., 2006), but this record could be truncated due to past forest management (Pèlachs et al., 2009).

Most mountain pine forests considered in this study and the pollen record from the lake sediments are located within the "Aigüestortes i Estany de Sant Maurici" National Park (PNAESM), situated in the Spanish Central Pyrenees (Figure 4.1). In this area, mountain pines are abundant and dominate the subalpine vegetation belt. Isolated individuals form the upper forest limit or subalpine-alpine treeline ecotone. The climate is continental with Atlantic influence. In the region around the Bassa Nera pond (hereafter BSN) the annual average precipitation reaches 1152 mm and is well distributed over the seasons. On the other hand, the mean annual temperature is 4.25°C, being January the coldest month and July the warmest one (Ninyerola et al., 2003). The subalpine vegetation is composed by conifer forests of mountain pine, silver fir and the understorey is dominated by *Rhododendron ferrugineum* L.*, Vaccinium myrtillus L.,* and Poaceae meadows (Carrillo and Ninot 1992). On the other hand, the montane belt, located below 1600 m a.s.l., is composed by deciduous oak forests of

Quercus petraea (Mattuschka) Liebl. with *Betula pendula* Roth., riverine forests (*Alnus glutinosa* L., *Fraxinus excelsior* L. and *Salix* spp.), broadleaf forests with *Tilia platyphyllos* Scop., *Prunus avium* L. and *Corylus avellana* L. and mixed forests of *B. pendula* with *Pinus sylvestris* L.

4.2.2 Tree-ring and pollen datasets

We used tree-ring width data from 423 trees sampled at 19 mountain pine forests located within the PNAESM (published in Gutiérrez et al. 1998, Tardif et al., 2003; Galván et al., 2014) (Figure 4.1). Pines were sampled at 1.30 m and two 5-mm-diameter cores were collected per tree using Pressler increment borers. Pines growing at elevations above 2200 m a.s.l. were sampled in open forests. Sampled trees include living and recently-dead dominant and mature mountain pine trees. The extracted cores were air-dried, sanded and visually cross-dated (Stokes and Smiley 1968). All cores were measured to an accuracy of 0.01 mm using an ANIOL measuring device (Aniol 1983) and cross-dating was validated with the program COFECHA (Holmes 1983). Series with abnormal growth or poorly correlated with the master chronology were discarded. Each individual ring-width series was standardized applying a spline function with a 50% frequency response of 32 years (Cook and Peters, 1981). A transformation of the ring-width value into a dimensionless ring-width index was done by means of detrending procedures (standardization) including the modelling and elimination of first-order autocorrelation (Fritts 1976). The program ARSTAN (Cook and Krusic 2013) was used to standardize all the tree ring series. In all subsequent analyses, the residual or pre-whitened ring-width indices were used.

We used the available pollen frequencies and the derived montane-subalpine pollen ratio obtained from a previous palaeoecological study focused on the vegetation dynamics of the last millennium (Garcés-Pastor et al., 2016). This sequence was obtained from the sediments of the BSN pond a small lacustrine system located in the Aiguamòg valley, in the boundary area of the PNAESM (42° 38' 18.5" N, 0° 55' 27.6" E, 1891 m a.s.l.) (Figure 4.1). Pollen analysis was performed according to standard procedures (Moore et al., 1991) with chemical treatment of sediments and mineral

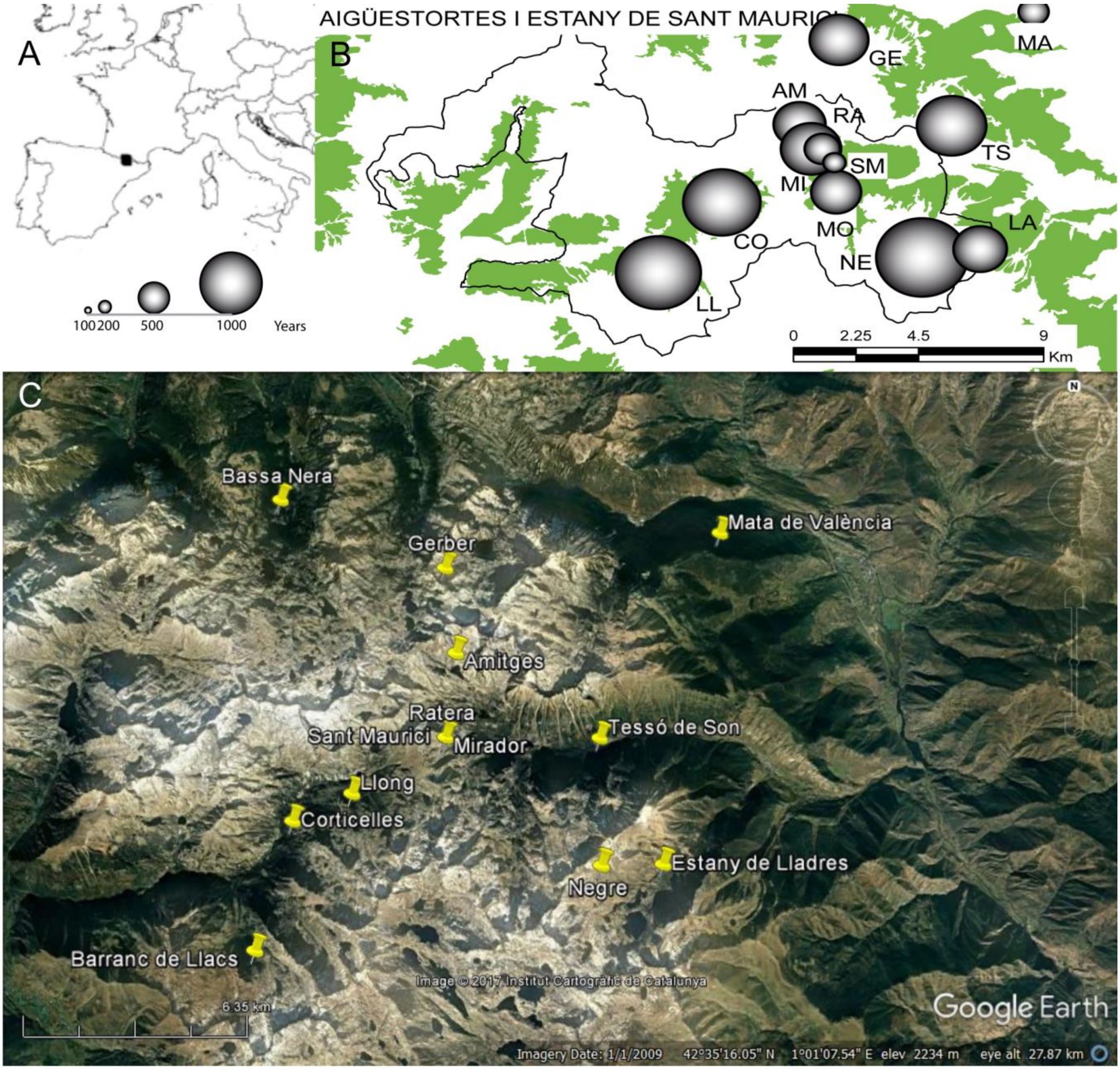

Figure 4.1. A) Location of the study area, B) Map showing the locations of the most relevant tree-ring width chronologies in the "Aigüestortes i Estany de Sant Maurici" National Park, Central Pyrenees. C) Topographic map with the relevant tree-ring chronologies and Bassa Nera pond.

separation in Thoulet solution. Details on pollen methods and relative abundances data can be found in Garcés-Pastor et al. (2016), but basically, the counting and identification of the grains were carried out at 400x magnification following Faegri et al. (1989) and Reille (1992). The montane-subalpine pollen ratio was calculated according to the pollen indicator taxa identified in the area by Cañellas-Boltà et al. (2009). Montane pollen types included *Alnus*, *Betula*, *Buxus*, *Corylus*, *Fraxinus*, deciduous *Quercus*, *Tilia* and *Salix*, while subalpine–alpine indicators included Asteraceae, *Calluna*, *Campanula*, Ericaceae, *Plantago* and Poaceae (see more information in Garcés-Pastor et al., 2016). In the present work we used the palynological data that overlapped with the period covered by most tree-ring chronologies (1286-1968, 31 sample depths) to explore correlations between both proxies.

4.2.3 Statistical analysis of pollen and dendrochronology

The pollen and dendrochronological data series used in this study have different temporal resolutions; the pollen record has a resolution from 8 to 26 years, while tree-ring width series are annually resolved. To allow statistical comparison of both datasets, we obtained the averages of the residual chronologies for the periods comprised between each pollen sample. In order to assess potential statistical correlations, we tested the normality of the data with Shapiro-Wilk test ($p < 0.05$). In the case they followed a normal distribution, the Pearson correlation coefficient (r) was used (Zar et al., 1999). When the variables presented a non-normal distribution, the Spearman non-parametric coefficient (rho) was used (Gibbons and Chakraborti, 2011). According to this, pollen frequencies followed a non-normal distribution, while the montane-subalpine ratio and the residual ring-width chronologies showed normal distributions. Therefore, individual pollen frequencies were compared with the residual ring-width chronologies using the Spearman *rho*, while the montane-subalpine ratio was compared with the residual ring-width chronologies using Pearson correlation coefficients.

To assess differences at local scale between the montane-subalpine ratio and tree-ring relationships, the values of the correlation coefficients (*r* de Pearson) were represented in relation to the geographical distance between the tree sampling sites and the pollen record coring BSN site, as well as the altitude of the tree sampling sites and the BSN site.

4.2.4 Climate and tree-growth relationships

In this study we focused on the response of the mountain pine forests located close to BSN (Gerber, Amitges, Mirador, Sant Maurici) to climate, in order to better understand the vegetation-climate-site complex. To differentiate between the influences of global and regional climate, the ring-width residuals were compared with the Northern Hemisphere Temperature Anomaly (NHTA, Mann et al., 2009) and the Pyrenees Temperature Anomaly (PTA, Dorado-Liñán et al., 2012). The PTA regional reconstruction is based on 22 maximum tree-ring density chronologies (MXD) distributed across the Pyrenees mountain range (from Bizkarze to Llipodère). The trees used by Dorado-Liñán et al (2012) were not the same used for the present study. Indeed, PTA reconstruction included data of maximum density (MXD), which is a different variable than the tree-ring width (TRW) used in this study. Even though a correlation between MXD and TRW may exist, this relationship has only been found for the series of growth and maximum density from the same trees. In fact, MXD is more sensitive to high temperatures in late summer whereas the TRW integrates the conditions throughout the growing period (from May to October) (Brifa et al., 2002). The last 700 years were divided by the well-known climatic periods (Medieval Climate Anomaly, Little Ice Age and Industrial Revolution) and also by 50-year sub-periods.

To determine the main climatic variables related to radial tree growth during the last century in Central Pyrenees (from 1901 to 2010), we assessed short-term growth responses by relating ring-width with monthly precipitation and mean monthly temperature, obtained from instrumental climatic data. Four localities for this analysis were chosen to cover a North-South gradient along 11 km (Gerber, Amitges, Corticelles and Barranc de Llacs, Figure 4.1). Gerber and Amitges are located in the eastern part of the PNAESM, while Corticelles and Barranc de Llacs are situated in the western PNAESM. All locations are above 2250 m a.s.l. in order to assure similar mountain environments, climatic conditions and growth characteristics. For the climatic data of the last century, gridded products were chosen since none of the meteorological stations in the area of the PNAESM had continuous measurements during the 20th century. CRU TS3.21 gridded total monthly precipitation and monthly average temperature covering the period 1901–2010 were used for the analyses (Harris et al., 2004). The CRU data for the study period were extracted from the 0.5° grid centred at 42.75°N 0.75°E, which included most of our study area in the PNAESM. However, in order to obtain more accurate measurements regarding to the elevation of our study site, the CRU data were interpolated with available measurements from the nearby meteorological station located at the "Estany de Sant Maurici" at 1920 m a.s.l (Figure S1, supplementary material). Thus, monthly-based CRU mean temperature and the total precipitation were rescaled using Estany de Sant Maurici local data. As a consequence, the new climatic series had lower temperatures and higher precipitation values than CRU values more similar to the Estany de Sant Maurici. However, the variability and trend of the CRU data was kept in the new climatic series over the study period.

Climate-tree-ring growth relationships were assessed by correlating monthly climate data with the mean site chronologies for months and seasons using the *treeclim* R package (Zang and Biondi 2015). These relationships were assessed by computing bootstrapped Pearson's correlation coefficients over the common period 1901-2010. Significance of the correlation coefficients was tested by the bootstrap method with a 95% confidence interval. To test whether the influence of precipitation and temperature on tree growth was stable or not over the studied period, we calculated bootstrapped moving climate-growth Pearson correlations.

4.3. Results

4.3.1 Relationships between pollen, the montane-subalpine ratio and tree growth

The correlations between BSN pollen of individual taxa and the ring-width chronologies showed that only some pollen taxa were related with the pine growth series (Table 4.1, Table S2 and Figure S2 Supplementary Material). Among the sites close to BSN (Table 4.1), we observed a higher number of specific pollen taxa correlations in Gerber and Mirador, while in Amitges no significant correlation was found for any single taxon. The pollen of *Fagus* positively correlated with the tree-ring growth series in three of the closest localities to BSN (Estany Gerber, Mirador and Estany de Sant Maurici), whereas significant correlations for *Corylus* and *Sanguisorba* were found at two localities (Estany Gerber and Mirador, Table 4.1). *Pinus* pollen frequencies did not show significant relationships with mountain pine growth (Table S1, supplementary material).

Comparing the montane-subalpine ratio of BSN record with ring-width chronologies, significant correlations were found for the closest locations to BSN, i.e. Gerber ($r = 0.46$, $p = 0.009$) and Amitges ($r = 0.63$, $p = 0.012$; see Tables 4.1 and Table S2, supplementary material). Figure 2 depicts the values of Pearson's (r) correlations between the montane-subalpine ratio and residual ring-width chronologies and altitude (m a.s.l.) (Figure 4.2a), and distance (Km) from BSN (Figure 4.2b). Our results showed that the correlations between the montane-subalpine ratio and ring-width chronologies were higher above 2000 m and for sites situated at distances shorter than 10 km from BSN (Figure 4.2).

Finally, in order to test the usefulness of the montane-subalpine pollen ratio to estimate the past growth of subalpine forests, we calculated the parameters of the linear functions relating the montane-subalpine ratio to Gerber and to Amitges chronologies:

$$\text{res\_Gerber} = 0.9637 + 0.02517 \cdot \text{Montane\_ratio} \quad (p\text{-}value=0.009) \quad \text{(equation 1)}$$

$$\text{res\_Amitges} = 0.9675 + 0.02204 \cdot \text{Montane\_ratio} \quad (p\text{-}value=0.012) \quad \text{(equation 2)}$$

	Gerber (5.04 km) (2268 m)		**Amitges** (6.42 km) (2390 m)		**Mirador** (7.83 km) (2180 m)		**Ratera** (7.95 km) (2170 m)		**Sant Maurici** (7.95 km) (1933 m)	
	rho	*p*	rho	*p*	rho	*p*	rho	*p*	rho	*p*
Montane-subalpine ratio	0.526	0.002	0.561	0.032						
Alnus	0.383	0.034								
Asteraceae non fenestrate							-0.928	0.008		
Corylus	0.376	0.037			0.472	0.023				
Fagus	0.393	0.028			0.443	0.034			-0.886	0.033
Sanguisorba	-0.466	0.008			-0.436	0.038				
Secale	0.498	0.004								

Table 4.1. Spearman rank correlation values (rho) calculated between the montane-subalpine pollen ratio, different pollen taxa and residual chronologies of ring-width indices considering forests located near the Bassa Nera site. The distance to the pollen site (Bassa Nera) and the elevation are indicated for each forest where chronologies were developed (first row).

where res_Gerber and res_Amitges are the respective values of the residual ring-width chronologies (Figure 4.3, Table S2 supplementary material). These functions are significant and could be used to infer past growth of subalpine forests in these two sites based on the montane-subalpine pollen ratio. About 21% and 39% of the variability in ring-width indices was explained by the values of the montane-subalpine ratio at a low temporal resolution, respectively.

4.3.2 Tree growth-temperature relationships during the last 700 years

Figure 4.4 shows the correlation of the pine residual dendrochronologies with Northern Hemisphere Temperature Anomalies (NHTA) (Mann et al., 2009) and with the Pyrenees May to September Temperature Anomalies (PTA) (Dorado-Liñán et al., 2012) for the sampled localities closer than 10 km to BSN. All the significant correlations between residual chronologies and temperatures (Figure 4) were positive meaning that subalpine trees growth is mainly limited by low temperatures. Focusing on the NHTA, positive and significant correlations were found for all localities in the 1650-1850 period, which matches the second phase of the Little Ice Age (LIA, 1700-

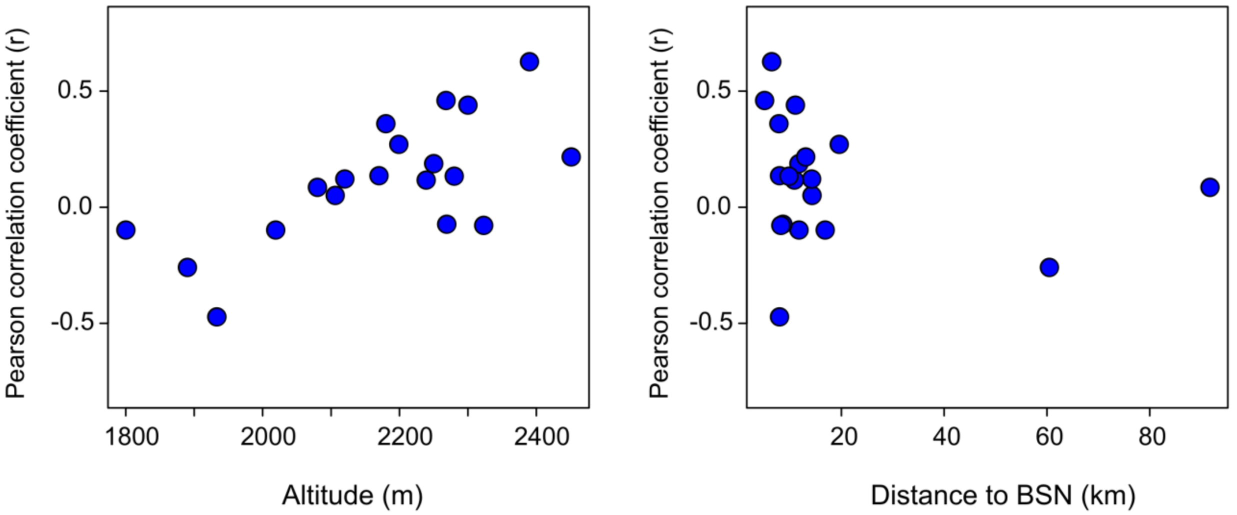

Figure 4.2 Plot depicting the correlation coefficient of Pearson's (r) of montane-subalpine ratio with residual ring-width chronologies according to altitude in m a.s.l. (a) and distance to Bassa Nera in Km (b)

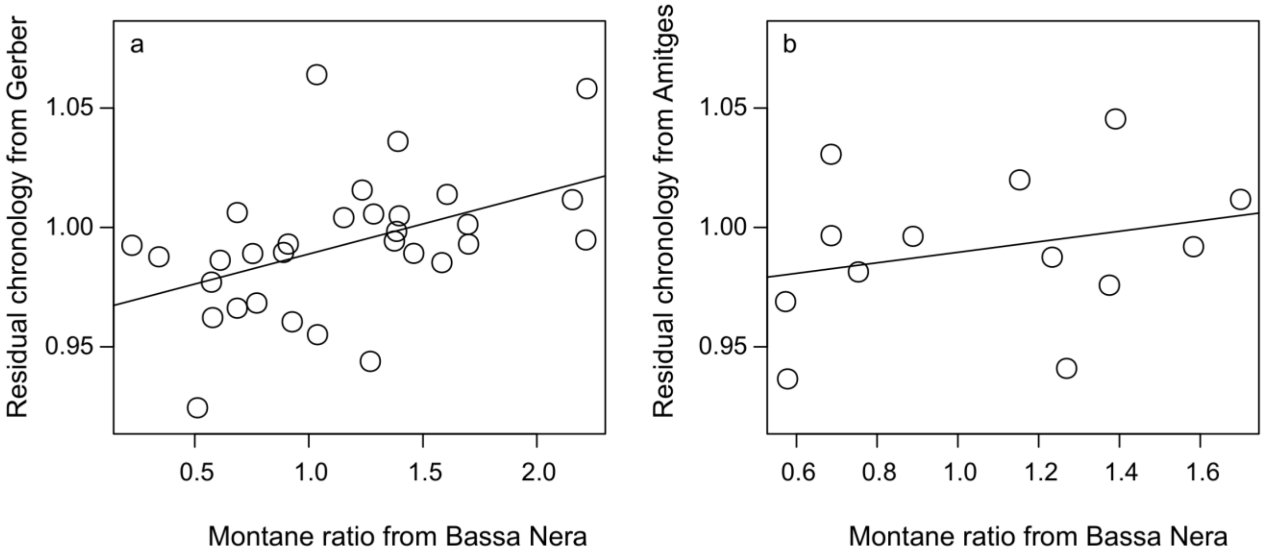

Figure 4.3. Linear models relating the montane-subalpine pollen ratio and ring-width indices (y axes) based on chronologies of Gerber and Amitges sites. res_Gerber = 0.9637 + 0.02517·Montane_ratio (*p*-value=0.009); res_Amitges = 0.9675 + 0.02204·Montane_ratio (*p*-value=0.012)

1850; Mann et al., 2009). The studied localities also correlated for the specific sub-periods of 1650-1699, 1700-1749 and 1800-1849. Other nearby localities such as Ratera (2170 m a.s.l.) and Sant Maurici (1933 m a.s.l.) have a shorter dendrochronological record (1811-2005) and did not show any significant correlation (data not shown). On the other hand, tree-ring chronologies showed better correlations with the reconstruction of the PTA (Dorado et al., 2012) than with NHTA. All the tree-ring chronologies strongly correlated for the periods of LIA (1600-1850) and Industrial Revolution (IR, 1850-2005), especially for the 50-year sub-periods of 1650-1699, 1700-1749, 1800-1849, 1850-1899 and 1950-1999. The correlated sub-periods with NHTA and PTA match with the solar grand minima of Maunder (1680-1715 AD) and Dalton (1800-1830 AD) (Bard et al., 2000), but only PTA was related to ring-width series for the sub-periods of 1850-1899 and 1950-1999. It must be borne in mind that the chronologies and the dendro-variable (maximum density, MXD) used for temperature reconstruction are independent from those tree-ring width chronologies (TRW) used in this study, avoiding a circular reasoning.

4.3.3 Tree growth-climate relationships during the 20th century

The relationships between tree-growth with monthly temperatures and precipitation are depicted in Figure 4.5. A common pattern to all localities can be observed, the majority of monthly mean temperatures are exerting a positive effect on growth both during the current and previous year of growth, meaning that growth is mostly limited by low temperatures. This positive effect is significant at the beginning of the growing period (months of April and/or May) and at the end of the growing period as most correlations are significant in previous fall (months of October and/or November) of the year of growth. However, some differences between localities can be appreciated. Pine growth from all the sites of study correlated with the previous March (t-1) temperatures. Indeed, for Corticelles and Barranc de Llacs, they also correlated with the previous November (t-1) temperatures. From all the study sites, only Barranc de Llacs showed a negative correlation with the temperatures of the previous September (t-1). For the current year, tree growth in Amitges and Barranc de

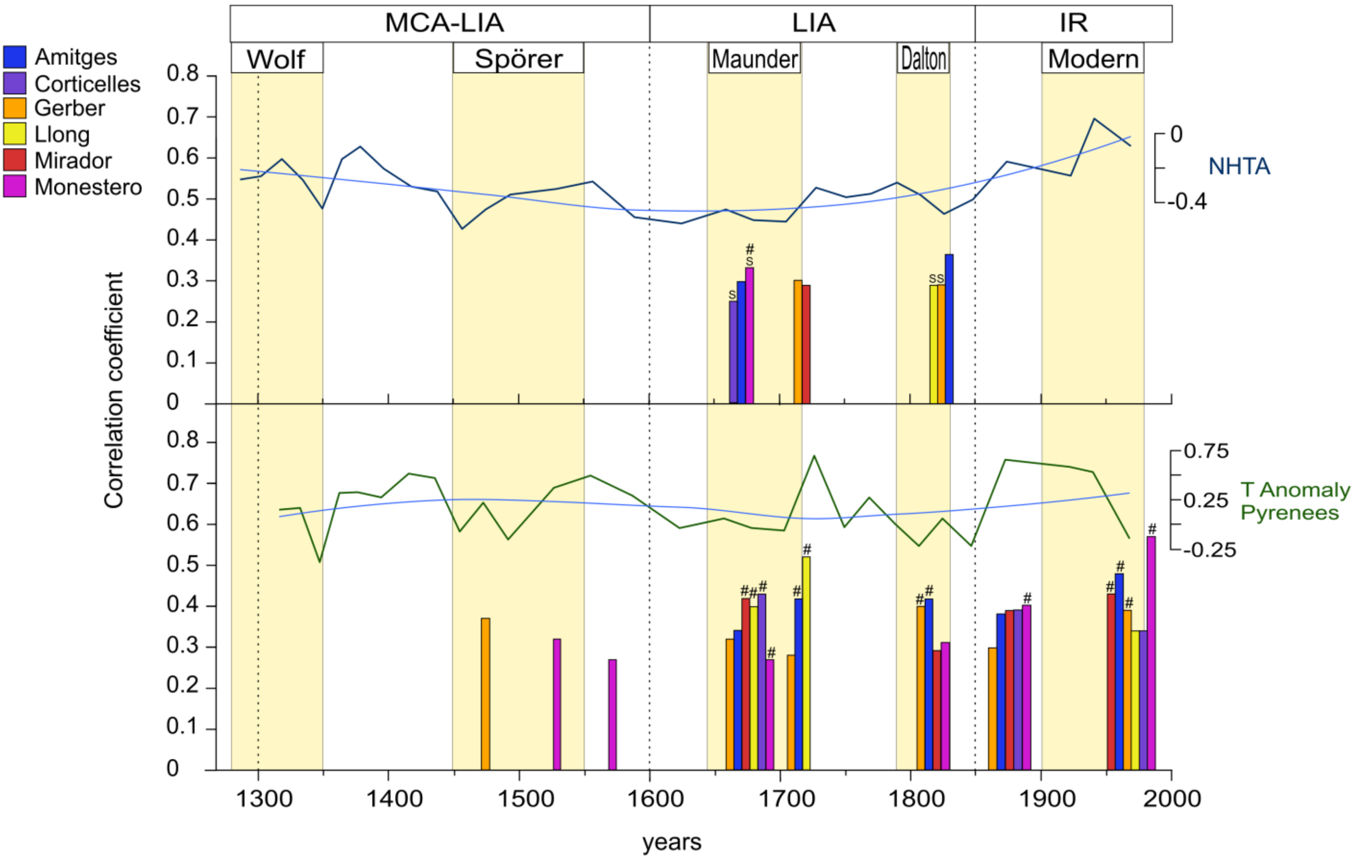

Figure 4,4 Associations (Pearson correlation ($p < 0.05$) between residual ring-width chronologies with Northern Hemisphere Temperature Anomalies (NHTA; Mann et al., 2009) and Pyrenees Temperature anomalies (PTA; Dorado-Liñán et al., 2012). # means $p < 0.005$, S: Spearman correlation. MCA-LIA: Medieval Climate Anomaly-Little Ice Age transition; IR: Industrial Revolution. Solar Grand minima are depicted in orange stripes.

Llacs correlated with April (t) temperatures. In general, there is a positive relation between tree-ring growth and the temperatures of previous March (t-1) and October-November (t-1) and current April- May (t). Regarding the growth-precipitation relationships, the signal strength is quite weak. The only significant and positive effect of precipitation is in June (t). On the contrary, Gerber, Corticelles and Barranc de Llacs presented a negative relation for the previous April (t-1) and current June (t) precipitations. Figure 4.6 shows the seasonal correlations of tree-growth with mean monthly temperatures and monthly precipitation. Monthly temperatures in all the sites are significantly positively correlated with tree-ring index for "seasons" of 3 months ending in December-January of the previous year limited. For the case of

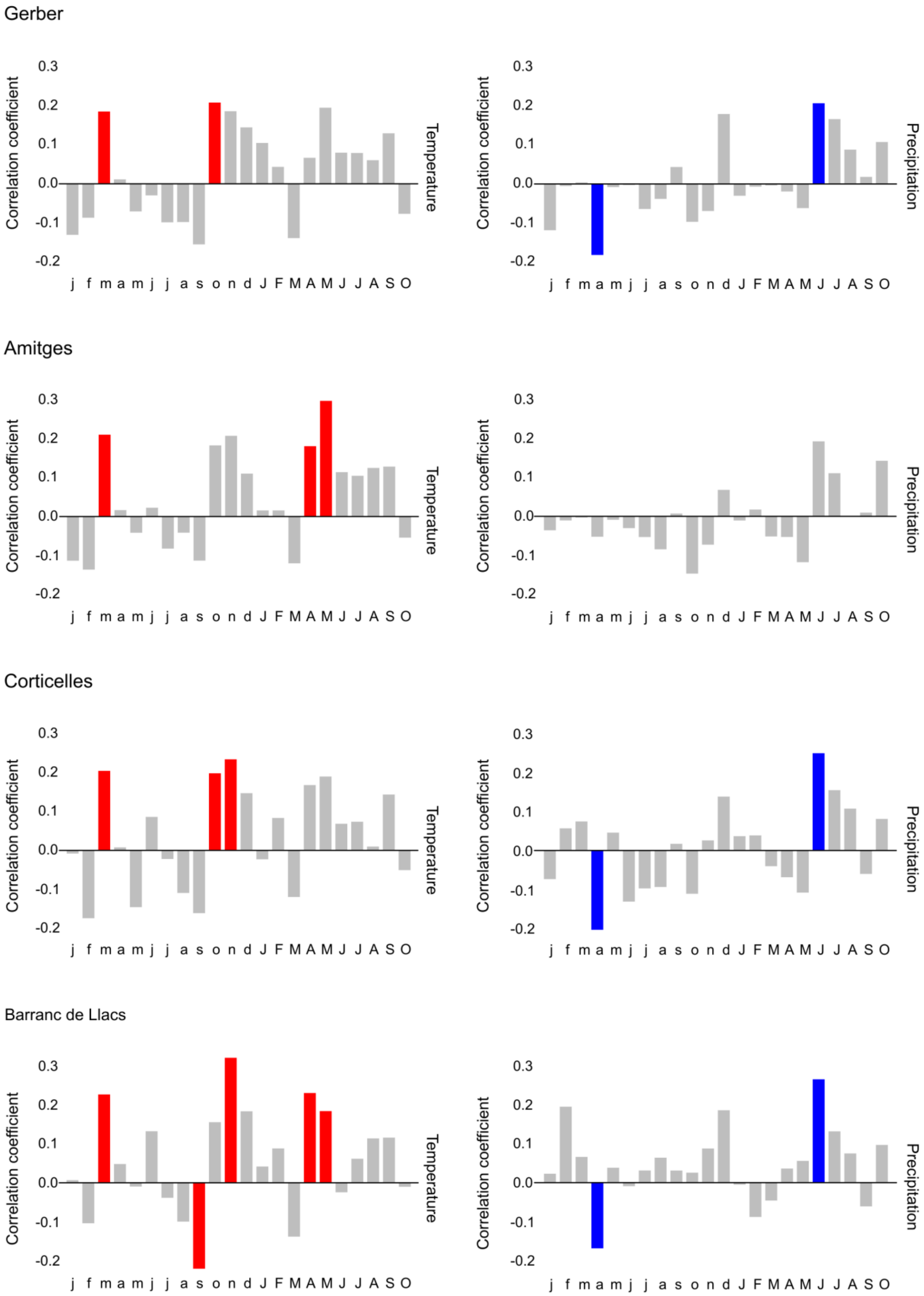

Figure 4.5 Correlation coefficients of residual annual tree-ring width chronologies against monthly mean temperatures and total precipitations. The months in lower case correspond to the last year, while the ones in capital letter correspond to the current year month. Significant coefficients ($p < 0.05$) are represented in red (temperatures) and blue (precipitation).

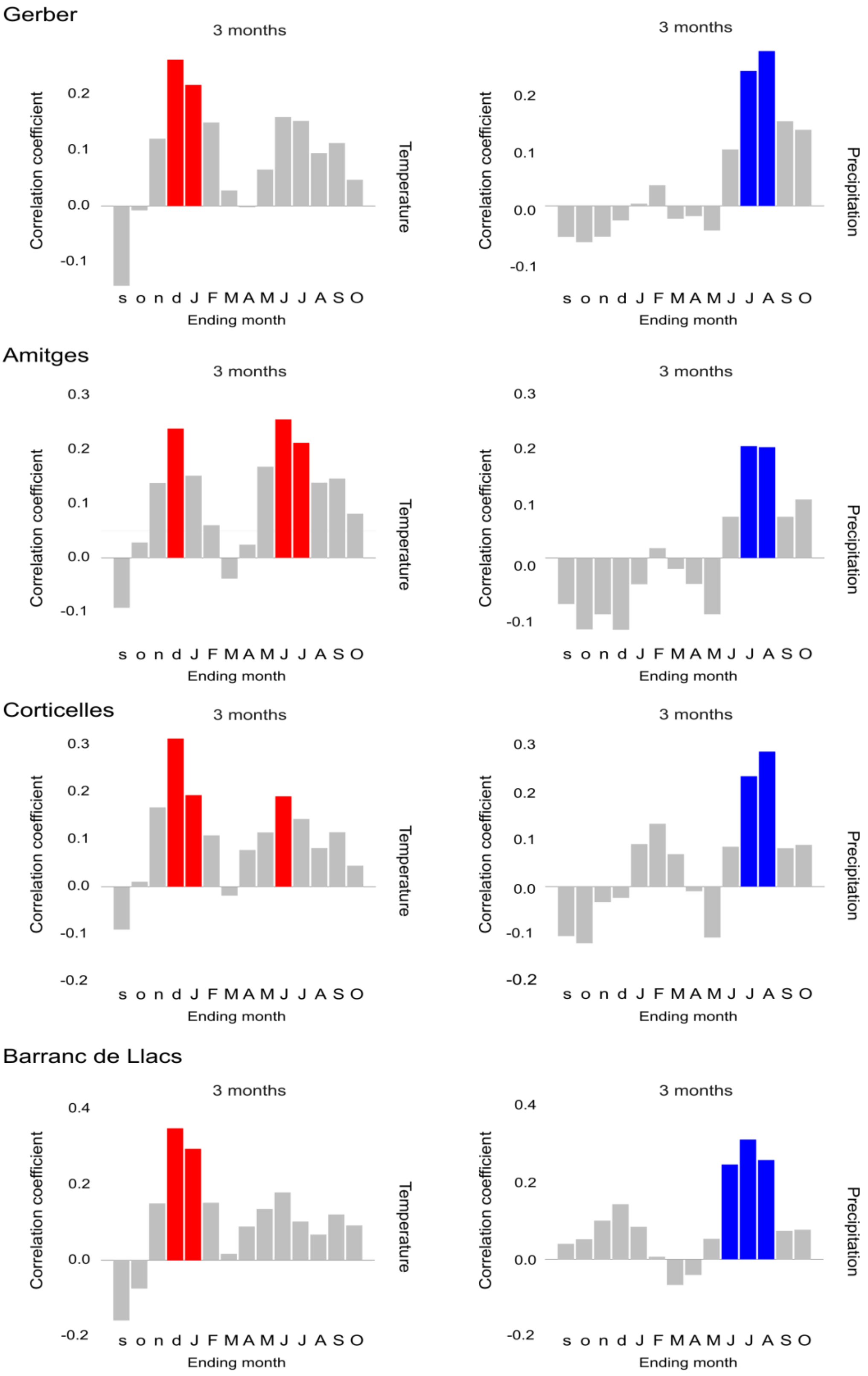

Figure 4.6 Seasonal correlation of tree-ring chronologies against mean temperatures and total precipitations. Season length are 3 months, where the months in the label are the last. Those months in lower case correspond to the last year, while the ones in capital letter correspond to the current year month. Significant coefficients ($p < 0.05$) are represented in red (temperatures) or blue (precipitation).

Amitges and Corticelles, seasons ending in June-July of the current year were also significant. On the other side, precipitation seasons ending in June, July and August months of the current year were significant for all the sites.

Since growth-climate relationships may not be stable over time and to consider the effects of the recent ongoing climate change, we performed a bootstrapped moving-window response function analysis to check in detail these relationships along the 20th century. Results are shown in Figure 4.7. A change in the most influencing months can be observed. For the first half of the century (1920-1950) pine growth responded positively to the current January and May temperatures. From 1950 onwards, only April-May temperatures had a broad positive effect on pine growth. On the other hand, results show a generally consistent response of tree growth to May (negative), June (positive), and October (positive) to precipitations. For the first half of the last century (1920-1950) pine growth responded negatively to May precipitation and positively to June. For the second half of the century, the October temperatures become more relevant, giving a positive pine growth response. No significant negative responses to precipitation are found in this period.

4.4. Discussion

4.4.1 The local subalpine forest

Our results suggest that the pollen records from some individual taxa from BSN correlate with pine growth for subalpine forests located at elevations higher than 2250 m a.s.l. such as those from Gerber and Mirador sites, which could have nearly similar ecological and climatic conditions (Table 4.1). On the other hand, mountain pine growth at Amitges, located at higher altitude (2390 m), only correlated with montane-subalpine ratio. Pines in Amitges are in the limit of their natural altitudinal distribution. Therefore, it is consistent that Amitges tree growth does not correlate with individual pollen taxa from BSN, since this locality has different climatic constraints and vegetation arrangements. From all the BSN pollen species that correlated with mountain pine growth (*Fagus*, *Corylus* and *Sanguisorba*) (Table 4.1), *Fagus* would provide a broad regional signal because trees are restricted to the lowermost part of the montane belt in the nearby Aran Valley (~1300-1600 m), but their pollen can reach the alpine belt (2300-2400 m) (Cañellas-Boltà et al., 2009). On the other hand, *Corylus* is considered a good indicator of the presence of parent taxa, since the pollen and parent trees are found in the same mountain belt. By contrast, *Sanguisorba* presents a negative correlation in Gerber and Mirador, but the ecological interpretation of this species is rather uncertain, because it could also be considered an indicator of human-induced vegetation changes (e.g. mowing, grazing and cultivation) (Court-Picon et al., 2006). A similar case is *Secale*, which is a cultivated species that cannot be easily dispersed and represents a local anthropic signal. The absence of correlation between *Pinus* pollen frequencies and *P. uncinata* tree-ring width could be explained by two reasons. First, pines produce high amounts of pollen that are dispersed by wind over long-distances, implying a significantly broad regional input (Mazier et al., 2006). Second, the studied fossil pine pollen frequencies from BSN include both *P. sylvestris* and *P. uncinata,* since it is uncertain to distinguish between them. The same applies to *Abies*; even though there is only one species in Central Pyrenees (*Abies alba*), it grows at intermediate altitudinal levels (1800-2000 m a.s.l.) and its pollen is also wind-dispersed over long distances (Cañellas-Boltà et. al., 2009).

Considering the correlation between the montane-subalpine pollen ratio and tree-ring width, a higher correlation of montane-subalpine ratio with Gerber and Amitges chronologies suggests that these localities share similar environmental conditions that might influence the vegetation communities. This might be at least partially related to the climate influence at local scale, which is conditioned by altitude and shows notable contrasts according to elevation and slope orientation (Ninot and Ferré, 2008). In general, our results suggest that BSN montane-subalpine pollen ratio better correlates with tree growth from sites located at the upper-subalpine stage (>2000 m a.s.l.) and at distances shorter than 10 km from BSN. This highlights the local nature of the montane-subalpine ratio (Garcés-Pastor et al., 2016). It is noteworthy that the highest correlations between montane-subalpine ratio and tree-ring width were found for the sites located at the highest altitudes instead of sites located at a similar altitude of BSN coring site (1891 m a.s.l.). The tree-ring width in

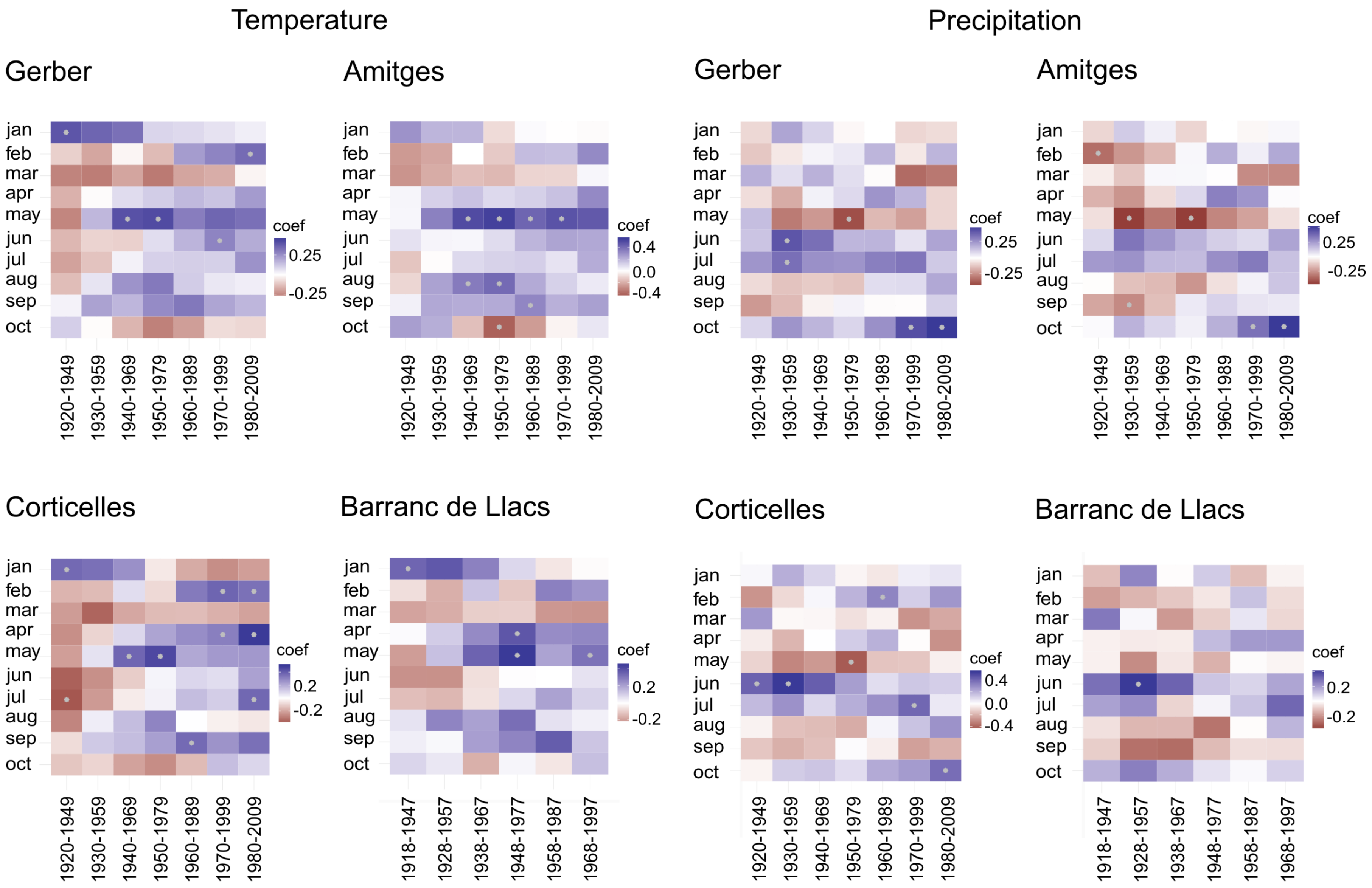

Figure 4.7 30 years bootstrapped moving-window with an offset of 10 years with Pearson correlations between current year monthly temperature and monthly precipitation from the current year with residual tree-ring chronologies from Gerber, Amitges, Corticelles and Barranc de Llacs. Asterisks indicate significant correlations at $p < 0.05$.

Amitges (2390 m a.s.l.) presents the highest correlation with montane-subalpine ratio (Table S2 supplementary material). Located in the alpine stage, these pines are found at the edge of their geographic altitudinal distribution area or growing under unfavourable conditions and limited by climatic factors such as low temperatures (Camarero and Gutiérrez, 2004, Batllori et al., 2012). As a result, Amitges tree-ring width series might be more sensitive to climatic variations (Fritts, 1976), thus showing a higher inter-annual variability and correlating better with the montane-subalpine pollen ratio. This is in line with studies by Kupfer and Cairns (1996) and Loehle (2000), who highlighted that the montane-subalpine ecotone is mainly controlled by temperature and sensitive to warming changes. The tree-growth from the highest altitudes and the montane-subalpine ratio are both mainly limited by low temperatures and, as a result, they are more sensitive to climate shifts than tree-ring chronologies from lower altitudes (see Tardif et al., 2003). The sensitivity of these two proxies makes them suitable for robust paleoecological reconstructions of high mountain forests. Moreover, given the fact that the altitudinal treeline ecotone position is also controlled by low temperatures (Batllori et al., 2012), it could be expected that both ecotones (montane-subalpine and treeline) could move upwards under higher temperatures if tree recruitment takes place at the same time. For the moment, we can say that tree-growth is enhanced by higher temperatures and that tree-growth positively correlates with the montane-subalpine pollen ratio. This is a clear hint to expect an upward shift of the ecotones and therefore, an excellent opportunity to verify if this movement is indeed a response to current global warming.

4.4.2 Global and regional climate influence in the Central Pyrenees forests

In general, our results suggest a high sensitivity of tree-growth to temperature. Since the correlations between them are positive, tree-growth would have been enhanced if temperatures had been higher in the past 700 years, and likely tree recruitment (Batllori et al., 2012). Tree-ring width chronologies and temperature anomalies, NHTA and PTA, showed a high positive correlation during the second phase of the LIA (1700-1850) (Mann et al., 2009), characterized by colder temperatures, higher humidity and maximum glacier advances in the Southern Pyrenees (González Trueba et al., 2008; Morellón et al., 2011). From both temperature anomaly reconstructions, PTA (Dorado-Liñán et al., 2012) presents a higher correlation with ring-width chronologies than NHTA, especially for the periods of LIA and IR. Although mountain pine TRW and the MXD chronologies from the same trees can show positive and significant correlations in the Pyrenees or Siberia (Galván 2014; Kirdyanov et al., 2008), other studies have not found any correlation for *P. sylvestris* in Sweden (Pritzkow et al 2014). These results are in agreement with the fact that *Pinus uncinata* growth is mainly limited by low temperatures near or above the treeline, and even under the recent climate change the positive relationship between tree growth and temperatures persists at high altitudes while at low altitudes temperature exerts a negative effect on growth (Gutiérrez 1991).

In general, tree-ring width correlated with the temperature reconstructions (NHTA, PTA) and with the solar grand minima of Maunder and Dalton, which is in line with other studies performed on the Pyrenees (Esper et al., 2015; Büntgen et al., 2017). Büntgen et al. (2008) and Dorado et al. (2012) found that the coldest summers in the Pyrenees were in-phase with the same solar minima. On the other hand, PTA was the only reconstructed temperature that correlated during the subperiods of 1850-1899 and 1950-1999, suggesting that pines responded to a more regional climatic signal. The subperiod of 1850-1899 coincides with the end of the LIA, which was characterized by significant fluctuation of water availability, as suggested by important water level oscillations in some Pyrenean lakes, e.g Lake Estanya and Basa de la Mora (Morellón et al., 2009; Pérez-Sanz et al., 2013). This subperiod also coincided with a period of high frequency of extreme precipitation events (1844-1894) registered in the nearby Montcortès lake and most rivers of the NE Iberian Peninsula (Corella et al., 2014). The intensity and frequency of extreme rainfall events together with an increase of temperature in the Pyrenees should have affected tree growth during the second half of 19th century. Regarding the subperiod of 1950-1999, a decreasing trend in NTHA and PTA is observed, with a more pronounced decline in Pyrenees temperature anomaly (Figure 4). This subperiod was characterised by lower lake levels in some Pyrenean lakes (Pérez-Sanz et al., 2013; Garcés-Pastor et al., 2016), low frequency of heavy rainfall events in the pre-Pyrenees (Corella et al., 2014) and especially high temperatures with a drastic climatic variability in the Iberian Peninsula (Pérez-Zanón et al., 2016; IPCC, 2013). These contrasting conditions prompted a change in tree-growth pattern of Pyrenean forests (Andreu et al., 2007, Agustí-Panareda et al., 2000). Thus, pine growth responded to a general climatic pattern (NHTA) modulated by local climatic forcings in the Iberian Peninsula and the Pyrenees (PTA).

4.4.3 Short-term growth responses to climate through the last century

The four localities selected for this study showed that tree-ring growth was significantly and positively related with the previous March (t-1) (Figure 4.5), previous autumn (October-November) (t-1) and current year of growth spring temperatures (April-May) (t), a result also corroborated by seasonal correlations (Figure 4.6). This suggests that low temperatures at the beginning and at the end of the growing season constrain tree-ring growth rates. Therefore, tree-rings growth would be wider if temperatures were warmer. The only positive and significant relationship between growth and precipitation is in June (t), when the growth rates are the highest (Camarero et al., 1998), indicating that lack of water is limiting tree-ring formation at high altitudes, in our study in three out of four studied sites. Summarizing, our results show that, although the climate warming, *P. uncinata* growth is mainly limited by low temperatures throughout the 20th century. Then, it is possible that, although the warming and at higher altitudes scale, this vegetation response has not reached any shifting threshold (Batllori et al., 2012). These results agree with Tardif et al. (2003), which found that the temperatures from November of the previous year and May of the same year of ring formation drove the pines growth-climate association. Additionally, our results also agree with those reported by Ninot et al. (2011). These authors found that low temperatures during the active physiological period (June-October) were the most limiting factor for the growth and regeneration of mountain pine in the timberline of the PNAESM. All these results might be explained by the study of Camarero et al. (1998), who pointed that the latewood cells lignification period extended from the beginning of June to October, and highlighted a delay between tree-ring formation and climate factors. In particular, the authors suggest a delay between the physiological activity and the resumption of cambial growth activity in spring (May) and the radial expansion of cambial derivatives and ring growth in June-July. On the other hand, the results of Ninot et al. (2011) and Batllori et al. (2012) are interesting for our discussion as they report that tree recruitment is also limited by low temperatures. In this regard, the warming trend during the 20th has favoured higher recruitment rates in the treeline ecotone and also an upward shift of the treeline ecotone in the Pyrenees as it has been reported by Camarero and Gutiérrez (2004) and Batllori and Gutiérrez (2008) although this is not a generalized phenomenon across the Pyrenees.

Comparing the effect of the studied climatic variables, we observed a high influence of temperature on radial growth, followed by summer precipitation (Figures 4.5 and 4.6). This is in agreement with the results of Tardif et al. (2003), which reported that temperature was the main factor related to radial growth in *P. uncinata*. For the whole period 1901-2009, only June (t) precipitation exerted a significant and positive effect on growth, meaning that pines growth is limited to some extent by the lack of water when growth rates are higher. The negative and significant effect of precipitation in April (t-1) must be interpreted as an indirect effect of precipitation lowering temperatures for growth resumption and for replenishment of carbohydrates reserves in late fall, which could be also accompanied by an indirect effect of lower solar radiation.

Moving correlations for shorter intervals, (30-year periods lagged 1 yr) in which we split tree growth-climate relationships throughout the 20th century, offer really valuable information about the effect of the recent warming trend. These results tell us that since the 1970 temperatures (see Figure S1) are not such a strong limiting growth factor as they used to be during the first half (Figure 4.7) and in the past centuries. In general, since the 1970 there are less significant correlation coefficients, *e.g.* in May, August or January. Thus, tree growth (and recruitment) of subalpine forest may benefit from warmer temperatures. Regarding precipitation, there is a similar trend as that described for temperatures. Thus, the lack of water in June (positive correlation with growth) is no longer significant since the 1970. On the contrary, October precipitation showed a significant and positive relationship with tree growth, probably due to the extended growing period at high altitudes as a consequence of warmer temperatures (Menzel et al., 2006).

4.4.4. Inference of tree growth from palynological indices

In this study, we introduce the possibility of inferring dendrochronological records (proxies of forest productivity) by using an index derived from pollen frequencies for periods longer than those covered by chronologies. Our models (Figure 4.3) show that such data could be a priori successfully derived for the two sites closest to the pollen sampling location and at the highest altitudes. The *r*-squared values for these regressions show that between 21 and 39% of the variability in tree growth can be explained by the variability in montane-subalpine ratio, which may be enough only for obtaining gross dendrochronological inferences at multidecadal resolution. It is important to remark that in this study there are few pollen data from the sedimentary core and because of that we lose temporal resolution. A denser sampling to achieve higher temporal resolution in future studies would provide a more accurate and useful relationship between tree growth and the montane-subalpine pollen ratio.

The montane-subalpine ratio has shown to be also correlated to other global climatic signals of the Northern hemisphere, such as the ice-rafted debris index (Garcés-Pastor et al., 2017). However, our results show that this index is more useful at local scales. Local environmental differences between sites may influence the sensitivity of trees to climate shifts as has been demonstrated in this study, and affect the parameter values of the models. Moreover, this sensitivity may vary over time, as shown by the results of the correlation values over the last 700 years and of the moving-window analysis for the last century. Therefore, carrying out more local studies will help to assess the relationships between radial growth and site conditions along ecological gradients, providing a proper background that will allow to improve the future climate-tree growth models and potential shifts of the forest belts across the altitudinal gradient.

4.5. Conclusions

This study highlights the importance of considering the local expression of general climatic patterns, modulated by site features, as the driver of forest dynamics. Even though no robust correlations have been found in this study between most of the pollen taxa (including pine) and tree-ring width, significant correlations exist between the montane-subalpine pollen ratio and the residual tree-ring chronologies from nearby localities. This relationship could potentially be used as a paleoecological tool to infer dendrochronological data (tree growth) for those periods that are not covered by tree-ring chronologies. This correlation is more reliable for trees located at high-elevation subalpine forests and treeline sites which are more sensitive to changes in temperature.

The association between tree-growth and climate fluctuations show that growth of mountain pine is mainly limited by low temperature. However, this sensitivity has varied along the last 700 years when temperatures were lower than today as shown by temperature reconstructions. Moreover, for the last century and using instrumental climatic data, we have been able to ascertain that tree-growth variability is still more constrained by low than by high temperatures at high altitudes in the Pyrenees. However, this effect seems to be fading in recent decades. Variations in sensitivity of tree species to climatic conditions should be taken into consideration when designing predictive models for inferring future forest responses to climate change.

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4.6 References

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Chapter 5

Genetic characterization of modern and past communities of a high mountain peat bog system using eukaryotic metabarcoding

The contents of this chapter has been submitted as:

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Abstract

We performed a metabarcoding study using universal 18S and COI markers to explore the diversity of eukaryotic communities of a Pyrenean peat bog ecosystem. We assessed the molecular diversity of four different micro-habitats along a flood gradient in Bassa Nera lentic system (Central Pyrenees). From the same study site, five sedimentary depths were also analysed in order to study the palaeoecological communities recovered from ancient DNA and to compare the detected sequences to those obtained from the modern communities. We also compared the information provided by the sedimentary DNA to the environmental reconstruction from morphological proxies such as pollen and macroremains from the same record. We successfully amplified ancient DNA with both universal markers from all the sedimentary samples, including the deepest one (10200 years old). Even though 18S could amplify a broader group of organisms, the taxonomic resolution was lower than that obtained from COI, and typically reached the family or genus levels. On the other hand, the taxonomic assignment of the detected COI sequences yielded mostly metazoans, probably due to gaps in reference databases. This first molecular approach has allowed to prove that the diversity of modern and past eukaryotic peat bog communities can be assessed using universal metabarcoding markers.

keywords: sedimentary-DNA, community-DNA, palaeoecology, eukaryotes.

5.1 Introduction

Depositional systems located in areas with low anthropogenic impact, such as mountain peat bogs, are invaluable palaeoenvironmental archives which allow to study local environmental processes and responses to climate variability (Smol et al., 2001). The communities living in these ecosystems can be considered sentinels of past and current climate shifts and the study of the historical changes in their biodiversity is crucial for understanding the dynamics of ongoing ecological processes driven by climatic forcings (Mann, 2002). Previous palaeoecological studies on peat land communities have traditionally used morphological assessment of a few taxonomic groups such as vascular plants, mosses, microalgae, chironomids or pollen (Charman, 2002; Godwin, 1981; Smol et al., 2001). These studies provide only partial views of the total biodiversity present, by analysing some selected taxa, depending on the availability of taxonomic expertise (Parducci et al., 2015).

Recently, the development of genetic techniques such as metabarcoding has allowed to perform ecological studies of broader taxonomical range (Hajibabaei et al., 2011; Taberlet et al., 2012a). These molecular techniques for biodiversity assessment are fast, objective and allow for the simultaneous detailed characterization of a wide array of diverse taxa present in the studied ecosystems. Beyond the use of metabarcoding for getting information on the extant biodiversity, the DNA obtained from sedimentary samples can represent an important source of information about past biodiversity (Pedersen, 2015). Most applications of metabarcoding in palaeoecological studies have focused on past vegetation communities by using chloroplast genetic markers such as *TrnL* or *rbcL* from frozen or lake sediments (Anderson-Carpenter, 2011; Jørgensen et al., 2012; Pedersen et al., 2013). Although these techniques have been successfully applied to study the modern community DNA of environments such as soils, marine benthos or lake sediments (Epp et al., 2012; Guardiola et al., 2016; Taberlet et al., 2012; Valentini et al., 2016), they have been rarely applied to the study of peat bogs. In recent years, Singer et al. (2016) studied the living diversity of Oomycetes in peat bogs using the nuclear 18S rRNA marker. This marker has also been used to study free-living soil Cercozoa r planktonic and sedimentary protists in lakes (Capo et al., 2016, 2015). However, to the best of our knowledge, other emerging universal metabarcoding markers for studying eukaryotic diversity, such as the mitochondrial cytochrome *c* oxidase subunit I (COI), have never been tested in peatland systems.

The DNA extracted from sedimentary samples is a mixture of modern DNA from living edaphic communities and ancient DNA from the remains of long-dead organisms (Bellemain et al., 2013; Epp et al., 2012; Lejzerowicz et al., 2013; Pawlowski and Holzmann, 2014). In general, sedimentary DNA studies rely on the assumption that the age of the DNA recovered is the same as the age of the sediments in which it is found, in absence of contaminations. This may be true when using specific primers that selectively amplify the DNA from remains of organisms not currently living in the deep strata of the soil (such as vascular plants or photosynthetic microalgae). However, universal primers commonly used for amplifying 18S and COI are able to detect a wide range of microbial taxa, many of which may belong to the living soil communities (Guardiola et al., 2015). Therefore, when using these markers for palaeoecological studies, the sequences recovered from edaphic organisms should be removed from the analyses in order to get more realistic reconstructions of the fossil communities and compare them with modern assemblages.

This study aims to obtain a more comprehensive picture of the eukaryotic diversity present in *Sphagnum* communities and sedimentary samples from mountain peat bogs, in order to assess its role in actual and past peatland ecosystems. In this paper, we offer a first molecular approach to the modern and past eukaryote communities of Bassa Nera, a unique wetland system in the Central Pyrenees (Pérez-Haase and Ninot Sugrañes, 2006; Pérez-Haase and Ninot Sugrañes 2017)⍰. This locality has been the subject of several previous taxonomical studies of modern and past communities (Pérez-Haase and Ninot Sugrañes, 2006; Cañellas-Boltà et al., 2009; Cambra, 2015; Garcés-Pastor, et al., 2016, 2017), which would allow for the comparison of the molecular results with other morphological approaches. We used an Illumina MiSeq platform to sequence two complementary metabarcoding markers: 18S and COI. The use of these molecular assessment techniques would ultimately allow to establish occurrence and abundance baselines for a wide array of taxa, necessary for characterizing the current diversity of peat bogs and for detecting and monitoring future changes in these communities. This is the first molecular characterization of the structure of micro-habitat communities in a peat bog of Central Pyrenees. In this study, we aimed 1) to check the suitability of 18S and COI markers to assess taxonomic and ecological eukaryotic diversity in peat bog communities and 2) to compare the knowledge about past communities inferred from ancient DNA with the palaeoenvironmental reconstructions based on morphological methods.

5.2 Methods

5.2.1 Study site

Bassa Nera is a lentic system located in the peripheral zone of Aigüestortes i Estany de Sant Maurici National Park at an altitude of 1891 m a.s.l (Figure 5.1). Previous paleoenvironmental studies allowed to reconstruct the historical development of the modern peat bog from a previous lacustrine environment (Garcés-Pastor et al., 2016, 2017). The vegetation of Bassa Nera catchment forms a complex mosaic that spans from a relatively mid-deep water body (max. depth 5 m) with flat shores surrounded by *Sphagnum* carpets, Cyperaceae fens and Sphagnum bogs, and steeper slopes covered by subalpine forest of *Pinus uncinata* and *Abies alba* (Carrillo et al., 2008; Pérez-Haase and Ninot 2017). The main habitats are geogenous fens (*Scheuchzerio palustris-Caricetea fuscae*) and ombrogenous bogs (*Oxycocco palustris-Sphagnetea magellanici*) (Pérez-Haase et al., 2010)□. Climate is subalpine with Atlantic influence and precipitation is well distributed along the seasons (annual average = 1152 mm) (Ninyerola et al., 2003). Mean annual temperature is 4.25 ºC, being January the coldest moth (-3 ºC in average) and July the warmest (14 ºC in average).

5.2.2 Field sampling and DNA extraction

To characterize the modern communities, four different microhabitats were sampled following a gradient of water flooding in August 2016 (Figure 5.1). Three replicates of 100 mL of a clump of the dominant mosses were obtained from each microhabitat and stored in 96% ethanol. We sampled sites next to the mire monitoring

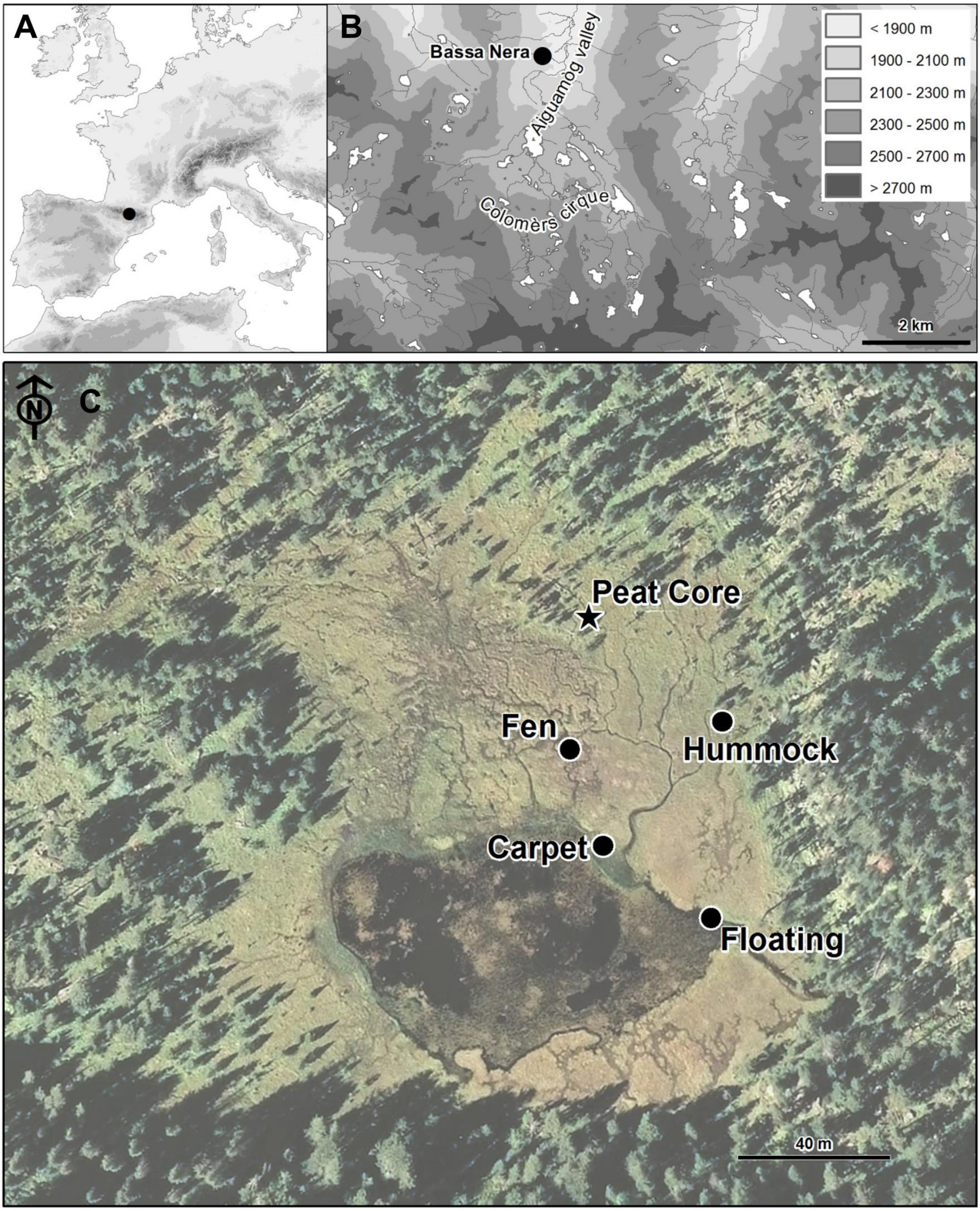

Figure 5.1. Sampling points of the modern and sedimentary samples of Bassa Nera. A) Location of the study area. B) Topographic map of the region surrounding Bassa Nera C) Location of the sampling points and core extraction.

plots used by Pérez-Haase and Ninot (2006) to measure the depth water table, so that the average moisture conditions, groundwater pH and electric conductivity are known for these sites (Pérez-Haase and Ninot 2017). The sampled microhabitats were: A) *Sphagnum* hummock (plant community *Carici fuscae-Sphagnetum magellanici* Bick 1985) B) *Sphagnum teres* carpet (community *Sphagno fallacis-Caricetum lasiocarpae* Steffen ex Passarge 1964), C) *Trichophorum cespitosum* fen (*Tofieldio calyculatae-Scirpetum cespitosi* Ballesteros, Baulies, Canalís et Sebastià ex Rivas-Martínez et Costa 1998), and D) *Sphagnum* and *Drosera longifolia* floating mire on the *Equisetum fluviatile* lake shore belt (*Equisetetum limosi* Steffen 1931) (Pérez-Haase et al., 2010). The sedimentary samples were obtained from the core BSN-6 (270 cm long), recovered from a hummock dominated by *Sphagnum magellanicum* and *S. capillifolium* in the littoral of Bassa Nera in 2011 (see more information in Pèlachs et al., 2015 and in Garcés-Pastor et al., 2017). The age-depth model was performed with 7 AMS radiocarbon dates obtained from peat and macroremains. It covers the last 10,211 cal yr BP in 270 cm with an average confidence interval error of ca. 220 yr and a sedimentation rate of 0.07± 0.21 cm yr^{-1}, ranging from 0.016 to 0.86 cm yr^{-1}. As a result, the age-depth model and the constant sedimentation rate provide a robust record for the interpretation of the molecular history (Garcés-Pastor et al., 2017). Five samples were studied from the following depths: 31; 109; 160; 220 and 265. cm, which correspond to 140, 3795, 6165, 8339 and 10094 cal yr BP, respectively.

All the samples were homogenized using a 600 W hand blender and stored at -20ºC until DNA extraction. All the equipment was cleaned with a 10% sodium hypochlorite solution and rinsed in deionised water between samples. The DNA extraction was performed in the laboratories of the University of Barcelona. 0.3 g of each homogenized sample were purified using Norgen Soil DNA Isolation Plus Kit (www.norgenbiotek.com). DNA concentrations of the purified DNA extracts were estimated with 1 μL of the final elution using the Qubit fluorometer (www.thermofisher.com).

5.2.3 PCR and sequencing

In order to get a wide taxonomic range of detected eukaryotic taxa, two different metabarcoding markers were used. The V7 region of nuclear-encoded ribosomal 18S RNA, which provides information of all eukaryotic groups, was amplified using the 18S_allshorts primers (5'-TTTGTCTGSTTAATTSCG-3' and 5'-TCACAGACCTGTTATTGC-3') (Guardiola et al., 2015). The shorter length of this region (around 100 bp) in contrast with V9 (180 bp) and V4 (350 bp) regions used in other 18S studies (Capo et al., 2015; Singer et al., 2016) is convenient for palaeoenvironmental studies where DNA may be fragmented. This primer set has been successfully used for assessing eukaryotic diversity of marine sediments (Guardiola et al., 2015, 2016) and shallow marine hard-bottom communities (Wangensteen et al., in review). A new degenerated primer set amplifying a 313 bp fragment of the mitochondrial marker COI (miCOIintF-XT 5'-GGWACWRGWTGRACWITITAYCCYCC-3'; Wangensteen et al., in review; and jgHCO2198 5'-TAIACYTCIGGRTGICCRAARAAYCA-3'; Geller et al., 2013) was also used. This marker is able to amplify almost all eukaryotic lineages with the exception of Viridiplantae and Ciliophora. The 18S marker has less natural sequence variability than COI (Wangensteen et al., in review). Therefore, the same sequence of 18S can be shared by several species belonging to related clades. This means that a 100% identity in 18S generally allows to assign a sequence to higher taxa such as family or order (Guardiola et al., 2016), but rarely to species level for metazoans or plants. Conversely, a 100% identity on the highly variable marker COI usually means a perfect match to a given species.

The PCR amplifications were performed at the environmental DNA laboratory at the University of Salford, UK. 8-base sample-specific tags for identifying the multiplexed samples and a variable number (2-4) of leading random bases, for increasing sequence diversity, were attached to the metabarcoding primers. PCR conditions followed Guardiola et al. (2015) for 18S and Wangensteen et al., (in review) for COI. After PCR, quality of amplifications was assessed by electrophoresis in agarose gel. The PCR products were pooled by marker and purified using Minelute PCR purification columns. Two Illumina libraries were built from the DNA pools using the NEXTflex PCR-free DNA library prep kit (www.biooscientific.com) and identified by different library tags. Both libraries were sequenced together in a single run of Illumina MiSeq using v3 chemistry 2x250 bp paired-end sequencing.

5.2.4 Bioinformatic pipelines and statistical analyses

The bioinformatic analyses were performed with the OBITools metabarcoding software suite (Boyer et al., 2016). The paired-reads were assembled using illuminapairedend. The resulting aligned datasets were demultiplexed using ngsfilter. A length filter (obigrep) was applied to the assigned reads (75-180 bp for 18S and 300-320 bp for COI). The obtained reads were dereplicated using obiuniq and chimeric sequences were detected and removed with the uchime_denovo algorithm implemented in vsearch (Rognes et al., 2016). The individual sequences were clustered into molecular operational taxonomic units (MOTUs) using the step-by-step aggregation clustering algorithm implemented in SWARM v2 (Mahé et al., 2015) with a resolution of d=1 for 18S and d=13 for COI (Wangensteen and Turon, 2016). The taxonomic assignment of the representative sequences for each MOTU was performed using ecotag (Boyer et al., 2016) on customized local reference databases, created from the sequences of 18S available from Genbank and a combination of COI sequences available from GenBank and the BOLD database. Both reference databases are available on-line from http://github.com/metabarpark/reference_databases. After taxonomic assignment, the list was manually checked. Those sequences assigned to bacteria or to the root of the tree of life were removed. Other sequences considered as potential contaminants related to human presence or activity were also removed. A summary of the pipelines is available in table S1 (supplementary material).

One main issue with the analysis of the DNA extracted from sedimentary samples is to distinguish the reads originated from living communities of edaphic organisms from those amplified from the remains of long-dead organisms (Bellemain et al., 2013; Coolen and Shtereva, 2009; Epp et al., 2012; Lejzerowicz et al., 2013; Pawlowski et al., 2014). There are groups of organisms that are known to dwell in soils down to several meters depth, such as Fungi, Cercozoa, non-photosynthetic Chrysophytes, Oomycetes, Ciliophora, Nematoda or Annelida (Fierer et al., 2003; Andersen et al., 2013; Asemaninejad et al., 2017). However, representatives of these groups may also live in surface bog habitats. Thus, detection of these phyla in sedimentary samples should be interpreted with caution. High abundances of sequences from these taxa, compared to non-edaphic taxa, in the sedimentary samples must be interpreted as the result of the living organisms in extant deep soil communities, rather than the prevalence of these taxa in ancient surface communities. To avoid this problem, when comparing ancient and living communities, these groups were removed from our analyses so that only those groups typical of non-edaphic environments, i.e. Bacillariophyta, Arachnida, Insecta, Rotifera, Tracheophyta, Bryophyta, were kept in order to get more realistic reconstructions. Calculations of Jaccard dissimilarity matrices and group representation nMDS diagrams were performed with the vegan package for R (Oksanen et al., 2016). The significance of dissimilarities between communities was assessed using the function ANOSIM in the same package.

For the case of Viridiplantae, only the results from 18S were used to assess modern and ancient plant communities. Given that the decay rate of ancient DNA may be different for diverse taxonomic groups (Zhu et al., 2005), the relative abundance of reads from ancient taxa shows high levels of uncertainty, so that only presence/absence data were used to compare ancient and living communities.

To compare the information provided by sedimentary DNA with the palaeoenvironmental reconstruction of Bassa Nera based on morphological methods, we used the available palynological and macroremain data from the palaeoecological study of Garcés-Pastor et al. (2017). Pollen and macroremain analyses were performed according to standard procedures (Moore et al., 1991; Mauquoy et al., 2010). Details on pollen and macroremain methods can be found in Garcés-Pastor et al. (2017).

5.3. Results

We metabarcoded a total of 27 samples (12 samples from four modern communities and 15 subsamples from sedimentary material). After the refining procedures, our final dataset for 18S comprised a total of 3,568,544 reads, from which 2,166,574 reads (60.7%) belonged to the modern communities (an average of 180,548 reads per sample) and 1,401,970 reads (39.3%) to the sedimentary ones (average: 93,464 reads per sample). In the case of COI, the final dataset included 1,778,003 reads, with 1,151,052 reads from modern communities (average: 95,921 reads per sample) and 626,951 reads from sedimentary samples (average: 41,797 reads per sample).

5.3.1 Read abundances and number of MOTUs for 18S and COI in modern samples

Figure 5.2 a and b shows the abundance of reads of 18S (a) and COI (b) from the modern communities assigned to major eukaryotic groups at a level of Phylum or lower. The proportions of sequences which could not be assigned to a phylum (unassigned Eukarya, Metazoa and Stramenopiles) were higher for COI (21.5%) than for 18S (1%). Remarkable differences can be observed between the groups detected with 18S and COI. The 18S yielded more reads from Bryophyta and Tracheophyta, while COI presented higher abundances of Arthropoda and Rotifera. Some replicates from the same micro-habitat yielded higher reads of a specific Phylum than other replicates. This is the case for Platyhelminthes in BSNCarpet-01 and BSNFen-02, and Tracheophyta for BSNFloating-03. These differences are due to a high abundance of reads from a particular MOTU in those samples, probably related to the presence of a single big-sized individual in that replicate (see supplementary material for abundance of individual MOTUs).

The relative diversity of the different groups is represented in Figure 5.2 c and d as the percentage of MOTUs assigned to the different groups in each sample by 18S (c) and COI (d). A higher dominance of small-sized MOTUs is remarkable, compared to the abundance barplots. A relatively homogeneous pattern of diversity can be observed among the different communities.

5.3.2 Comparison between modern and ancient samples

Figure 5.3 a and b shows the relative diversity of non-edaphic taxa from modern and sedimentary samples for 18S (a) and COI (b), according to the presence/absence of MOTUs belonging to each phyla, after removal of edaphic taxa. The 18S detected high diversities of Ciliophora, Tracheophyta, Arthropoda and other

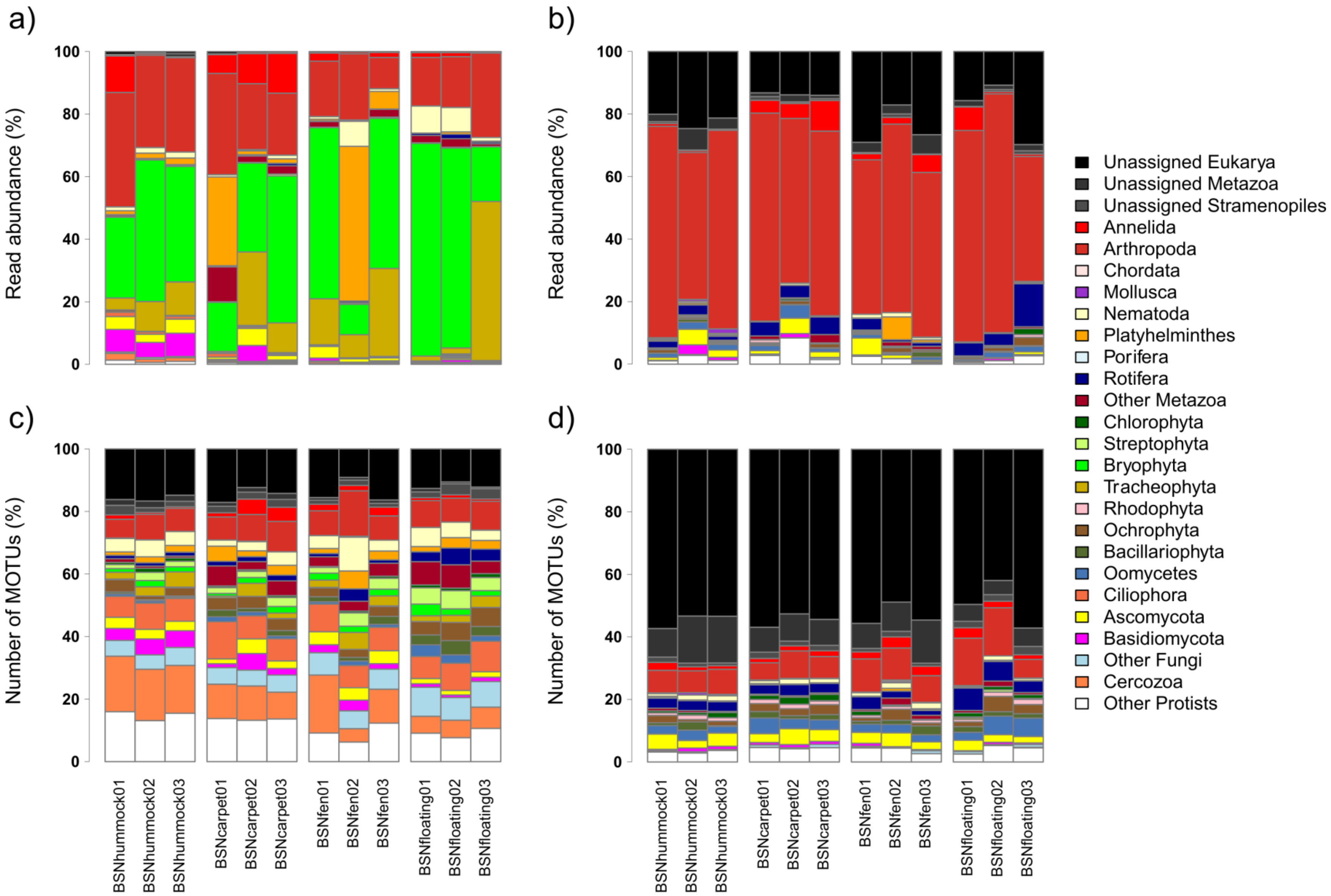

Figure 5.2. Patterns of read abundance (a, b) and relative MOTU diversity (c, d) per sample using 18S (a, c) and COI (b, d) in the four modern communities.

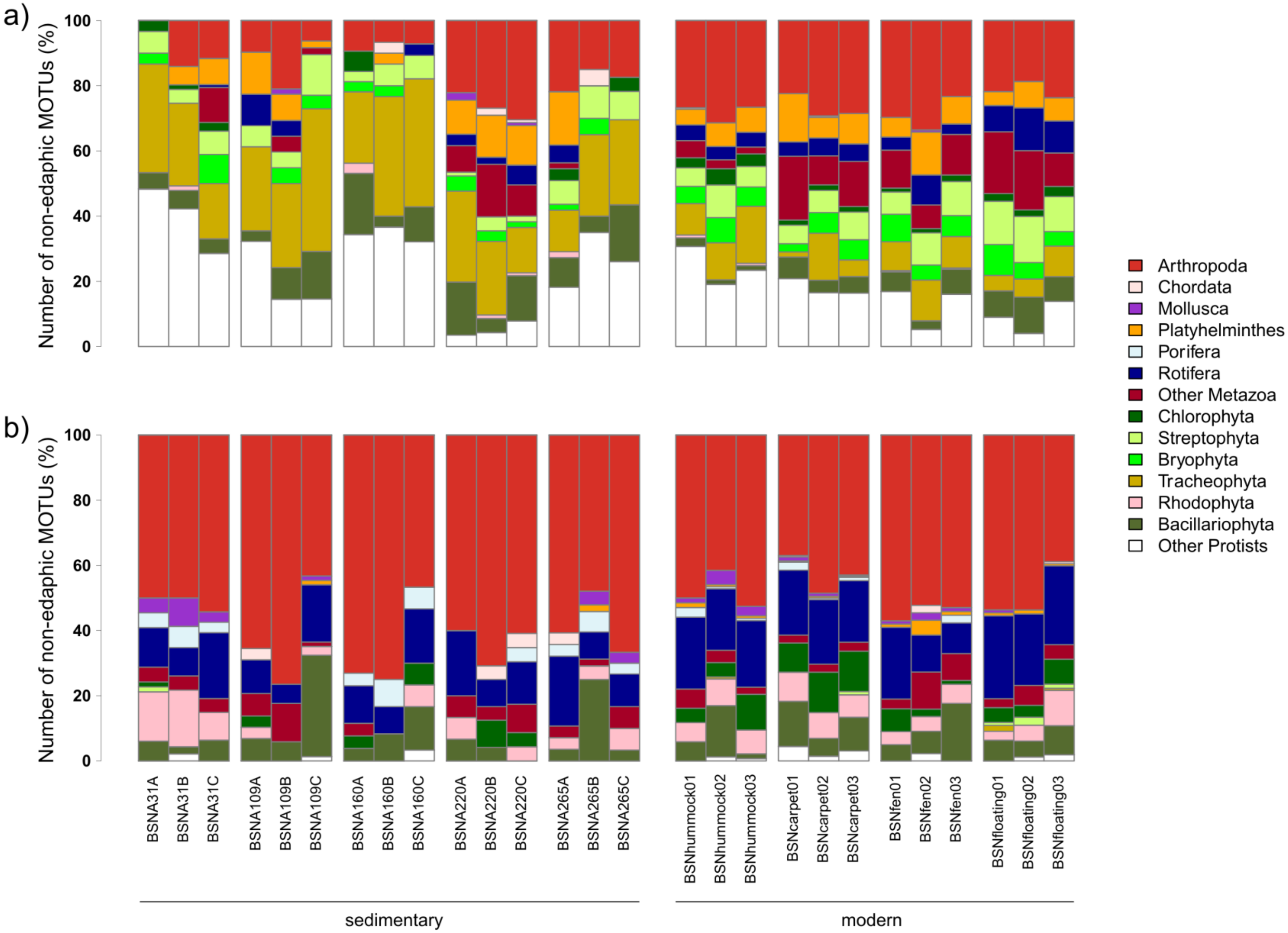

Figure 5.3. Relative diversity of non-edaphic taxa from sedimentary and modern samples, according to the detected presence of MOTUs of 18S (a) and COI (b), after removal of edaphic taxa.

protists. On the other hand, COI detected a higher number of Arthropoda, Rotifera and Bacillariophyta. Comparing the modern and past samples of 18S, we can observe a higher prevalence of Tracheophyta and other protists in the sedimentary samples, with a lower number of Arthropoda and other Metazoa. For COI, in general, the relative diversity of non-edaphic MOTUs by phylum seems more regular over time.

Figure 5.4 shows the non-metric multidimensional scaling ordination for the non-edaphic communities of modern and sedimentary samples using Jaccard dissimilarities. Results showed significant differences between communities for 18S (ANOSIM R=0.98, p-value $< 10^{-4}$) and COI markers (ANOSIM R=0.97, p-value $<10^{-4}$).

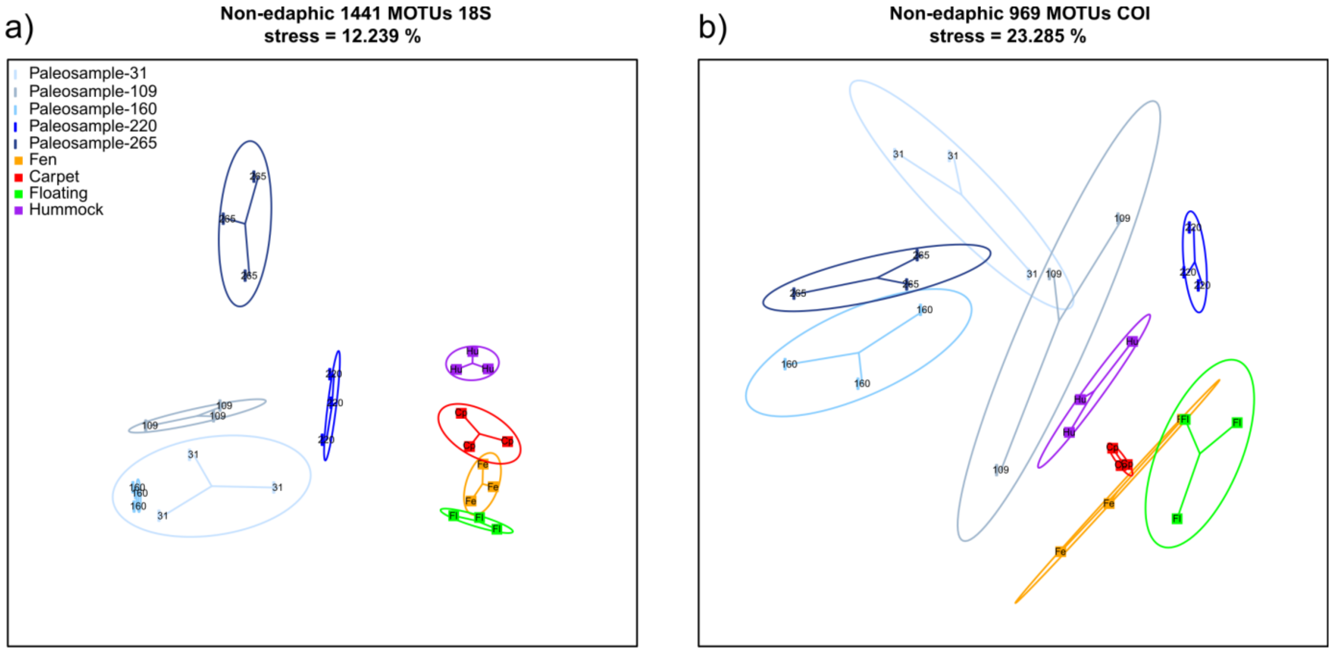

Figure 5.4. Non-metric multidimensional scaling ordination using Jaccard dissimilarities with non-edaphic MOTUs of samples for 18S (a) and COI (b) markers.

5.3.4 Viridiplantae

Figure 5.5 shows the abundances and relative MOTU diversity of 18S for the divisions of Viridiplantae, which represent a 54.54% of the sequences of the modern samples and a 4.52% of the sedimentary samples. The abundances present different patterns for the modern communities. The BSN-hummock is similar to BSN-carpet, with high abundances of Sphagnopsida and some Liliopsida and Magnoliopsida. While BSN-fen is similar to BSN-floating with a higher proportion of Bryopsida and Magnoliopsida. Table S2 shows the rank of the 20 most abundant 18S MOTUs for each modern community. A MOTU assigned as Bryophyta had the highest reads in all modern communities. *Sphagnum* dominated BSN-hummock and BSN-carpet while BSN-fen and BSN-floating are rich in Bryopsida. The hummock community also presents some Magnoliopsida, such as *Sanguisorba* and *Parnassia,* with some Liliopsida, such as Petrosaviidae. The carpet community presents a higher proportion of Liliopsida such as Cyperoideae and Petrosaviidae, while Magnoliopsida are represented by Asterales and *Filipendula*. The fen community is mainly composed by Bryopsida with some Magnoliopsida such as *Utricularia* and Liliopsida (Petrosaviidae). Finally, the floating community has higher amounts of Bryopsida and Droseraceae, followed by *Sphagnum*, Cyperoideae and *Utricularia.* On the other hand, COI failed to amplify any Tracheophyta or Bryophyta within the most abundant MOTUs (Table S3).

The sedimentary samples showed higher abundances of Liliopsida and Magnoliopsida, with some Pinopsida and other Streptophyta (Figure 5.5). The relative MOTUs diversity also presents some differences between modern samples, where BSN-hummock and BSN-carpet are similar and have high diversity of Sphagnopsida. BSN-fen and BSN-floating have more Bryopsida, other Bryophyta and Charophyceae. On the ancient samples, there are higher presence of Pinopsida and Magnoliopsida. Table S4 shows the rank of the 20 most abundant MOTUs for sedimentary samples after removing the edaphic taxa. All samples reflect a relatively high number of Tracheophyta, such as Cyperoideae, Pooideae, Mesangiospermae or rosids. We can observe a shift between Cyperoideae and Pooideae over time, with Cyperoideae dominating the samples BSN-31 to BSN-160 and Pooideae being more abundant in the oldest samples (BSN-220 and BSN-265). BSN-31 has a 14.28% of Viridiplantae sequences, this sample is dominated by Cyperoideae, with some Mesangiospermae (Magnoliopsida) and Bryopsida. It also presents some proportions of Pooideae and Saxifragales. BSN-109 (2.58% Viridiplantae) is also dominated by Cyperoideae, Pooideae and Mesangiospermae, jointly with Petrosaviidae, lamiids and *Equisetum*

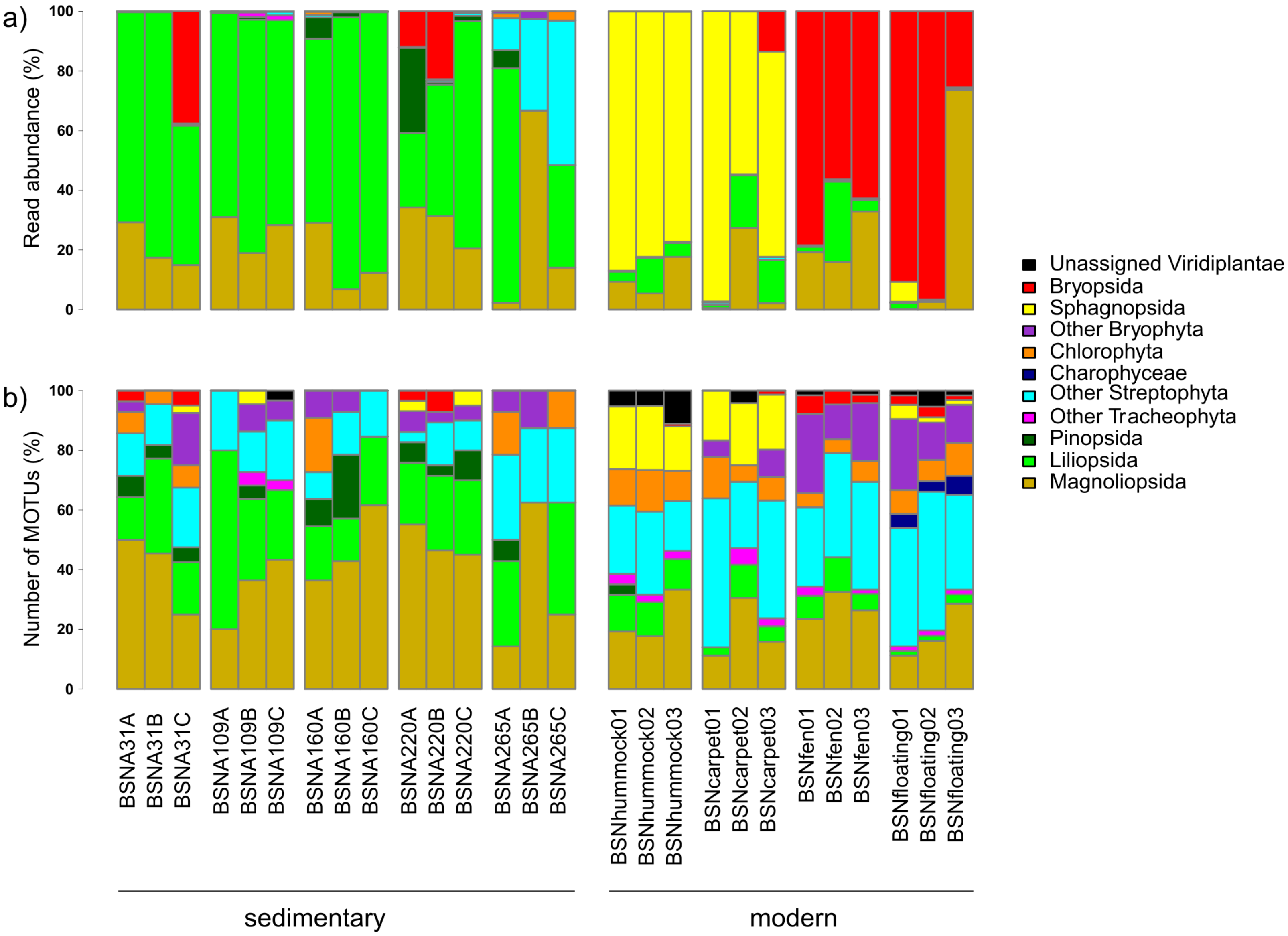

Figure 5.5. Abundance (a) and relative MOTU diversity (b) for the divisions of Viridiplantae detected using 18S.

sp. BSN-160 (1.79% Viridiplantae) has also high prevalence of Cyperoideae and Mesangiospermae, followed by Pinidae and Cupressaceae. Surprisingly, BSN-220 (11.99% Viridiplantae) presents a higher proportion of Tracheophyta in the most abundant MOTUs. It is dominated by Pooideae, accompanied by Pinidae and Bryopsida. It also presents some rosids, lamiids and Betulaceae. BSN-265 (0.07% Viridiplantae) is still dominated by Pooideae, with some presence of Pinidae. In general, the total amount of reads recovered from plants is lower in the sedimentary samples compared to the modern samples, and it decreases with depth. Figure S1 (supplementary material) shows the non-metric multidimensional scaling ordination for the Viridiplantae (18S marker) in modern and sedimentary samples using Jaccard dissimilarities. Results showed significant differences among communities for Viridiplantae (ANOSIM R=0.92, p-value $< 10^{-4}$).

5.3.5 Arthropoda

Figure 5.6 shows the read abundance and relative MOTU diversity of COI for the phylum Arthropoda. The relative abundance of the diverse orders strongly differ between the modern samples and the ancient ones. The modern samples present a higher abundance of mites (Sarcoptiformes and Trombidiformes), whereas the sedimentary samples present a large variability among them. The BSN-265 yielded a larger proportion of Opiliones and Copepoda, while BSN-160 had very low abundances. On the other hand, BSN-220 presents similar proportions to modern samples. Finally, BSN-31 and BSN-109 have outstanding proportions of Copepoda and Branchiopoda. Some orders, such as Ostracoda, only appeared in the modern samples. Table S3 (supplementary material) shows the rank of the 20 most abundant MOTUs for COI modern samples. Half of the most abundant MOTUs are from Arthropoda, specially the mites Sarcoptiformes and Trombidiformes, basal Hexapoda (Collembola), Insecta (Diptera, Coleoptera), Maxillopoda (Cyclopoida, Harpacticoida) and Ostracoda. In some cases, the taxonomic assignment could reach the species level. The hummock community presented a higher proportion of Arachnida.

Table S5 (supplementary material) shows the rank of the 20 most abundant MOTUs for COI ancient samples without the edaphic taxa. Most of the MOTUs could

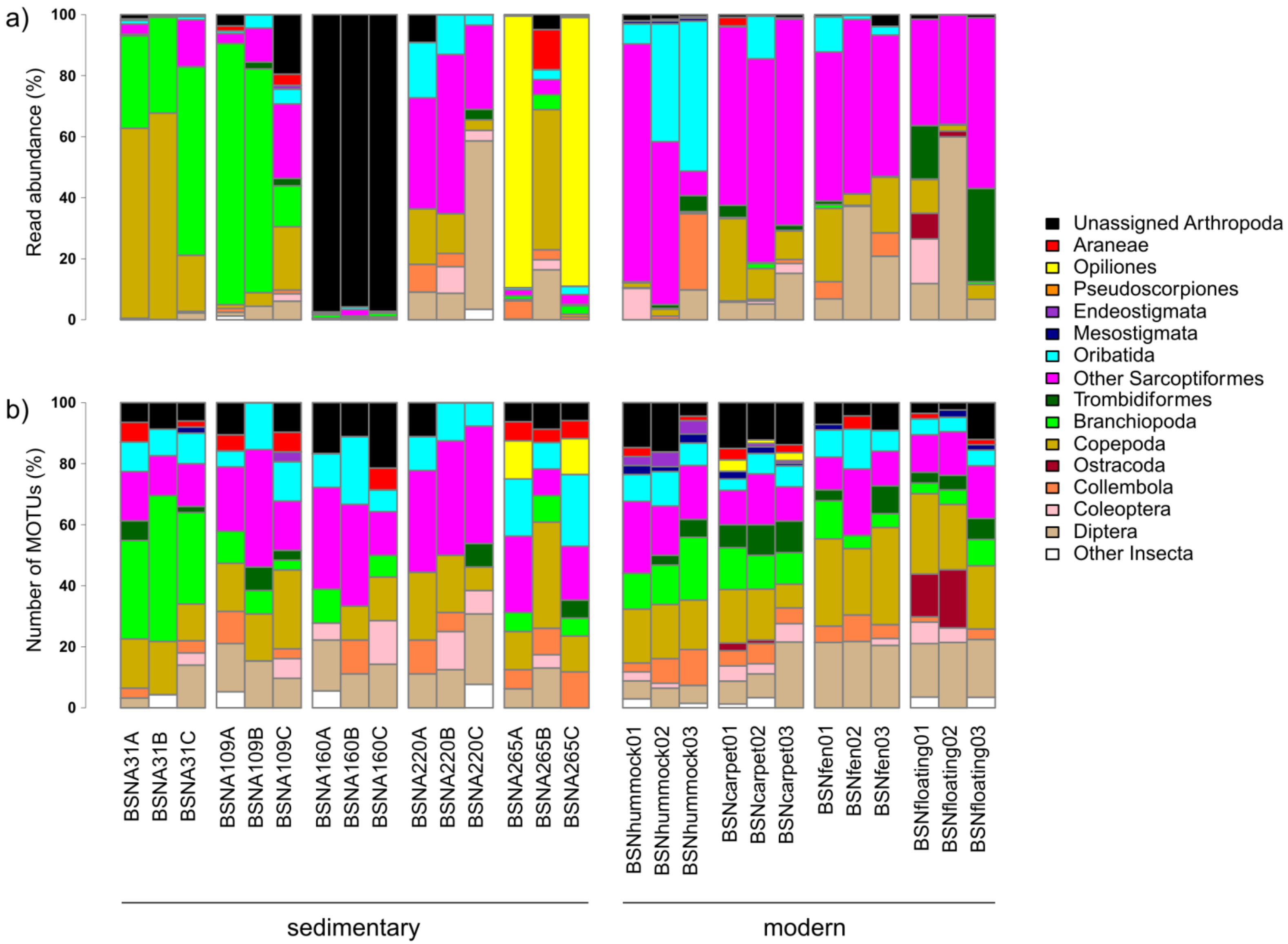

Figure 5.6 Read abundance (a) and relative MOTU diversity (b) for orders of the phylum Arthropoda detected by COI.

be identified only to the ranks of kingdom to order. From all the MOTUs, a higher number of Metazoa can be appreciated. From the Arthropoda that could be identified, most were Branchiopoda, Maxillopoda and Arachnida. There is a community shift from Branchiopoda and Maxillopoda in the most superficial samples (BSN-31, 109, 160) to an Arachnida community with Insecta and Collembolla (BSN-220, 265). Figure S2 (supplementary material) shows the non-metric multidimensional scaling ordination for the Arthropoda (COI) in modern and sedimentary samples using Jaccard dissimilarities. Results showed significant differences among communities for Arthropoda (ANOSIM R=0.89, p-value < 10^{-4}).

5.3.6 Comparison of sedimentary DNA to pollen and macroremains

We compared the Viridiplantae 18S from sedimentary DNA results with the environmental reconstruction of the same record by Garcés-Pastor et al. (2017). Figure 5.7 shows the diagram with the presence/absence of the 18S DNA sequences for the five sedimentary depths compared with the pollen and macroremains. We can observe the presence of conifer and Bryophyta sequences in all samples, while dicots and monocots are mainly in BSN-220, On the other hand, green algae are more present in BSN-31.

BSN-31 presents high proportions of Cyperoideae and Bryopsida with a MOTU assigned to Mesangiospermae (Magnoliopsida) that also appears in BSN-109 and BSN-160 (BOG2_000000149). It also presents some proportions of Pooideae, Saxifragales, Petrosaviidae and Pinidae and traces of *Vaccinium* sp (Table S3). In Table BOG_18S (Supplementary material) we can also observe the presence of Desmidiales, Streptophytina, Chlorophyceae and Scenedesmaceae. The macroremains presented low proportion of *Sphagnum*, with Ericaceae, *Polytrichum* and *Equisetum* in that depth. Regarding the pollen, *Pinus,* Ericaceae and Poaceae frequencies are well represented while Apiaceae have its highest values.

BSN-109 also presents high proportions of Cyperoideae, Pooideae and the former Mesangiospermae sequence, accompanied by Poaceae and asterids. The presence of Betulaceae, *Betula* and Pinidae is also remarkable, with *Equisetum,* Bryophyta and Desmidiales. Garcés-Pastor et al. (2017) also found *Equisetum* and

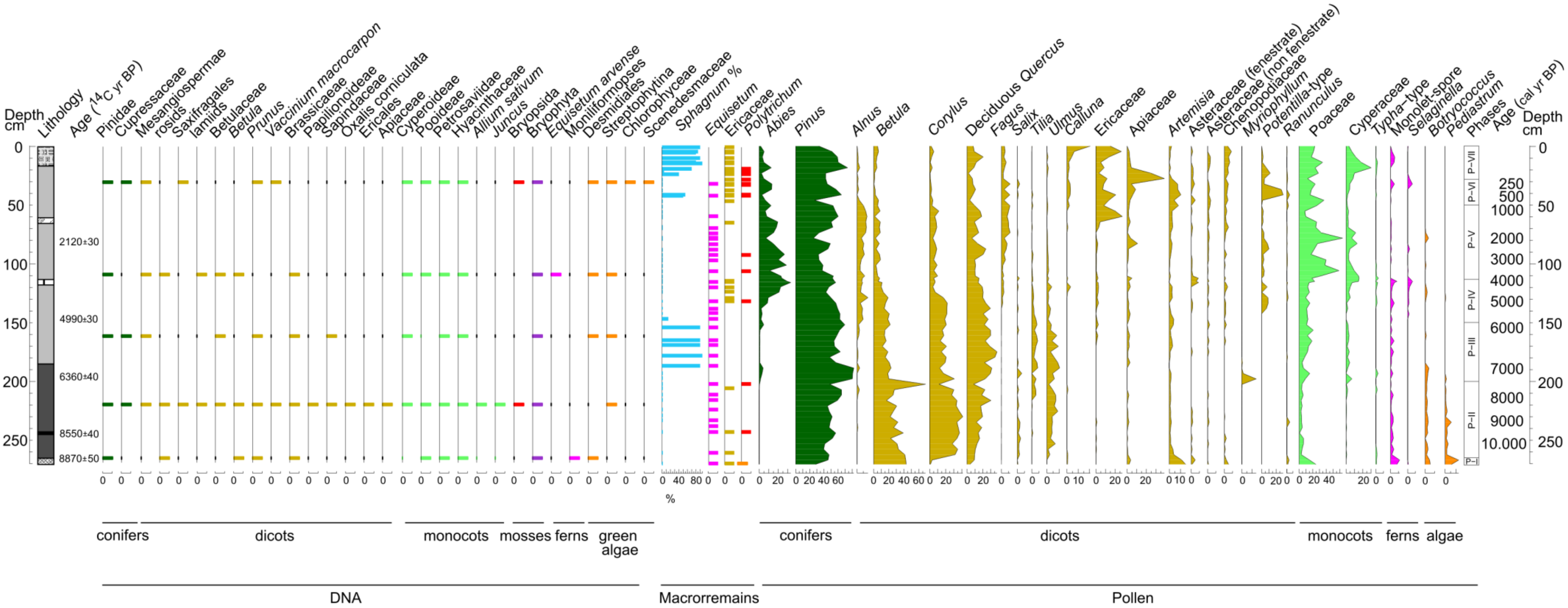

Figure 5.7. Diagram with the presence/absense of detected DNA sequences of Viridiplantae and the abundances of pollen and macroremains from the morphological study by Garcés-Pastor et al. (2017).

Sphagnum macroremains at this depth. Pollen presented the highest amounts of *Abies*, Poaceae and Cyperaceae. On the other hand, *Pinus* is well represented and *Betula* has relatively low frequencies. Some ferns (Monolet-spore and *Selaginella*) are observed.

BSN-160 also has high amounts of Cyperoideae and the former Mesangiospermae sequence, followed by Pinidae, Cupressaceae and some Betulaceae. It also presents Desmidiales, Rhodophyta and Bryophyta. COI detected a MOTU assigned to Porifera (Table S5). Although freshwater Porifera are in minor proportion, its presence has been reported in Bassa Nera (Garcés-Pastor et al., 2017). In the morphological palaeoenvironmental reconstruction, no *Sphagnum* macroremains were found at this depth, whereas pollen presented high amounts of *Pinus, accompanied* by *Betula* and some Poaceae.

BSN-220 community has high abundance of Pooideae, Pinidae and Bryopsida accompanied by some Betulaceae, Sapindaceae and *Pinus*. Some traces of Cyperoideae and Ericales were also detected, with some COI sequences assigned as Viridiplantae (probably green algae) and Rhodophyta. This depth did not present *Sphagnum* or other macroremains. Regarding the pollen, we can appreciate high proportions of *Pinus*, *Betula* and *Corylus*. However, Poaceae and Cyperaceae have its lower values. Some *Botryococcus* was observed.

BSN-265 has the lowest DNA abundances, but high proportions of Pooideae and Desmidiales, with some presence of Pinidae and traces of *Betula*, Brassicaceae and *Prunus*. There are also Bryophyta, while COI detected one MOTU of Rhodophyta, a Porifera and a Bacillariophyceae (Table S5). Regarding the environmental reconstruction, there is no available data of macroremains. By contrast, pollen present some *Pinus* and Poaceae proportions while *Betula* and *Artemisia* reach the highest values. Some ferns (Monolet-spore) and algae (*Botryococcus* and *Pediastrum*) could also be appreciated.

5.4. Discussion

5.4.1. Modern communities in Bassa Nera

Our results suggest that the 18S marker is more suitable to identify a broader range of eukaryotes and to assess the relative abundances of Viridiplantae. However, due to its lower natural variability, this marker has a lower taxonomic resolution than COI. Therefore, most MOTUs could be identified only to the rank of Family or higher. On the other hand, COI is more suitable for Metazoa, allowing to characterize many Arthropoda, Annelida and Rotifera, some of them at the species level. Nonetheless, COI yields a higher proportion of unassigned sequences that could correspond to the ones that 18S identified as Cercozoa or Ochrophyta, highlighting the gaps in the current COI reference databases for other groups different from Metazoa (Wangensteen et al., in review).

The obtained MOTUs for the vegetation communities broadly correspond to the communities observed during the sampling. For the case of the BSN-Floating03 subsample, which showed a high abundance of Magnoliopsida in comparison with the other subsamples, a *Drosera* was observed during the sample processing. Another species found in the floating and fen communities was *Utricularia* sp, which was also observed in the catchment (Pérez-Haase and Ninot, 2006; 2017). The MOTU classified as Petrosaviidae include sequences of Poaceae, Typhaceae and Cyperaceae (Suplementary material). All the modern samples present Petrosaviidae or Cyperaceae reads, which is coherent with the catchment vegetation. The communities shift from Sphagnopsida to Bryopsida as samples get closer to the pond. The presence of Tracheophyta such as *Sanguisorba*, *Parnassia* and Violaceae in the Hummock together with *Filipendula* in the carpet suggest a less humid micro-environment. On the other hand, the presence of *Utricularia* and Droseraceae in the fen and floating communities indicates wetter conditions and probably nutrient deficit, since both are carnivorous.

On the other hand, Table S3 (supplementary material) shows a high amount of assigned sequences of Arthropoda by COI, with a higher proportion of Diptera. Even though Sarcoptiformes are the most abundant order, different freshwater taxa dominate each community (Thorp and Covich, 2009), such as Camisiidae, Malaconothridae and Limnozetidae (Suplementary material). Hummock has *Nothrus* *pratensis*, while carpet presents *Tyrphonothrus maior*. In the case of fen and floating communities, there is no lower taxonomic identification below the order level. However, Diptera and Harpacticoida abundances increase with proximity to the floating area. The only Diptera with high abundances in the hummock is *Limnophyes*, which is a chironomid. Carpet presents the Tabanidae *Atylotus fulvus* and another genus of chironomid (*Paracricotopus*). The fen community has the ceratopogonid *Stilobezzia ochracea* and *Culicoides kibunensis* and the chironomid *Corynoneura*, all of which present aquatic larvae. The Floating community has a higher abundance of Diptera, the ceratopogonid *Dasyhelea modesta* and *Palpomyia lineata*, and the chironomids *Monopelopia tenuicalcar* and *Polypedilum tritum*. The harpacticoid copepod *Bryocamptus pygmaeus* is found in the carpet, fen and floating communities. This species inhabits freshwater environments in mountain regions and presents a wide ecological plasticity (Jersabek et al., 2001). With 18S we also obtained good taxonomic resolution for some Arthropoda (Table S2, supplementary material). As occurs with COI, the order Sarcoptiformes (Desmonomata, Brachypylina) has the higher dominance in all communities (Figure 6, Arthropoda) but in general, the taxonomic resolution is lower. There are some taxa that could be assigned to genus, such as *Hydrozetes*, an aquatic mite in the fen and floating communities or the freshwater copepod *Acanthocyclops* in fen.

The strengths of both markers have allowed to reproduce the extant community diversity of Bassa Nera. In order to use the MOTUs as ecological indicators, a high taxonomic resolution, at the genus or species level, is desirable. Therefore, COI would be more suitable than 18S for obtaining detailed ecologically relevant information, whereas the better assignment rates of 18S might make this marker more suitable for detecting changes in the relative abundances of higher taxonomic groupings.

5.4.2. Ancient DNA in sedimentary samples

Once the possible living edaphic taxa are removed from the communities, we observe that the patterns of diversity of MOTUs for 18S and COI become more similar between the sedimentary and modern samples (Figure 5.4). The non-metric multidimensional scaling ordination showed that modern and sedimentary communities are statistically different. At first sight, none of the reconstructed assemblages from ancient communities that we have studied can be considered to reproduce the modern assemblages. This suggests that more exhaustive studies must be performed to create modern community-DNA analogues for all the Phyla. However, some differences in abundances between sedimentary and modern samples might be a result of a differential preservation rate of the nuclear and mitochondrial DNA along time in the different taxa. Our results suggests that the 18S fragment is degraded earlier for plants than for animals, being COI better preserved. For example, the low detection rate of 18S from Bryophyta sequences cannot be due to gaps in reference databases, since they have been abundantly identified from the modern samples. This would reinforce the idea that mitochondrial DNA is more protected than nuclear DNA from degradation. But the differential detectability could also be just a result of the DNA abundance. As mitochondrial DNA, COI has in general a higher number of copies per cell (Pääbo et al., 2004). On the other hand, the copy number of tandem rRNA sequences for 18S may considerably vary between different eukaryotic groups (Zhu et al., 2005). As a result, the quantitative comparison of ancient community composition based on sequence abundances must be interpreted with caution. Although their time scales were broader, Epp et al (2012) also found a lower amount of bryophyte DNA on sedimentary samples while the recent soil samples had high abundances. They suggested that Bryophytes contain secondary metabolites that enhance DNA degradation (e.g. Xie and Lou, 2009) and this could potentially cause proportionally higher DNA degradation rates after cell lysis in bryophytes compared to other organism groups. RNA metabarcoding would be a suitable technique to be used in future studies for assessing just communities of living organisms, whose results could be then compared with the results from total DNA (Guardiola et al., 2016; Lejzerowicz et al., 2013).

5.4.3. Palaeoenvironmental reconstruction and ancient DNA

In general, the reconstruction obtained from 18S ancient-DNA (Figure 5.3) cannot reach high taxonomical resolution and, with some exceptions, most of the recovered taxa are in the level of family or above. This might constrain the interpretation and comparison to macroremain and pollen data. However, we find that the studied proxies offer complementary information. In this section the discussion will be structured into the five studied depths.

BSN-31: In this case, the *Sphagnum* macroremain proportions could be related with the Bryophyta sequences while *Polytrichum* might correspond to the MOTU assigned to Bryopsida. On the other hand, *Vaccinium* sequence also fits with the Ericaeae. The high amounts of *Pinus* and *Abies* pollen match with the sequences of Pinidae, which are detected along the whole record. Indeed, the relatively high proportions of Poaceae pollen also coincide with the sequences of Pooideae and Petrosaviidae. On the other hand, the presence of Desmidiales, Streptophytina, Chlorophyceae and Scenedesmaceae suggest a moist environment, also corroborated by the presence of COI sequences of Bacillariophyceae and Porifera (Table S5, supplementary material).

BSN-109: The sequences of Cyperoideae, Pooideae and Poaceae would match with the higher amounts of pollen from Cyperaceae and Poaceae. The detection of *Betula* and Pinidae agrees with the presence of the mixed forest based on the pollen frequencies. The sequences of *Equisetum* and Bryophyta would match the *Equisetum* and *Sphagnum* macroremains. Regarding the aquatic system, Desmidiales might indicate some water table, which is in line with the pond scenario that describes Garcés-Pastor et al (2017) for this period.

In BSN-160, both DNA sequences and pollen point to a mixed pine and deciduous forest. On the other side, the high amounts of Cyperoideae pollen and aquatic green algae would match with the palaeoenvironmental interpretation of a shore environment. This is corroborated by the sequences of Desmidiales, Rhodophyta, Bryophyta and the freshwater Porifera, which might also suggest more moisture.

For BSN-220, Betulaceae and Sapindaceae sequences suggest the presence of a montane forest with some Pinidae, coinciding with the higher pollen frequencies of *Betula* (Garcés-Pastor et al., 2017). On the other side, the low abundances of Cyperoideae sequences match with the low proportions of Cyperaceae pollen. Regarding the aquatic system, the presence of Rhodophyta sequences and *Botryococcus* agree with some water presence.

Even though BSN-265 has the lowest DNA abundances, we could extract some information. The Pooideae sequences agree with the steppe-like grasslands represented by Poaceae and *Artemisia*. While the low proportions of *Betula* sequences coincide with the *Betula* pollen. On the other hand, the sequences of Desmidiales agree with the open water environment also reported by high proportions of *Botryococcus* and *Pediastrum* by Garcés-Pastor et al. (2017).

Our results highlight that the interpretation of ancient sedimentary DNA does not entirely overlap with the reconstruction based on pollen and macroremains. However, the combination of both reconstructions reveals more detailed information of plant palaeocommunities than that achieved by each individual approach (Jørgensen et al., 2012). Pollen analysis may provide information at a more regional level, while macroremains and sedimentary DNA may provide more local details. A higher taxonomic resolution for plant species could be probably obtained from using different metabarcoding markers, such as chloroplast markers (Parducci et al., 2017).

5.4.4. Future uses of peat bog metabarcoding for palaeoenvironmental reconstructions. Pros and cons and things to improve

Our results suggest that 18S and COI markers are useful to study modern and past peat bog communities. A multi-marker approach is recommendable in order to cover the entire community biodiversity (Epp et al., 2012). Currently, the most significant limitation in the analysis of community and sedimentary DNA is the lack of exhaustive reference databases. Such collections must contain a broad range of barcode sequences derived from accurately identified species and covering all the major lineages of Eukaryota. Nevertheless, DNA identifications can be more easily standardized and are more objective in comparison with morphology-based identification approaches (Jørgensen et al., 2012).

In this study, 18S has provided more useful information about past communities than COI, which was mainly restricted to Metazoa. The use of COI would allow a precise assignment of animal communities provided that a complete reference database is available (Wangensteen and Turon, 2016). However, with the current reference database available for Pyrenean peat bog communities, the taxonomic results from COI are scarcely better than those from 18S. This issue will undoubtedly be solved in the future by improving the barcoding efforts. In order to obtain a more accurate description of the vegetation palaeoenvironments, it would be desirable to use some chloroplast marker which allows for a better taxonomic resolution than 18S for higher plants. However, the markers used in this work have allowed to obtain some insights on ancient communities and results which broadly agree with those obtained from morphological analysis of pollen and macroremains. The present work is an exploratory study with 18S and COI makers on a small number of sedimentary samples. More extensive studies with higher resolution will undoubtedly allow for a more detailed comprehension of the information provided by the DNA from past communities.

Morphological palaeoenvironmental studies to date are mostly based on the identification of vegetal remains. Studies in palaeoecology of other organisms such as Arthropoda or other Metazoa have been limited to the scarce biological traces that remain in the sediment. With the proper analysis of metabarcoding data based on modern analogues, this DNA technique has the potential to offer a new palaeoenvironmental multi-approach of diverse taxa from the same period. Such approach would allow a better understanding of the relationships between animal and vegetation communities and their response to past climatic shifts. The advantages of metabarcoding to study a large number of taxa simultaneously without previous morphological expertise is obvious in the case of understudied or complex groups, where finding taxonomic expertise is often impossible.

Although the use of metabarcoding does not depend on taxonomic expertise, it requires some bioinformatic skills. The laboratory procedures and data collecting may be considerably shorter than for morphological analyses, but the use of contrasted bioinformatic pipelines and reliable reference databases is crucial for obtaining accurate results. Further investigations are also needed in order to study how DNA degradation affects the different markers for different taxa. For example, in this study we have seen that the DNA from *Sphagnum* and other mosses is probably not well preserved and might be undetectable in ancient samples with the used markers.

Another limiting factor is the scarce knowledge about the autoecology of many small-sized metazoan groups, with some notable exceptions such as chironomids. Once the reference databases are improved and the sequences are assignable to the genus or species level, the ecological interpretation of this data will need current information on species distribution and autoecological preferences. This knowledge would allow the acquisition of reliable ecological information from a number of independent taxonomical sources such as mites, collembola, copepoda, ostracoda and many other small arthropods and metazoans, which would provide robust inferences of palaeoecological reconstructions from the detection of metazoan species (Pansu et al., 2015).

This is the first attempt to sequence DNA of ancient samples from the Pyrenean peat bogs. We were able to amplify DNA and get useful sequencing information from samples spanning a period of 10000 years. However, the number of sedimentary samples analysed in this preliminary work is scarce and does not allow to obtain robust conclusions. It would be desirable to perform more exhaustive samplings in the future.

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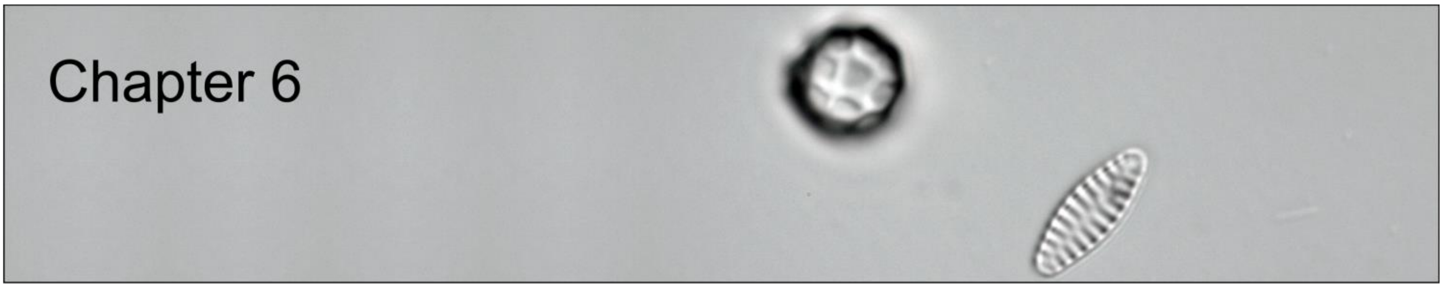

Chapter 6

General discussion

“Cuando creíamos que teníamos todas las respuestas, de pronto, cambiaron todas las preguntas"

- Mario Benedetti -

In this section, a general discussion of all the results obtained in the preceding chapters will be presented together with former palaeoenvironmental analyses carried out in the Pyrenees. The temporal framework ranges from the early Holocene to the present. Chapter 2 studies the last millennium at a high resolution and proposes a palaeoenvironmental tool that is applied in Chapter 3 to study vegetation dynamics during the whole Holocene. Whereas Chapter 4 focus in the relationship between the pollen of Bassa Nera, regional mountain pine forests and climate from the Little Ice Age to the 21th century. Chapter 5 provides a description of current and past eukaryotic communities in Bassa Nera. As a result, this section has been divided into six subsections, corresponding to the main topics covered in this thesis: palaeoenvironmental history, anthropic pressure, climate and vegetation interactions, palaeoenvironmental tools, DNA techniques in palaeoecology and palaeoenvironmental reconstructions. Finally, possible directions of future work for palaeoecological research in the Pyrenees will be proposed in this section.

6.1 Palaeoenvironmental history of Bassa Nera

The palaeoecological reconstructions of the Bassa Nera sequences studied in this thesis have provided a robust background on vegetation dynamics and ecological changes in this lacustrine system since the onset of the Holocene. Vegetation responses to environmental shifts have been addressed in Chapters 2 to 4, based on different temporal frames and using diverse palaeoecological techniques. From the onset of the Holocene to Mid-Holocene, environmental changes in Bassa Nera have been mainly driven by climatic variability (Chapter 3). However, the appearance of charcoal around 6700 cal yr BP suggest that fires might have also influenced the vegetation dynamics and resilience.

6.1.1 Early Holocene (10.000-7000 cal yr BP)

The results of Chapter 5 have revealed that by the onset of the Holocene (10.211 cal yr BP), Bassa Nera was an open water lake surrounded by steppe-like grasslands with a relatively nearby birch forest. This scenario might be the transition from the steppe taxa that abounded during the colder and arid Younger Dryas to the expansion of deciduous forest in Central Pyrenees (González-Sampériz et al., 2006; Jalut et al., 1992; Reille and Lowe, 1993). On the other hand, the high proportions of *Betula* agrees with the early phase of birch colonization in the Pyrenees during the Late Glacial-Holocene transitional period (Cunill et al., 2013; Gil-Romera and González-Sampériz, 2014; Reille and Lowe, 1993). Shortly after, between 10.070 and 7343 cal yr BP, a mixed *Corylus* and *Betula* forest established in BSN catchment, prompted by warmer summer temperatures (Anderson et al., 1988; Cacho et al., 2010; Heiri et al., 2003). This is in line with the *Betula*-*Quercus*-*Corylus* succession registered by several authors (González-Sampériz et al., 2006; Jalut et al., 1992; Montserrat, 1992; Reille and Lowe, 1993) in all records from both slopes of the Pyrenees. However, our results suggest a poor *Quercus* development, agreeing with González-Sampériz et al. (2006) in Portalet. Probably *Corylus* dominated over *Quercus* because of the altitude and strong oceanic influence.

Some fires occurred between 9968 and 8548 cal yr BP in Bassa Nera. Given the lack of other anthropic indicators, they might be attributed to natural fires prompted by the large amounts of deciduous biomass, as occurred in the nearby Portalet peat bog and Basa de la Mora Lake in the same period (Lasheras-Álvarez and Sanz, 2013; Pérez-Sanz et al., 2013) (Figure 6.1).

6.1.2 Mid-Holocene (7000-4000 cal yr BP)

Deciduous forest decreased between 7343–5832 cal yr BP, while *Abies* appeared and rose by 6356 cal yr BP. The appearance of *Abies* is coherent with the east-to-west colonization of the Central Pyrenees (Pèlachs et al., 2009; Resina et al., 2016), with some chronological differences that might be related with their location and altitude (Figure 6.1). Around 6800 cal yr BP, the records of Bassa Nera show periods of subaerial exposure or hydric fluctuations (Chapter 3) that might point to a transition between aquatic and littoral scenarios. This period has been recorded by a transition from lakes to peat systems in nearby localities such as Portalet and Estanilles peat bogs and Burg lake (González-Sampériz et al., 2006; Pèlachs et al., 2011; Pérez-Obiol et al., 2012).

A higher fire frequency is inferred from the noteworthy increment of charcoal around 6200 cal yr BP. By 5832-3912 cal yr BP, a downward shift of deciduous forests

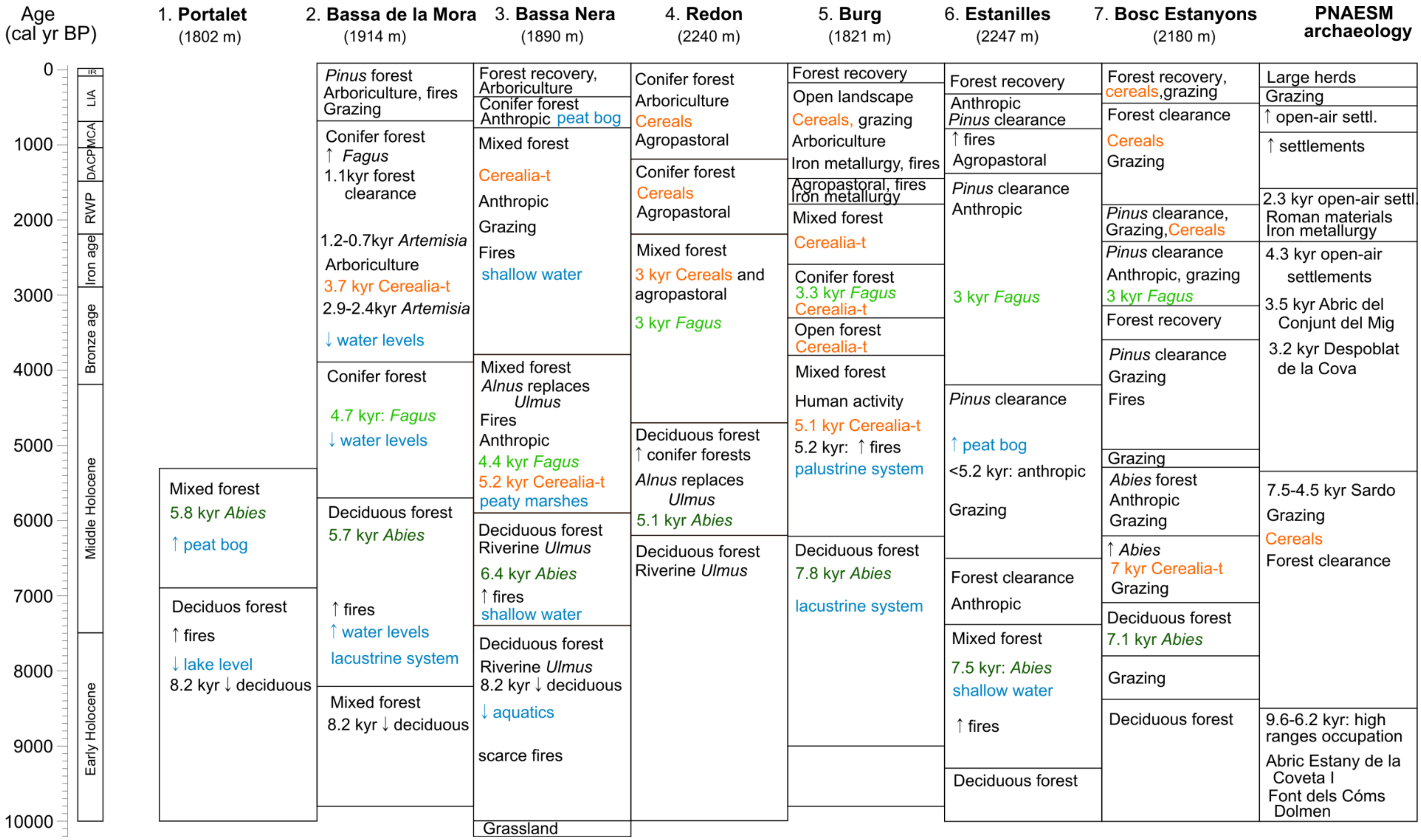

Figure 6.1. Overwiev of sediment results (pollen, algae, macrorremains) and resultant climate and environmental inferences of Bassa Nera and nearby lacustrine and peat bog sequences from the Central Pyrenees and pre-Pyrenees recording the last 1000 years. Dark Ages Cold Period (DACP), Medieval Climate Anomaly (MCA), 'Little Ice Age (LIA)' and Industrial Revolution (IR). Vegetal associations – Mixed forest: *Pinus, Abies, Betula, Corylus* and deciduous *Quercus*; Conifer forest: *Pinus* and *Abies*; Montane forest: *Corylus* and deciduous *Quercus*. Human pressure Crops: Cerealia-t and *Secale cereale*; Grazing: *Rumex*, Chenopodiaceae, *Urtica* and *Potentilla*; Human-related taxa: *Plantago*, Asteraceae and *Artemisia*. References: Portalet (González-Samperiz et al., 2006;Gil-Romera et al., 2014); Bassa de la Mora (Pérez-Sanz et al., 2013); Bassa Nera (Garcés-Pastor et al., 2016, 2017); Redon (Pla and Catalan, 2005); Burg (Bal et al. 2011;Pèlachs et al. 2007;2011); Estanilles (Pérez-Obiol et al., 2012;Cunill et al., 2013); Bosc Estanyons (Ejarque et al., 2010; Miras et al., 2007); PNAESM (Catalan et al., 2012; Rodríguez 2011)

prompted a transition from deciduous to coniferous taxa in Bassa Nera catchment. The *Abies* expansion and *Fagus* appearance by 4492 cal yr BP might have been a result of a shift to rainy and warm summers. Where *Abies* rose in altitude to avoid the warming while a change to greater precipitation might have promoted a downward displacement of optimal deciduous habitats (Alba-Sánchez et al., 2010; Pèlachs et al., 2011). A replacement of *Ulmus* by *Alnus* in 5286–4054 cal yr BP in Bassa Nera might have been favoured by the establishment of emerged lands and lakeshore environments (Pérez-Obiol et al., 2016; Revelles et al., 2015) and is coherent with the decline of elm in nearby Redon lake (Pla and Catalan, 2005) and the rest of the Pyrenees (Montserrat, 1992; Reille and Lowe, 1993) (Figure 6.1).

6.1.1 Late Holocene

During the last part of the Holocene Bassa Nera was mostly dominated by coniferous taxa with some montane forest. However, changes in the montane ratio have allowed to infer upward shifts of the montane boundary along this period. By 3000 cal yr BP, fires and agropastoral indicators evidence anthropic pressure though forest clearance (Chapter 3). On the other hand, the aquatic system remained as a pond with shallow waters.

The last millennium could be studied with pollen and diatoms (Chapter 2). As a result, more information about the aquatic environment was obtained. Results suggest that aquatic habitats progressively shrank through time due to changes in hydrological conditions. During the Medieval Climate Anomaly (1149-653 cal yr BP) montane forest was close to Bassa Nera catchment and there were some anthropic influence through forest clearance and local to regional crops. Regarding the aquatic system, changes in water level can be appreciated, possibly as a result of strong seasonality and hydric fluctuations. The unstable, cold and humid conditions during the MCA–LIA transition prompted a downward shift of the montane vegetation and a continuity of hydrological fluctuations, with some regional fires for crops and grazing. The second phase of the LIA was characterised by an intensification of human disturbance through grazing. On the other hand, the aquatic system presented shallower waters probably because of the infilling process and the development of the peat bog in the pond edges. The colder temperatures of this phase of LIA might have favoured the peat accumulation in Bassa Nera (Martınez-Cortizas et al., 1999). By the onset of the warmer and more arid period of the Industrial Revolution, which gave way to the Current Global Warming, a mixed forest dominated the catchment, with frequent regional fires and a continuity of shallow waters.

6.2 Anthropic influence in Bassa Nera

Bassa Nera has passed through short exploitation phases interspersed with periods of land abandonment and grazing, possibly as a result of the synergic effects between climate changes and human activity (Gassiot et al., 2012; Jalut et al., 2009). Our results suggest that the farming activities in BSN and their influence in the environment became evident during the Bronze Age and intensified in the Roman period and Middle Ages.

The first evidences of anthropic influence in Bassa Nera catchment are grazing activities, inferred by agropastoral indicators and charcoal from 7343 to 5832 cal yr BP. This is coherent with agropastoral activities and forest clearance recorded in the nearby records of Sardo cave (Gassiot et al., 2012), Bosc dels Estanyons and the Estanilles peat bogs (Cunill et al., 2013; Miras et al., 2007) (Figure 6.1). Between 5190 cal yr BP and 2000 cal yr BP societies established a cereal based agriculture in Bassa Nera. Although the first record is a punctual event (5190 cal yr BP), it coincides with cereal seeds found in Sardo cave and Cerealia-t pollen recorded in the Burg lake and Madriu valley records (Gassiot et al., 2014; Miras et al., 2007; Pèlachs et al., 2007). In agreement with the appearance of cereals in other studies of the Central Pyrenees and coinciding with Magny (2004), the development of cereal-based subsistence in BSN could have been prompted by the rise of the regional population and by dry conditions. The following period (5190–4300 cal yr BP) gave way to a raise in grazing activites and matches with an intensification of archaeological settlements found in PNAESM (Sardo cave, Estany de la Coveta I, Obagues de Ratera and Saboredo)(Gassiot et al., 2014). These period of high mountain exploitation in the Central Pyrenees (Gassiot et al., 2014; Jalut et al., 2000; Pèlachs et al., 2007) was probably prompted by the increased frequency of dry summers around 4600–4300 cal yr BP in the

Mediterranean area (Azuara et al., 2015; Jalut et al., 2000). A rise of fires and forest management through forest clearance and occasional crops in Bassa Nera and nearby valleys characterise the Bronze Age (4230–3500 cal yr BP) (Figure 6.1). The coeval spread of *Fagus* fits with its expansion in the Pyrenees and Cantabrian mountains (Magri, 2008; Montserrat, 1992; Pérez-Sanz et al., 2013). The rise of *Fagus* might have been favoured by the combination of the resulting open spaces from anthropogenic disturbance with a change to higher precipitation (Miras et al., 2007; Pèlachs et al., 2009). During the Late Bronze Age (3150–2650 cal yr BP), the rise of cereals and agropastoral indicators suggest the establishment of agricultural landscapes in BSN. This agrees with the record of higher anthropic pressure through farming and pasturing activities found in the nearby Burg, Estanilles and Bosc dels Estanyons peat bogs (Figure 6.1). Since that moment, Cerealia-t did not reappear in BSN until the Roman Period (2000 cal yr BP). By the Medieval Climate Anomaly (1100 cal yr BP) an intensification of agriculture and livestock occurred in Bassa Nera, where societies used fire to maintain open spaces (Bal et al., 2011; Pérez-Obiol et al., 2012). This coincides with an increase of human settlements in PNAESM and the Central Pyrenees (Catalán et al., 2012)(Figure 6.1 and 2.6). Around 800 cal yr BP, the appearance of *Secale cereale* together with agropastoral and arboriculture indicators suggest crop diversification and an increase of pastures. Also recorded in adjacent regions (Cunill et al., 2013; Miras et al., 2010; Pérez-Obiol et al., 2012) (Figure 2.6). By the end of the MCA-LIA transition (~ 450 cal yr BP), the drop of crops and the continuity of some grazing evidences suggest that people might have abandoned the farming activities in high ranges because of the increasingly colder conditions while some livestock persisted. However, around 120 cal yr BP, the frequent fires, forest clearance and a peak in Poaceae might be the result of an increased need for supplies and raw materials during the Industrial Revolution. Later, the disappearance of cereals and the reduction of agropastoral pollen indicate crop abandonment, probably as a result of the social and economic changes. The abandonment of rural lands and the establishment of Aiguestortes i Estany de Sant Maurici National Park in AD 1955 and the protection of its surroundings in AD 1990 favoured the expansion of arboreal taxa, such as *Pinus* (Améztegui et al., 2010; Bal et al., 2011).

6.3 Climate pressures in vegetation

In this thesis we have studied the dynamics of subalpine forests of Central Pyrenees during the last 700 years to assess the relationships between palynological and dendrochronological records (Chapter 4). Our results suggest a significant correlation between the integrative montane pollen ratio and the pine tree-ring growth of subalpine and alpine forests located less than 10 km from Bassa Nera. A high correlation between the ring-width and temperature reconstructions (NHTA and PTA) during the second phase of the LIA (1700-1850) suggest that radial growth of pines is more sensitive to the low temperatures during the past cold periods than to recent warming. In general, the tree-ring growth correlated with the temperature reconstructions (NHTA, PTA) and the solar grand minima of Maunder and Dalton, which it is in line with other studies performed on the Pyrenees (Büntgen et al., 2008; Dorado-Liñán et al., 2012).

On the other hand, PTA was the only reconstruction that correlated during the subperiods of 1850-1899 and 1950-1999, coinciding with periods of significant fluctuation of water availability and extreme precipitation events. This suggests that pine growth responded to a general climatic pattern (NHTA) modulated by local climatic forcings in the Iberian Peninsula and the Pyrenees (PTA). Regarding the climatic data of the last century, our results suggests that low temperatures before and at the end of the growing season do affect the tree-ring growth. Comparing the effect of the studied climatic variables, we observed a higher influence of temperature in radial growth, followed by summer precipitations (Tardif et al., 2003). Moving correlations for shorter intervals showed a shift in the most influential months between the first and second half of 20th century. The spring temperatures prevailed as the most important drivers of pine tree growth over the second half of the century, as a result of the increase of warmer conditions (López-Moreno et al., 2010) and reduced frequency of extreme winter cold events (Andreu et al., 2007; Tardif et al., 2003).

6.4 Uses of the montane ratio as a palaeoenvironmental tool

6.4.1 Altitudinal vegetation shifts

The montane-subalpine ratio has proven to be a good indicator of local vegetation altitudinal shifts with potential to be used as a palaeoenvironmental tool (Chapter 2) (Garcés-Pastor et al., 2016). However, high mountain landscapes are characterised by a contrast of vegetation mosaics (Loidi, 2017) and the current definition of the montane ratio might not include all of them. Moreover, studies such as Cañellas-Boltà et al (2009) have proven that some kinds of vegetation communities such as open landscapes might lead to concentrate higher amounts of pollen from lowlands transported by the upward flow. As a result, more studies should be performed in order to improve the calibration of this ratio. For example, more pollen studies should be performed with other altitudinal transects with diverse valley orientations of Central Pyrenees and vegetation diversity to find additional suitable pollen indicators. Calibrating that information with the current montane ratio would allow to embrace a higher vegetation diversity with more regional information. After creating a solid baseline of knowledge in the Pyrenees, this approach could be compared with other mountain ranges of the Iberian Peninsula or the Alps. Furthermore, it could be also interesting to explore the relationship between this ratio and temperature, in order to perform inferences. A further step could be to introduce a similar ratio using results from environmental DNA. Given that the DNA gives a more local information than pollen (Jørgensen et al., 2012), it might be less useful to detect altitudinal shifts in vegetation. But it could be very interesting to establish baselines for whole eukaryotic communities, to relate those communities to environmental parameters in order to detect good indicator eukaryotes and to monitor changes due to the ongoing climate warming, as well as to apply them as modern analogs to past DNA communities (Chapter 5).

6.4.2 Vegetation response to the North Atlantic influence

Furthermore, the relationship between the montane ratio and global climatic signals for the Northern hemisphere, such as the ice rafted debris (IRD), has allowed to better understand the vegetation response to North Atlantic influence and other pressures such as fires or anthropic influence (Chapter 3). During the Holocene Optimum (10.200-5300 cal yr BP) montane ratio and IRD trends were similar, and this might be related to the growth of deciduous taxa in response to a regional rise of moisture in the Northern Hemisphere (Pèlachs et al., 2011). By the end of Bond event 4, the montane ratio starts to decouple from IRD. Fires and climate might have affected the vegetation resilience of Bassa Nera and hampered its response to climate, prompting a progressive downward shift of montane vegetation in the catchment.

Although significant environmental changes occurred in BSN during the entire record, our results suggest that the Loss on Ignition (LOI) at BSN is less sensitive to global climate changes than at Burg. This shows a high influence of local factors in BSN, which might have hidden any direct relationship between IRD oscillations and BSN peat bog development (Mäukiläu, 1997). These different scenarios show how local influence could limit the suitability of LOI as a paleoclimate indicator in the case of peat bog systems.

6.4.3 Inference of tree growth from palynological indices

Montane ratio has also showed a significant correlation with ring-widths of pines from closer localities (Chapter 4), suggesting a sensitivity of those two proxies to temperature changes at local scales. This relationship could be used as a palaeoenvironmental tool to infer dendrochronologies for periods longer than those covered by chronologies at multidecadal resolution.

Given that the montane index is more useful at local scales, it could be interesting to carry out more local studies in order to better understand the relationships between radial growth and site conditions along ecological gradients. This would provide a proper background that would allow to improve the future climate tree-growth models in high mountain ranges.

6.5 DNA techniques in palaeoecology

Metabarcoding techniques have allowed to perform fast and objective ecological studies of broader taxonomical range (Hajibabaei et al., 2011; Taberlet et al., 2012), allowing the simultaneous detailed characterization of a wide array of diverse taxa present in the studied ecosystems. Our results have showed that this technique is potentially useful to study past and modern Eukaryotic peat bog communities, but it still presents some limitations. One of them is the natural sequence variability of the marker, that would allow to identify the detected sequences to a higher taxonomic resolution. In this study we have used COI and 18S. COI has a high taxonomic resolution, while the latter usually assigns sequences to higher ranks such as family or order (Guardiola et al., 2016). The second issue is the taxonomic gaps in current reference databases. This is a matter of economic resources and time to barcode more organisms and improve the databases. By improving the barcoding efforts, this issue will undoubtedly be solved in the future. There are additional problems related to possible contaminations during the DNA extraction and amplification procedures. But one crucial issue that needs to be considered for palaeoecological molecular studies is the presence of living edaphic communities in sedimentary samples.

In general, sedimentary DNA studies rely on the assumption that the age of the DNA recovered is the same as the age of the sediments in which it is found. This might be true when studying macroorganisms that are not part of the edaphic community. For examples with plants, even though roots can reach a certain depth. But universal primers such as 18S and COI are able to amplify a wide range of microbial taxa, which may belong to living communities. This problem is worsened by the fact that DNA from living organisms may be present at much higher concentrations than DNA from old remains. Therefore, sequences from edaphic organisms should be removed from the analyses in order to get more realistic reconstructions of the fossil communities and to compare them with the modern assemblages.

The results with edaphic DNA might compromise the use of some proxies that were used in palaeoecology, since we cannot assure if they come from living or dead communities. For example, parasitic and saprophyte fungi, mites, testate amoebae, and other non-photosynthetic organisms that feed on bacteria. Therefore, more studies must be performed to better understand the biology of those organisms for a proper interpretation of those proxies.

6.6 Palaeoenvironmental reconstructions

Morphological palaeoenvironmental studies from aquatic sediments are mostly based on the identification of pollen and vegetation remains. However, these proxies present some issues that could compromise the palaeoecological interpretation if they are not taken with caution.

Pollen assemblages comprise a distance-weighted integration of surrounding vegetation. They incorporate pollen grains from a variety of local and distant sources and are affected by the transit of ascending air currents that transport pollen from lower-altitude vegetation belts (Jackson and Lyford, 1999). As a result, pollen records could present an overrepresentation of some taxa, as we have observed with *Pinus* in Bassa Nera (Chapters 2, 3 and 4), while other taxa might be underrepresented or absent. Therefore, it is important to perform local and regional studies with modern vegetation analogs in order to better understand local palaeoenvironmental pollen reconstructions (Cañellas-Boltà et al., 2009). On the other hand, although pollen analyses have recently reached better taxonomic resolution because of improved identification keys, the work is still time-demanding, and the occurrence of morphological similarities allow taxonomic resolution only to the genus or family level in most cases, rarely to the species level (Faegri and Iversen, 1989; Jackson and Williams, 2004). On the other hand, macrofossils identification can reach lower taxonomic levels but the identification of the remains depends on the nature of the preserved material. There is also a bias due to differences in the tissues preservation, where seeds and buds use to be the best preserved and identifiable. By contrast, macroremains are much less common than pollen (Birks and Birks, 2000), and a species absence in the record does not necessarily indicate its absence from the local vegetation. Then, pollen has been mainly used to assess regional environmental questions, while macroremains allow a more local study.

On the other hand, metabarcoding techniques may use chloroplast markers like *TrnL* to obtain a broad register of the past vegetation communities. This DNA technique can help to overcome the problems in taxa identification providing higher taxonomic resolution and, in addition, elucidate the ancestry and genetic composition of ancient plant populations. Given that chloroplast sequences are essentially absent from angiosperm pollen (Birky, 1995), most of the amplified plant sequences come from locally deposited seeds or somatic tissues and represent flora from within the catchment area (Willerslev et al., 2003). So, sedimentary DNA seems to have an origin more similar to macroremains than pollen (Jørgensen et al., 2012; Parducci et al., 2017; Pedersen et al., 2013), thus providing better information on local biodiversity in palaeoenvironmental reconstructions.

Many studies (Jørgensen et al., 2012; Parducci et al., 2015; Pedersen et al., 2013) have revealed that sedimentary DNA data combined with other proxies such as pollen and macrofossils show a wider diversity of species than using those proxies separately. Therefore, DNA should be viewed as a complementary, rather than alternative, approach to assays of more traditional environmental proxies (Pedersen, 2015).

Metabarcoding allows to study other eukaryotic organisms that leave scarce biological traces in the sediment and could not be studied at a morphological level. With the use of proper markers, this technique has the potential to offer a new palaeoenvironmental multi-approach of diverse taxa from the same period. For example, using COI and *TrnL* markers together we would be able to obtain data from proxies that have been used in morphology such as oribatid mites, chironomids, diatoms, cladocera or vegetation communities. This would allow to obtain a huge amount of information in a short time with high taxonomic resolution, and would open a vast range of possibilities to palaeoecology research. Another advantage of this technique is the traceability of the information, meaning that those sequences that could not be taxonomically assigned with current reference databases will be stored in public repositories, and they could be objectively identified in the future using updated databases. This is not possible when using partial morphological identification data which are recorded as unknown taxa in classical studies.

6.7 Future work

This study highlights the use of independent and complementary proxies for further palaeoecological studies. The incorporation of new techniques such as DNA metabarcoding and the use of palaeoenvironmental tools like modern analogs and vegetation ratios will provide valuable information that will help to perform more robust interpretations of past environmental dynamics.

This thesis opens some topics which deserve to be more deeply studied in the future. In this section we have seen the potential of montane ratio and the need to perform further studies regarding modern analogs, to calibrate this ratio with other high mountain vegetation assemblages and even with eukaryotic communities.

It would also be interesting to perform more palaeoenvironmental reconstructions of the Pyrenees peat bogs, in order to have more data about the influence of Northern Hemisphere Climate in the vegetation and the aquatic systems, as well as to infer patterns in the expansion of high-mountain forest taxa through the Pyrenees, such as *Abies* or *Fagus*.

Future studies for assessing the communities of living organisms are an important step for DNA palaeonvironmental studies. For this aim, RNA metabarcoding is a suitable technique that could be used. The same sedimentary samples can be analysed for RNA and DNA. Then, results could be compared with the results from total DNA to distinguish the living organisms from the dead (Guardiola et al., 2016; Lejzerowicz et al., 2013). The knowledge of living edaphic communities would provide a baseline to properly study the past eukaryotic communities.

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Chapter 7

General conclusions

Overall, the study of the environmental changes occurred in Bassa Nera catchment along the Holocene presented in this dissertation contributes to a better understanding of the palaeoecology of Central Pyrenees. This study highlights the sensitivity of high-mountain vegetation of the Central Pyrenees to climate changes and anthropic pressures.

In this sense, the main conclusions of this dissertation are grouped according to the general objectives described in the Introduction.

Chapter 2:

- Vegetation of the Bassa Nera catchment responded strongly to climate with altitudinal shifts and is most likely currently responding to current global warming. From the Medieval Climate Anomaly to the Medieval Climate Anomaly – Little Ice Age transition, the montane–subalpine ecotone reached the Bassa Nera catchment.

- The montane ratio has proven the usefulness of good pollen indicators for revealing vegetation trends, providing a suitable tool for palaeoecological studies and for monitoring regional changes in natural communities in response to current global warming.

- Human management of natural resources has changed over the past millennium. Through the Medieval Climate Anomaly and Medieval Climate Anomaly – Little Ice Age transition, people of the region used fires to open the forests for cultivating and grazing. With the Little Ice Age cooling, grazing was the main form of resource exploitation. During the Industrial Revolution, some farming activities were still conducted until the authorities restricted resource exploitation by creating the National Park.

- Aquatic taxa, diatom communities and sedimentary units allowed to describe the peat bog development at the coring site and its infilling at approximately AD 1565.

- Consistent shifts in vegetation, fire activity and aquatic communities throughout the sequence are clearly related to climatic signals such as the Medieval Climate Anomaly and Little Ice Age phases.

Chapter 3:

- Changes in aquatic taxa, macroremains and sedimentary units show a non-linear development of the peat bog over the larger previous lake.

- Vegetation strongly responded to climate during the first half of the Holocene (10200–6700 cal yr BP) with punctual episodes of downward shift in the montane forest. Then, a transition period took place (5700–5250 cal yr BP) when climate and fires prompted a progressive downward shift of montane vegetation and its replacement by coniferous taxa, which has dominated the catchment with some mixed montane forest since 3912 cal yr BP.

- The montane ratio has enabled the assessment of the potential correlations between changes in vegetal communities and the climatic forcing indicated by the Ice Rafted Debris index, and highlighted the different responses of the vegetation to the North Atlantic influence in Bassa Nera during the Holocene.

- From 7300 cal yr BP onwards, charcoal and pollen indicators evidence human disturbance through grazing, pointing to the use of fire as a tool for forest clearance or maintaining open spaces.

- The first cereal crops in Bassa Nera occurred around 5190 cal yr BP and coincided with dry climate conditions until the cultivation of cold resistant species like *Secale cereale*. Notable periods of anthropic pressure include the Late Bronze Age, with the establishment of agricultural landscapes, followed by the Roman Period and Middle Ages.

Chapter 4:

- Significant correlations exist between the montane-subalpine pollen ratio and the residual tree-ring chronologies from nearby localities. This correlation is more reliable for trees located at high-elevation subalpine forests and treeline sites which are more sensitive to changes in temperature.

- The association between tree-growth and climate fluctuations shows that the growth of mountain pine is mainly limited by low temperature.

- The sensitivity of mountain pine has varied along the last 700 years, when temperatures were lower than today as shown by temperature reconstructions.

- For the last century and using instrumental climatic data, we have been able to ascertain that tree-growth variability is more constrained by low than by high temperatures. However, this effect seems to be fading in recent decades.

Chapter 5:

- Our results suggest that 18S and COI markers are useful to study modern and past peat bog communities.

- In this study, 18S has provided more useful information about past communities than COI, which was mainly restricted to Metazoa.

- The markers used in this work have allowed to obtain some insights on ancient communities and results which broadly agree with those obtained from morphological analysis of pollen and macroremains.

- The DNA from remains of *Sphagnum* and other mosses is probably not well preserved and might be undetectable in ancient samples with the used markers.

Annex 1

Supplementary materials

This section contains supplementary information for chapters 2, 4 and 5.

Supplementary material of Chapter 2

Montane / subalpine ratio

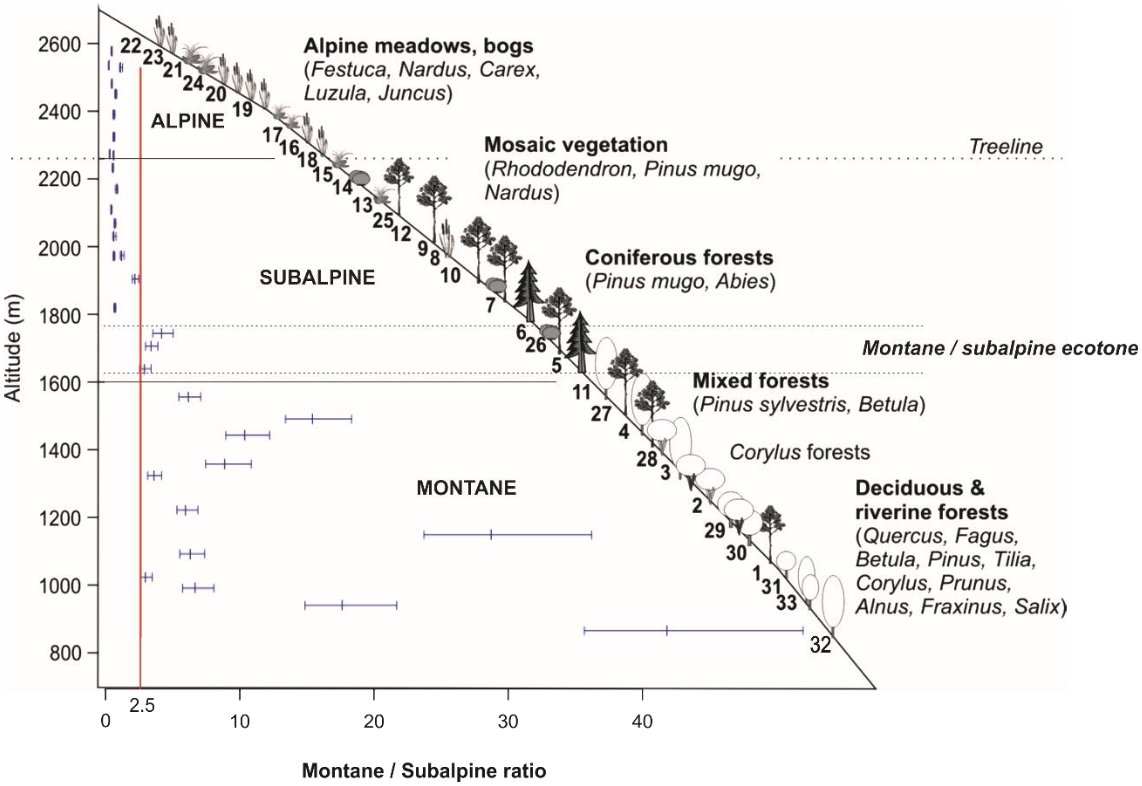


Figure S1. Altitudinal arrangement of vegetation belts determined in the study of Cañellas-Boltà et al. (2009) with the montane / subalpine ratio calculated for every sampling site. The mean value and the 95% bootstrap confidence intervals (5,000 replicates) are shown for every sampling site. The red line separates the ratio values from montane to subalpine samples. (Modified from Cañellas-Boltà et al. 2009).

In order to improve the palaeoecological interpretation of Bassa Nera and the Aiguamòg Valley, we introduce a pollen ratio based upon a modern proxy-calibration study of Cañellas-Boltà et al (2009) done in the same region. In that study, the relationships among modern pollen rain, vegetation and altitude were analysed along a transect ranging from 800 to 2600 m and including the headwaters of Garona river, a sector of Aiguamòg Valley and several sites of Garona Valley, Valarties Valley, and Circ de Colomèrs. All those sites are located within the peripheral zone of the ‘Aiguestortes i Estany de Sant Maurici’ National Park, created in 1955 and extended in 1990. From the analysis of the pollen content of 33 moss samples collected along the altitudinal transect, four main pollen groups were distinguished in relation to their usefulness as vegetation and altitudinal indicators: very good indicators, good indicators, non indicators and allochthonous pollen types. The first two groups were useful as indicator taxa for palaeovegetational and palaeoaltitudinal reconstruction, while the two latter should be interpreted with caution in palaeoenvironmental studies.

The montane / subalpine ratio was calculated using only the “very good” and “good” pollen indicators of montane and subalpine-alpine belts, which have a good correspondence between abundance of pollen and local occurrence of parent taxa. Montane pollen types included *Alnus, Betula, Buxus, Corylus*, *Fraxinus*, deciduous *Quercus*, *Tilia* and *Salix*, while subalpine-alpine indicators included Asteraceae, *Calluna*, *Campanula*, Ericaceae, *Plantago* and Poaceae. The percentages of the montane pollen were summed and divided by the sum of the percentages of subalpine pollen.

The utility of this ratio for discriminating both vegetation belts was assessed by calculating its values and their 95% confidence intervals for the 33 moss samples from the altitudinal transect studied by Cañellas-Boltà et al. (2009). Figure S1 shows the analysis of 5,000 bootstrap replicates per sample with a resampling size equal to 80% of the original sample sizes and using the rarefy function implemented in R package vegan (Oksanen et al. 2016). The 95% confidence intervals for subalpine is 0.16 – 2.12, while for montane stage is 2.72 – 43.42. According to this altitudinal transect, the values between 2.12 – 2.72 correspond to the real montane / subalpine ecotone.

In order to determine a functional threshold value for the ratio, we plotted the frequency histograms (Figure S2) obtained from 60,000 further bootstrap replicates of samples from the montane belt (800-1700 m) and from 60,000 bootstrap replicates of samples from the subalpine belt (1700-2300 m). From this analysis, a threshold value of 2.5 can be inferred, which indicates the close presence of the montane-subalpine ecotone, whereas higher values in the paleoenvironmental series would imply the occurrence of upward montane migration.

It is important to highlight that this montane ratio has been proved in one well studied altitudinal transect, and therefore it gives a regional interpretation. This ratio has been useful for the palaeoenvironmental interpretation of Bassa Nera catchment but should be interpreted with caution in other areas with similar vegetation. It would be interesting to calibrate this ratio with other altitudinal transects in order to have a better comprehension of the vegetal stages in the forests of Central Pyrenees.

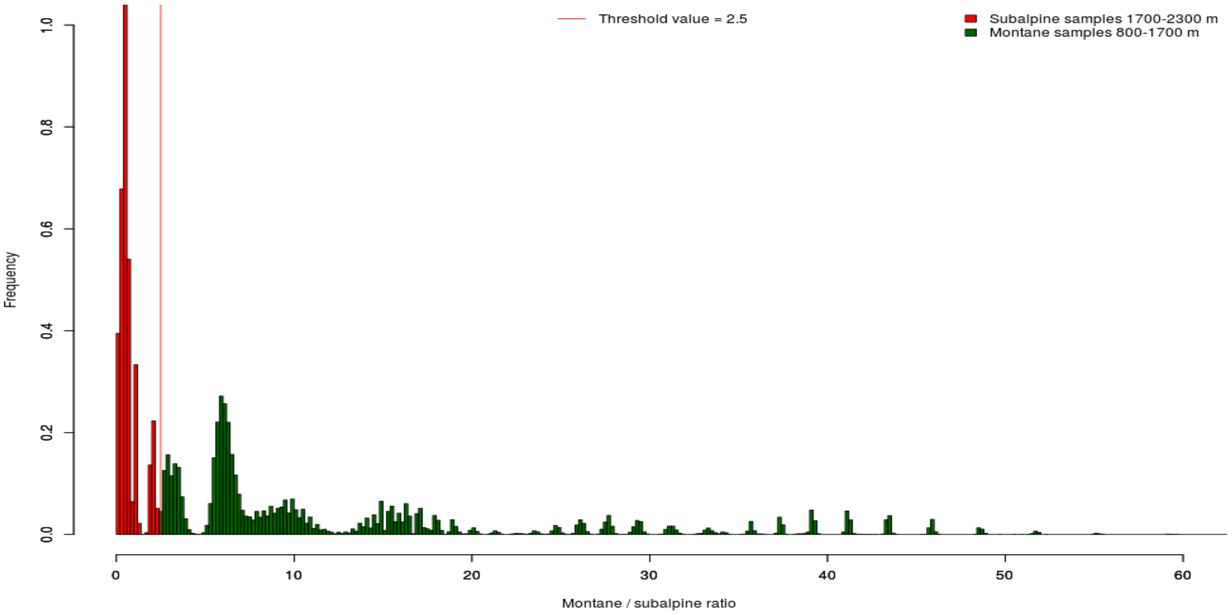


Figure S2. Frequency histograms of 60,000 bootstrap replicates of montane / subalpine ratio values from modern samples belonging to both vegetation belts, montane (green) and subalpine (red). The threshold discriminating value of 2.5 is indicated.

References

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H (2016). *vegan: Community Ecology Package*. R package version 2.3-3, URL https://CRAN.R-project.org/package=vegan.

Supplementary material of Chapter 4

Tables

Table S1 Statistical correlations between pollen of Bassa Nera and Gerber chronologies

	Gerber	Amitges	Portell	Barranc dels Llacs	Vall de Mulleres	Lladres	Airoto	Conangles	Coticelles	Mirador	Monestero	Mata de Valencia	Negre	Ratera	Sant Maurici	Tessó de Son	Bielsa	Cutas	Llong
Montane-subalpine ratio	0.526	0.561																	
Abies												0.088							
Alnus	0.383																		
Artemisia									-0.482										
Asteraceae fenest.					0.486	0.478													
Asteraceae non fenest														-0.928					
Chenopodicaceae/ Amarantaceae											0.474								
Corylus	0.376									0.472									
Ericaceae						-0.574		-0.542				-0.288							
Evergreen *Quercus*			0.654	0.438															0.622
Fagus	0.393							0.587		0.443					-0.886				
Fraxinus																-0.550			
Galium																	-0.740		
Helianthemum																		-0.895	
Juglans							-0.620												
Olea								-0.492											
Parnassia					-0.478							-0.478							
Plantago				-0.403															-0.604
Potentilla																	-0.645		
Rumex								0.584											
Sanguisorba	-0.466		-0.620							-0.436	-0.696								-0.602
Secale	0.498								-0.407										
Tilia																-0.497			
Urtica							-0.577						-0.477						

Table S2. Statistical correlations between montane-subalpine pollen ratio of Bassa Nera and residual dendrochronologies tree-ring chronologies (res). Significance level = 0.05. BSN = Bassa Nera pond.

				Pearson correlation			
Forest	**Distance to BSN pond (km)**	**Elevation Altitude** (m a.s.l)	**Aspect**	***r***	***p-value***	**No. Sampled trees**	**Period**
Gerber	5.04	2268	W	0.460	0.009*	31	1286-1968
Amitges	6.42	2390	S-E	0.627	0.012*	15	1624-1968
Mirador	7.83	2180	SE	0.360	0.091	23	1417-1968
Ratera	7.95	2170	N	0.136	0.796	6	1825-1968
Sant Maurici	7.95	1933	S-SE	-0.472	0.344	6	1825-1968
Llong	8.2	2323	W-NW	-0.078	0.791	14	1658-1968
Corticelles	8.61	2269	W-NW	-0.073	0.727	25	1378-1968
Monestero	9.76	2280	SE	0.134	0.584	19	1493-1968
Tessó de Son	10.83	2239	N-NE	0.117	0.654	17	1556-1968
Airoto	11.03	2300	W	0.440	0.131	13	1679-1968
Barranc dels Llacs	11.72	2250	N-NW	0.188	0.345	27	1349-1968
Mata de València	11.73	2019	N-NW	-0.098	0.566	13	1679-1968
Negre	13.04	2451	SE	0.217	0.319	23	1417-1968
Lladres	14.19	2120	NW	0.122	0.578	23	1417-1968
Conangles	14.27	2106	S-SW	0.051	0.838	18	1528-1968
Vall de Mulleres	16.83	1800	N-NE	-0.098	0.687	19	1493-1968
Portell	19.56	2199	W	0.271	0.369	13	1679-1968
Bielsa	60.47	1890	E	-0.259	0.440	11	1727-1968
Las Cutas	91.74	2080	S-SW	0.086	0.824	9	1769-1968

Figures

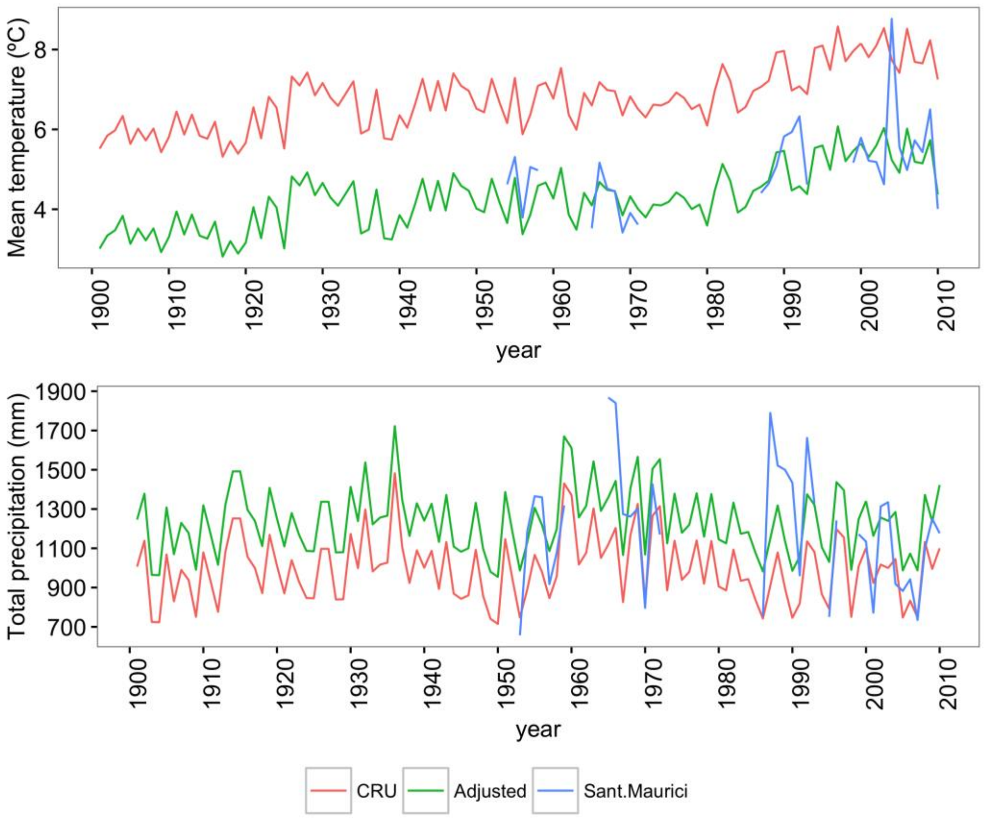


Figure S1. Annual mean temperature and total precipitation covering the period 1901-2010. Month-based CRU mean temperature and the total precipitation were rescaled using local data from Estany de Sant Maurici data weather station.

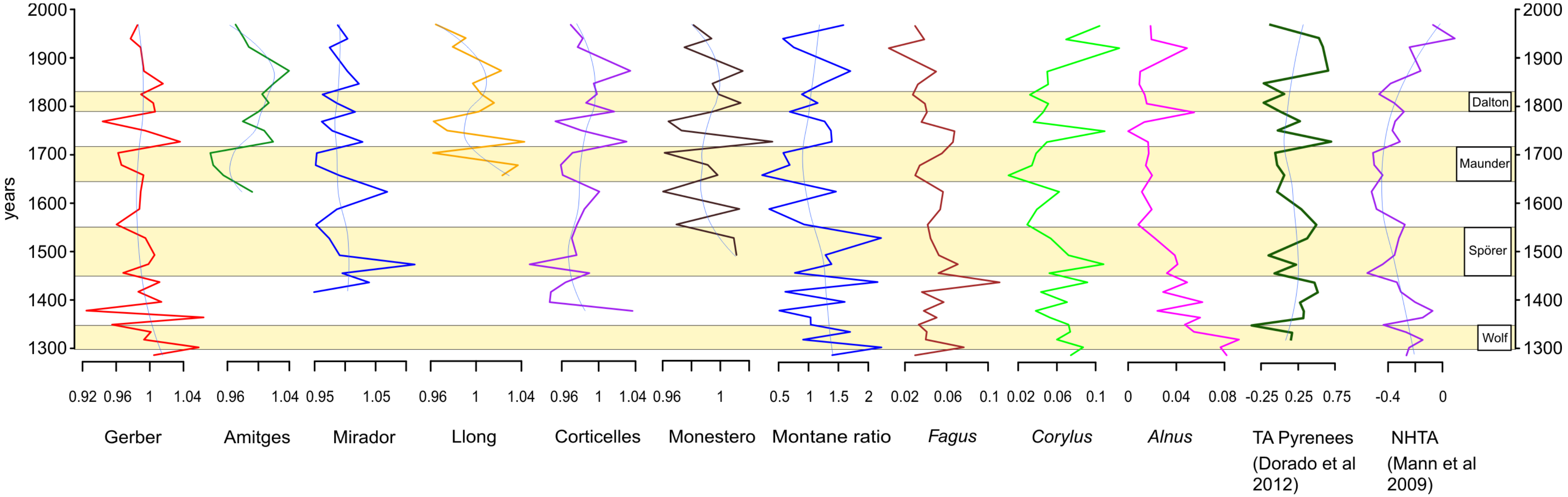


Figure S2. Diagram with residual ring-width chronologies, Bassa Nera pollen frequencies of *Fagus*, *Corylus* and *Alnus*, the montane-subalpine pollen ratio (montane ratio), temperature reconstruction from the Pyrenees and North Hemisphere Temperature Anomaly. Solar Grand minima are also depicted in orange stripes.

Supplementary material of Chapter 5

Tables

Table S1. Summarized pipeline for the complete metabarcoding procedure including two markers: COI and 18S. Software names beginning in “owi_” are custom R scripts available at http://github.com/metabarpark.

Sampling (preservation in ethanol)		
Pre-processing: (homogenization of samples)		
DNA extraction: (Norgen Soil DNA Isolation Plus Kit)		
	PIPELINE FOR COI	PIPELINE FOR 18S
PCR-1Tagged	Tagged Leray primers	Tagged Allshort primers
Library	NEXTflex (BIOO)	NEXTflex (BIOO)
HT Sequencing	Illumina MiSeq 2x250 bp	Illumina MiSeq 2x150 bp
Raw sequences QC	fasttqc fastx_trimmer	fasttqc
PE alignment	illuminapairedend	illuminapairedend
Demultiplexing	obiannotate/obisplit ngsfilter	obiannotate/obisplit ngsfilter
Length filter	obigrep 300-320 bp	obigrep 75-180 bp
Dereplication	obiuniq	obiuniq
Rename identifiers	obiannotate	obiannotate
Chimera removal	vsearch uchime_denovo	vsearch uchime_denovo
Clustering	SWARM v2 d=13 obitab owi_recount_swarm delete singletons	SWARM v2 d=1 obitab owi_recount_swarm delete singletons
Taxonomic assignment	ecotag using db_COI_BOLD	ecotag using db_18S
Add higher taxa	owi_add_taxonomy	owi_add_taxonomy
Final refinement	Blanks correction Abundance renormalization Minimal abundance filtering Removal of contamination MOTUs	Blanks correction Abundance renormalization Minimal abundance filtering Removal of contamination MOTUs
Community analyses and integration of the results		

Table S2. 20 most abundant 18S MOTUs for modern samples

Hummock			Carpet			Fen			Floating		
Scientific name	**Best id**	**Total reads**	**Scientific name**	**Best id**	**Total reads**	**Scientific name**	**Best id**	**Total reads**	**Scientific name**	**Best id**	**Total reads**
Sphagnum	1	211999	Sphagnum	1	144277	Bryopsida	0.99	212126	Bryopsida	0.99	249266
Desmonomata	1	82196	Bothrioplana semperi	1	62581	Bothrioplana semperi	0.99	77189	Droseraceae	1	126639
Tectocepheus sarekensis	1	40149	Rhynchoscolex simplex	1	25935	Utricularia	1	76531	Anystina	0.93	37122
Hygrocybe	0.99	20472	Cyperoideae	1	22051	Brachypylina	1	24896	Desmonomata	1	28406
Acrogalumna longipluma	1	20360	asterids	1	18330	Desmonomata	1	16016	Rhabdolaimus aquaticus	1	27565
Cernosvitoviella atrata	1	19699	Bryopsida	0.99	16462	Poaceae	0.99	14507	Hydrozetes lacustris	1	17888
Sanguisorba	1	17635	Aeolosoma sp. GG-2011	0.98	11302	Rhabdolaimus aquaticus	1	11974	Enochrus quadripunctatus	1	9841
Poaceae	0.99	17069	Poaceae	0.99	10116	Hydrozetes lacustris	1	11882	Podocopida	1	8806
Hydrophilinae	1	12190	Harpacticoida	1	10086	Geocentrophora sphyrocephala	1	9342	Sphagnum	1	8590
Brachypylina	1	11678	Cernosvitoviella atrata	1	8355	Calyptostoma velutinus	0.94	8145	Calyptostoma velutinus	0.94	4164
Fungi	1	11436	Fungi	1	7457	Harpacticoida	0.96	7488	Tubificina	1	3032
Agaricomycetes	1	7709	Tubificina	1	7060	Podoplea	0,88	5362	Cyperoideae	1	2639
Violaceae	1	4612	Filipendula vulgaris	0.99	6371	Fungi	1	4934	Parasitengona	0,94	2503
Helicoon fuscosporum	1	4525	Brachypylina	1	6083	Harpacticoida	1	4382	Macrobiotidae	0.99	2456
Catenulida	0.95	4026	Chamaedrilus cognettii	1	5502	Lumbriculidae	1	4115	Zygoptera	1	2238
Steganacaridae	1	3886	Chaetonotidae	1	5376	Limnognathia maerski	1	2516	Utricularia	1	2126
Harpacticoida	0.98	3423	Entelegynae	0.98	4964	Acanthocyclops	1	1862	Harpacticoida	0.98	1949
Parnassia	1	2957	Tubificina	0.99	4753	Aeolosoma sp. GG-2011	0.98	1804	Chaetonotidae	1	1931
Prismatolaimus	1	2944	Lepidochaetus zelinkai	1	4494	Leotiomycetes	0.98	1737	Lumbriculus	1	1886
Fungi	1	2765	Naididae	0.97	4472	Peniophorella praetermissa	0.99	1724	Brachypylina	1	1584

Table S3. 20 most abundant COI MOTUs for modern samples

Hummock			Carpet			Fen			Floating		
Scientific name	**Best id**	**Total reads**	**Scientific name**	**Best id**	**Total reads**	**Scientific name**	**Best id**	**Total reads**	**Scientific name**	**Best id**	**Total reads**
Nothrus pratensis	0.99	47933	Malaconothridae	0.84	108107	Malaconothridae	0.84	10258	Malaconothridae	0.84	64168
Malaconothridae	0.84	32706	Tyrphonothrus maior	1	75834	Sarcoptiformes	0.85	4409	Trombidiformes	0.81	30644
Poduroidea	0.8	16875	Maxillopoda	0.82	38042	Stilobezzia ochracea	1	2286	Dasyhelea modesta	0.99	17267
Limnophyes sp.7SW	1	7517	Atylotus fulvus	0.98	15599	Maxillopoda	0.82	2210	Rotifera	0.78	10853
Microtrombidiidae	0.87	3937	Sarcoptiformes	0.85	14431	Corynoneura	0.99	1432	Enochrus ochropterus	0.99	10006
Sarcoptiformes	0.83	2588	Scheloribatidae	0.88	13342	Mycobatidae	0.91	1332	Sarcoptiformes	0.89	8325
Sordariomycetes	0.86	2489	Paracricotopus	1	10232	Eukaryota	0.79	1293	Eukaryota	0.76	5803
Tectocepheus	0.88	2324	Pristina	0.85	9395	Isotomidae	0.98	1220	Podocopida	0.88	5649
Neelipleona	0.89	2227	Didymium	0.85	7599	Platyhelminthes	0.84	968	Lumbriculus variegatus	0.99	5612
Anacaena lutescens	1	1973	Malaconothridae	0.99	7191	Bryocamptus pygmaeus	0.98	896	Eukaryota	0.73	5250
Eukaryota	0.76	1779	Sarcoptiformes	0.82	6714	Neocopepoda	0.82	725	Palpomyia lineata	1	5134
Planorbidae	0.8	1574	Eukaryota	0.79	6290	Culicoides kibunensis	0.97	636	Monopelopia tenuicalcar	1	4652
Eukaryota	0.78	1568	Bryocamptus pygmaeus	0.98	6275	Malaconothrus	0.84	565	Eukaryota	0.76	3972
Adineta	0.9	1437	Murrayon pullari	0.99	6041	Leohumicola	0.9	546	Eukaryota	0.78	3687
Eukaryota	0.71	1390	Cognettia glandulosa B SM2014	0.98	5978	Harpacticoida	0.84	518	Lecane cornuta	0.87	3630
Eukaryota	0.8	1342	Eukaryota	0.78	5767	Ploima	0.83	442	Polypedilum tritum	0.98	2703
Eukaryota	0.76	1311	Trombidiformes	0.81	5726	Sordariomycetes	0.88	440	Trebouxiophyceae	0.79	2605
Eukaryota	0.75	1148	Philodinidae	0.9	4961	Pristina	0.85	417	Ochrophyta	0.75	2287
Eukaryota	0.79	1114	Bdelloidea	0.9	4741	Cyclopoida	0.86	316	Bryocamptus pygmaeus	0.98	2225
Leohumicola	0.9	1085	Leotiomycetes	0.88	4325	Eukaryota	0.75	288	Maxillopoda	0.84	2216

Table S4. 20 most abundant 18S MOTUs for sedimentary samples

31 cm			109 cm			160 cm			220 cm			260 cm		
Scientific name	**Best id**	**Total read**	**Scientific name**	**Best id**	**Total read**	**Scientific name**	**Best id**	**Total read**	**Scientific name**	**Best id**	**Total read**	**Scientific name**	**Best id**	**Total read**
Cyperoideae	1	28063	Cyperoideae	1	2955	Cyperoideae	1	6802	Bicosoecida gen. 1 sp. EK-2010a	0.9	2335	Dinophyceae	0.87	3999
Mesangiospermae BOG2_000000149	0.97	9089	Pooideae	1	1795	Bicosoecida gen. 1 sp. EK-2010a	0.92	5505	Pooideae	1	619	Heterophryidae	0.89	3965
Bryopsida	0.99	7058	Mesangiospermae BOG2_000000149	0.97	1564	Bicosoecida	0.82	1645	Pinidae	1	377	Pooideae	1	80
Pooideae	1	776	Poaceae	0.99	178	Mesangiospermae BOG2_000000149	0.97	748	Bryopsida	0.99	277	Desmidiales	0.94	50
Paramonas globosa	0.87	585	Bicosoecida gen. 1 sp. EK-2010a	0.92	151	Paramonas globosa	0.87	554	Navicula	1	216	Bicosoecida gen. 2 sp. EK-2010a	0.85	46
Paramonas globosa	0.87	547	asterids	1	143	Bicosoecida gen. 1 sp. EK-2010a	0.9	412	Salamandroidea	1	127	Adeleidae	0.91	19
Chaetonotus acanthodes	1	281	Bicosoecida gen. 1 sp. EK-2010a	0.88	97	Bicosoecida	0.82	369	rosids	0.99	114	Polypodiidae	1	17
Saxifragales	0.98	267	Equisetum arvense	1	96	Salamandroidea	1	132	rosids	1	110	rosids	1	16
Bicosoecida gen. 1 sp. EK-2010a	0.92	218	Paramonas globosa	0.87	84	Pinidae	1	79	asterids	1	109	Petrosaviidae	1	13
Poaceae	0.99	202	rosids	1	80	Cupressaceae	0.99	39	Desmonomata	0.99	101	Dysderidae	1	13
Gregarinasina	0.89	171	Petrosaviidae	1	76	Paramonas globosa	0.91	27	Fragilariaceae	0	91	Salamandroidea	1	12
Pyrenomonadales	0.77	140	Sapindales	1	43	Bicosoecida gen. 1 sp. EK-2010a	0.88	22	Betulaceae	96	76	Paramonas globosa	0.89	9
Pinidae	1	121	Streptophytina	0.89	41	Desmidiales	0.91	21	Petrosaviidae	1	74	rosids	0.99	8
Nuclearia	0.86	88	Desmidiales	0.94	29	Mesangiospermae BOG2_000077141	0.97	19	Mesangiospermae	1	61	Pinidae	1	8
Nuclearia	0.76	82	Navicula	1	27	Dysteridae	1	13	Sapindales	0.99	47	Bacillariophyta	0.88	7
Chaetonotidae	1	79	Betulaceae	1	24	Mesangiospermae BOG2_000010361	0.96	12	Rhizidiomyces apophysatus	1	40	Prunus	1	6
Prunus	1	63	Brassicaceae	1	20	Navicula	1	9	Cymbellales	0.9	37	Petrosaviidae	0.98	5
Microdalyellia	1	62	rosids	0.99	18	Paramonas globosa	0.92	9	Chaetonotidae	0.93	37	Navicula	1	5
Chaetonotidae	1	59	Petrosaviidae	0.98	17	Lauraceae	1	7	Papilionoideae	1	34	Sinella curviseta	1	5
Paramonas globosa	0.88	45	Pinidae	1	15	Sapinadaceae	1	7	Pinus	0.99	33	Atripliceae	1	4

Table S5 20 most abundant COI MOTUs for sedimentary samples

31 cm			109 cm			160 cm			220 cm			260 cm		
Scientific name	**Best id**	**Total read**	**Scientific name**	**Best id**	**Total read**	**Scientific name**	**Best id**	**Total read**	**Scientific name**	**Best id**	**Total read**	**Scientific name**	**Best id**	**Total read**
Bacillariophyceae	0.81	4774	Branchiopoda	0.73	251	Arthropoda	0.82	2867	Psychoda alternata	0.93	15	Rhodophyta	0.79	9248
Porifera	0.82	2032	Bacillariophyta	0.81	51	Porifera	0.75	333	Tyrphonothrus maior	1	9	Porifera	0.75	1841
Planorbidae	0.8	1897	Navicula	0.84	24	Arthropoda	0.76	41	Malaconothridae	0.84	6	Opiliones	0.77	540
Maxillopoda	0.81	1028	Thalassionema	0.86	14	Branchiopoda	0.73	31	Rhodophyta	0.81	6	Bacillariophyceae	0.81	93
Branchiopoda	0.73	396	Bacillariophyta	0.83	14	Arthropoda	0.8	15	Nothrus pratensis	0.99	5	Ceratophysella denticulata	0.83	25
Florideophyceae	0.84	362	Sellaphora	0.85	13	Bacillariophyta	0.84	13	Sarcoptiformes	0.85	3	Opiliones	0.77	24
Branchiopoda	0.73	166	Bacillariophyceae	0.82	13	Eimeria	0.72	12	Murrayon pullari	0.99	3	Mollusca	0.72	15
Branchiopoda	0.72	127	Bacillariophyceae	0.82	12	Naviculaceae	0.83	9	Rotifera	0.82	3	Branchiopoda	0.75	13
Porifera	0.82	110	Haslea	0.86	11	Bacillariophyceae	0.82	7	Coccomyxa	0.76	2	Tyrphonothrus maior	1	11
Rhodophyta	0.81	80	Bacillariophyceae	0.83	11	Tyrphonothrus maior	1	6	Bacillariophyceae	0.85	2	Araneae	0.92	8
Pyropia	0.82	78	Nitzschia	0.83	10	Harpacticoida	0.83	6	Crotoniidae	0.9	2	Diptera	0.9	8
Banchiopoda	0.75	74	Sellaphora	0.84	10	Murrayon pullari	0.99	6	Sarcoptiformes	0.85	2	Harpacticoida	0.79	8
Tyrphonothrus maior	1	73	Bacillariophyceae	0.83	10	Malaconothrus	0.87	4	Limoniidae	0.94	2	Haslea	0.84	7
Pyropia	0.83	58	Bacillariophyta	0.83	10	Malaconothridae	0.84	4	Harpacticoida	0.84	2	Naviculaceae	0.83	7
Maxillopoda	0.82	57	Arthropoda	0.76	10	Othius angustus	0.99	4	Rhodophyta	0.8	1	Sellaphora	0.84	7
Branchiopoda	0.73	54	Sellaphora	0.87	9	Nothrus pratensis	0.99	3	Acutodesmus	0.75	1	Harpacticoida	0.82	7
Rhodophyta	0.83	44	Bacillariophyta	0.85	9	Asplanchna	0.81	3	Sellaphora	0.86	1	Bos	0.99	7
Rhodophyta	0.82	44	Tyrphonothrus maior	1	9	Rhodophyta	0.99	2	Banksinoma	0.95	1	Platyhelminthes	0.75	7
Branchiopoda	0.74	41	Ovatella vulcani	0.92	9	Dysdera	0.86	2	Malaconothridae	0.99	1	Porifera	0.82	7
Maxillopoda	0.81	35	Stylochoidea	0.78	9	Malaconothrus mollisetosus		2	Sarcoptiformes	0.82	1	Porifera	0.82	7

Figures:

Figure S1. Non-metric multidimensional scaling ordination using Jaccard dissimilarities with Viridiplantae.

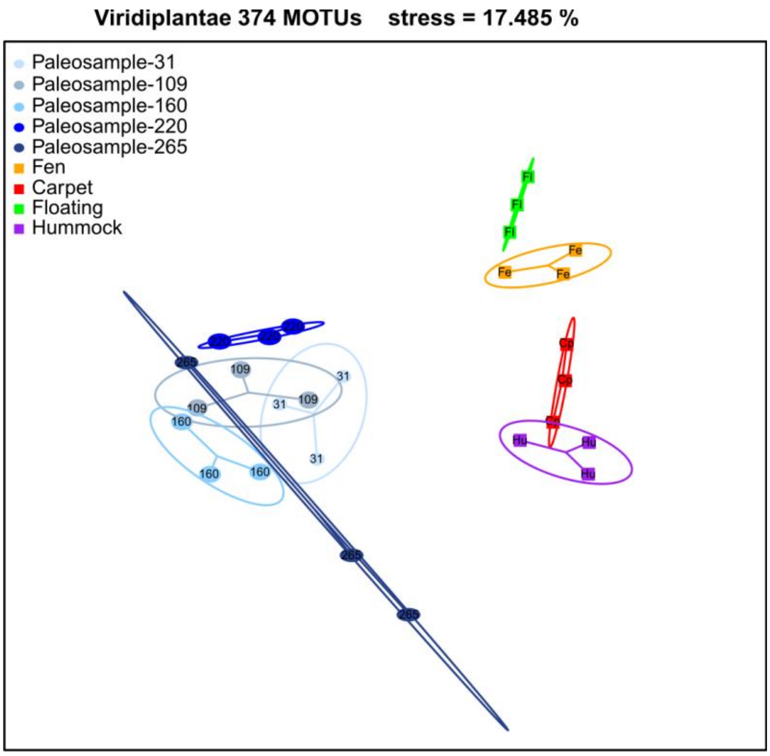
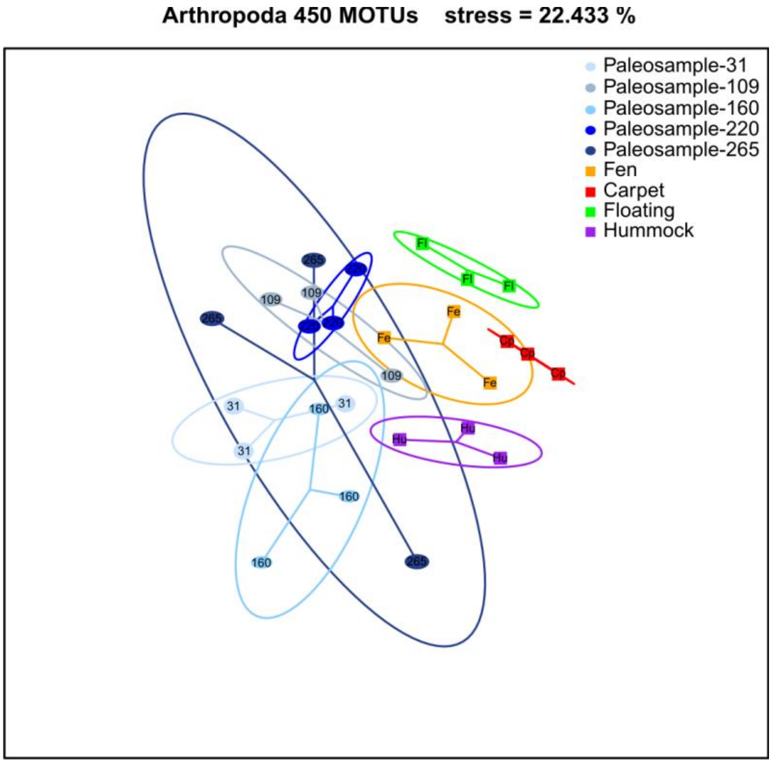


Figure S2. Non-metric multidimensional scaling ordination using Jaccard dissimilarities with Arthropoda.



Annex 2

This annex provides the original publications of:

Chapter 2: Garcés-Pastor, S., Cañellas-Boltà, N., Clavaguera, A., Calero, M.A., Vegas-Vilarrúbia, T., 2016. Vegetation shifts, human impact and peat bog development in Bassa Nera pond (Central Pyrenees) during the past millennium. *The Holocene*, *27*(4), 553-565.

Chapter 3: Garcés-Pastor, S., Cañellas-Boltà, N., Pèlachs, A., Soriano, J-M., Pérez-Obiol, R., Pérez-Haase, A., Calero, M-A., Andreu, O., Escolà, N., Vegas-Vilarrúbia, T. 2017. Environmental history and vegetation dynamics in response to climate variations and human pressure during the Holocene in Bassa Nera, Central Pyrenees. Palaeogeography, Palaeoclimatology, Palaeoecology, 479, 48-60.