

Maceral composition and molecular markers of two condensed Middle Holocene peat profiles in N Spain.

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ABSTRACT

This study deals with the palaeohydrological information obtained from lipids composition and vegetal tissue preservation of two peat cores from Asturias, North Spain. The two profiles differ in the type of peatland (raised bog in La Borbolla and blanket bog in Buelna) and the type of organic matter being more bryophytic in the raised bog and more herbaceous in the blanket bog. The peatlands are located close to the coast on impermeable, old flat erosion surfaces which favoured peat accumulation within a distance of 3.5 km from each other. The accumulation rate varied between 0.05 and 0.07 mm/yr and the records extend from around 9000 to 2500 cal. yr BP.

The main differences between the two peat sites can be summarized as follows: the raised bog has lower mineral matter content and H/C atomic ratio and higher C/N ratio and extraction yields compared to the blanket bog. It has also a higher Tissue Preservation Index for Huminite macerals and increasing Inertodetrinite Index with depth. Regarding biomarkers, the raised bog has a relatively higher concentration of *n*-alkane-2-ones compared to the corresponding *n*-alkane of similar carbon number, higher concentration of medium- vs. high-molecular-weight-methyl-ketones and higher triterpenoids/steroids ratio than the blanket bog.

Alternating humid/dry periods have been recorded in both profiles based on lipid concentrations indicating that the *Sphagnum*-rich intervals in the raised bog are more sensitive than the herbaceous blanket peat record to climatic variations. The main humid intervals identified are dated as 5000-7500 cal. yr BP in the blanket bog and as 6000-3500 cal. yr BP and last 2500 cal. yr BP in the raised bog and correlate with the humid periods traced in other peatlands locations in this region. The transition Middle-to-Late

Holocene is characterized by humid conditions in the region which favoured the *Sphagnum* growth in the peats.

The reflectance of huminite in these records appears to be more related with differences in the oxidative conditions than with variation of peat maturity. It is higher in the layers with high mineral matter content at the beginning of organic matter accumulation and also in the upper part of the profile subjected to seasonal desiccation. A more intense biochemical gelification coincides with higher concentration of hopanoids derived from bacteria in the upper part of the profile (acrotelm).

High amount of nonadecan-2-one (K₁₉) and high concentrations of triterpenyl acetates, which are also determined in other peat profiles from Northern Spain are also detected in these profiles. This indicates that the specific conditions required for the formation of these compounds, probably associated to wet temperate climate, dominated throughout the region.

Keywords: raised bog, blanket bog, tissue preservation index, *Sphagnum*, biomarkers, palaeohydrology

1. Introduction

Since the early work of Blytt (1876) and Sernander (1909), who established a climatostratigraphic division of the Holocene based on alternating dark peat layers containing wood associated to drier and warmer climate, and lighter-coloured, *Sphagnum*-rich horizons indicative of wetter cooler conditions, there has been an increasing number of palaeoclimate studies using peat-bog archives (e.g. Blackford, 2000; Charman, 2002; Barber et al., 2003; Bindler, 2006). The main assumptions on which the peat-based climatic reconstructions rely are: that the vegetation remains are an accurate record of the original vegetation on the mire surface at the time of deposition and that the assemblage composition responds to changes in the water table driven by changing climate. Although any peat deposit can offer palaeoenvironmental information, those with a higher palaeoclimate potential are rain-fed ombrothrophic bogs, the evolution of which is closely linked to meteoric precipitation without any influence from surface water or aquifers (Barber et al., 2003). Both raised bog (van Geel 1978; Barber et al., 1994, Galka et al., 2013) and blanket mire records (Blackford and Chambers, 1991; Chambers et al., 1997; Ellis and Tallis, 2000) have been used for palaeoenvironmental studies. Both kinds of deposits, even being essentially ombrothrophic, have a number of peculiarities that should be taken into account when used as archives. Blanket bogs are developed in more open areas and even on gentle slopes that might be responsible for their slightly higher mineral matter contents compared to raised bogs. The latter are typically confined and owe their origin to low relief and impeded drainage, having domed surfaces hydrologically isolated from ground water. High precipitation sustains the blanket bogs that generally have a lower

Sphagnum contribution than raised bogs. Their overall characteristics may result in different sensitivity to climate variation.

Numerous studies allowed having independent and continuous peat records of climate variability, which have permitted the identification of climate events of different relevance at regional or global scale (Chambers et al., 2010). Efforts focus now in the correlation of different deposits (Mauquoy and Barber, 1999; Hughes and Barber, 2004; Barber and Langdon, 2007; Loisel and Garneau, 2010; Lopez-Dias et al., 2013b) and in the refinement of the methodology to make results more comparable (Charman et al., 2009). Many of the palaeoenvironmental studies on peat bogs have been based on palynological profiles, which provide information not only on the peat-forming vegetation in the palaeomire but also on the vegetation in the surrounding area, as pollen and spores are blown by wind. The macrofossil analysis focus on the peat-forming vegetation but it is not always possible to be carried out, when intense humification processes affect the peat. Although peat has been commonly studied using transmitted light (Cohen and Spackman, 1972; Cohen, 1982), reflected light microscopy offers a number of procedures that are very useful for investigating the degree of humification. Koch (1969) distinguished five degrees of humification which can be identified on the basis of their appearance under reflected light microscopy. Esterle (1994) distinguished various types of peat at megascopic and microscopic scale accompanied by chemical signature. Spectral analysis of the fluorescing components (cellulose tissues, some huminites and liptinite components) may provide valuable information not only about the degree of humification but also about the alteration and oxidation processes prior to burial (Hagemann and Dehmer, 1991; Dehmer 1993). In addition, maceral analysis of peat reflects the degree of preservation of tissues

(Sykorova et al., 2005) and quantifies the impact of wildfires (Glasspool and Scott, 2013).

The Cantabrian region of Spain is the southernmost area in Europe where the climate conditions are adequate for the development of ombrotrophic mires. These have been described in mountainous and littoral locations throughout the Cantabrian region (Fernandez Prieto et al., 1987, Pontevedra-Pombal et al. 2006). Organic geochemical studies on Asturian peats have increased in recent years (Ortiz et al., 2010, 2016; López-Días et al., 2010a and 2010b, 2013a, 2013b). This work presents an integrated biomarker and petrographic study of two peat profiles which cover the middle Holocene and the transition to the Late Holocene in the oriental part of Asturias. Both are located very close to each other and can be defined as blanket and raised bogs. Their organic input and the record of arid/humid events are discussed on the basis of preferential lipid concentrations, and degree of preservation of tissues providing an opportunity for discussing the sensitivity of both profiles to palaeoenvironmental variation.

2. Geological setting

The coastal Asturian mires have typically developed on top of a series of sub-parallel, flat, east-west trending ridges (“rasas”) over a quartzitic substrate (Mary, 1983). The densest area of littoral mires is located in the eastern part of Asturias close to the boundary with the province of Santander. The flat ridges on which the peat bogs have developed are the result of marine erosion surfaces over the Ordovician quartzite (Mary, 1983). The plane relief and an impermeable surface have prevented drainage, thereby favouring the accumulation of ombrotrophic peat. The studied bogs developed onto two different erosion surfaces situated at different altitudes. La Borbolla is a raised

bog (UTM 30 T 365813 4804949) with an extension of 8 ha situated at 227 m above sea level (masl) on a flat surface formed during the Miocene, whereas Buelna blanket bog (UTM 30T 369898 4805333) is located over a Lower Pliocene erosion surface at a height of 133 masl and covers an area of around 9 ha. A general view of both sites, located within a distance of 3.5 km, is shown in Figure 1.

The climate in this region is of the Atlantic type with humid temperate winters and occasional droughts in the summer. Identified flora comprises *Sphagnum*, heather: *Erica tetralix* and *Calluna vulgaris*, grass as *Molinia caerulea* and sedges as *Eriophorum angustifolium* and *Carex nigra*. The pollen analysis revealed a significant amount of *Betula*, *Alnus* and *Erica* pollen (Menedez Amor, 1950).

3. Methods

3.1. Sample collection and preparation

A semi-cylindrical peat core sample was taken with a manual Russian probe 5 cm in diameter and 50 cm in length. The sampling site was chosen making transects and selecting the core with the thickest peat layer. The core was then cut into portions approximately 1 cm thick on the same day of collection and kept at -18°C. The samples were freeze-dried prior to analysis to prevent peat alteration. In general, one out of 4 samples was analysed, except in cases where there was significant variation in the parameters. In this case one out of two samples was selected. Only one sample was analysed from the uppermost and lowermost intervals corresponding to living vegetation and bottom sediment, respectively.

3.2. Radiocarbon dating

Radiocarbon chronology based on Accelerator-Mass-Spectrometry (AMS) ^{14}C dating was performed at the Centro Nacional de Aceleradores, Seville, Spain. Seven samples were analysed from La Borbolla and four from Buelna profiles. Prior to analysis identifiable roots which could belong to various levels of vegetation were removed. The sample was then treated with HCl to eliminate carbonates, NaOH to remove secondary organic acids and finally it was acid rinsed to neutralize the solution prior to drying. AMS results were obtained by reducing the carbon sample to graphite and using the corresponding standards and backgrounds. The conventional radiocarbon age represents the measured radiocarbon age corrected for isotopic fractionation, which was calculated from the $\delta^{13}\text{C}$ values. Calibrated dates (2σ) were calculated using the CALIB RADIOCARBON CALIBRATION PROGRAM (Stuiver et al, 2005) version CALIB 6.0 (Reimer et al., 2009) and the calibration curve INTCAL09 (Reimer et al., 2009).

3.3. Elemental analysis and ash yield

The mineral matter content of the samples was calculated as ash yield by heating the samples up to 600°C under air (100 mLmin^{-1}) at a heating rate of $10^{\circ}\text{Cmin}^{-1}$, after confirming the total absence of carbonates. The carbon, nitrogen and hydrogen contents were determined using a LECO CHN-2000 device. The carbon could be assumed to be organic as no carbonates were detected. Before analysis the samples were ground to $212\ \mu\text{m}$ in an agate mortar

3.4. Organic geochemical analyses

The samples were ultrasonically extracted for 1 h in dichloromethane/methanol solution (3/1). The sample/solvent ratio was kept at 1:20. After extraction, the solvent solution was filtered and the solvent was removed in a rotary evaporator and then dried under a nitrogen flow. The extracts were analysed using a gas chromatograph (GC) Agilent 7890A attached to a mass spectrometer (MS) Agilent 5975C equipped with a HP-5 capillary column with He as carrier gas. The GC oven temperature was programmed to rise from 35 to 300°C at a rate of 3°C min⁻¹. This was followed by an isothermal period of 15 min at 300°C. The internal standard used was *n*-Tricosane-d48. Patterns of *n*-alkanes were identified and quantified in the GC traces by comparing the retention times with an external standard prepared for this purpose. Other classes of compounds were identified on the basis of their mass spectra and their retention times by comparing them with previously published results (Philp, 1985; Peters et al., 2005).

3.5. Organic petrography analyses

Samples were embedded in epoxy resin and polished according to standard procedures for coal analysis (ISO 7404-2, 2009). The nomenclature applied for the classification of components followed that developed by the International Committee for Coal and Organic Petrology-ICCP (ICCP, 2001, for inertinite macerals, and Sykorova et al., 2005 for huminite macerals). In the case of huminite macerals the definitions have to be extended to include recent plant tissues and therefore, well-preserved transparent tissues under white light with strong fluorescence are recorded as textinite, and often attrinite is also transparent in white light. Maceral analysis were carried out following essentially the ISO 7404-3 (2009) standard but under blue-light excitation, since a significant amount of the peat material was translucent and therefore

indistinguishable under reflected white light. The identification was confirmed under white light too. The amount of fluorescent components would probably be overestimated since particles or portions of fluorescent particle slightly under the surface would likely be counted. Reflectance was measured on the highest reflecting huminite macerals according to ISO 7404-5 (2009) and separate records were performed for corpohuminite, telohuminite and detrohuminite.

4. Results

4.1. Description of the profiles

La Borbolla profile reaches a depth of 56 cm and consists of around 8 cm of living vegetation dominated by *Sphagnum*, followed by an interval of bryophytic reddish peat with abundant roots. From 28 to 42 cm the peat becomes darker, more compact and clayey. The following 6 cm are dark grey and silty and from this point to the end of the profile the sediment becomes lighter (Fig. 2). The Buelna blanket bog is placed on gentle slope. The sampled core reaches a depth of 87 cm. The first 15 cm consists of unconsolidated living vegetation with many roots. Up to a depth of 24 cm, roots are still abundant but the peat becomes more consolidated. The following 36-cm-thick layer consists of compact dark reddish peat. This interval is followed by 8 cm of dark clayey material. The last 20 cm are a light grey siltstone with low organic content.

4.2. Chronology

Table 1 shows the results of the AMS ^{14}C dating of samples selected from the La Borbolla and Buelna profiles. The oldest sample dated from La Borbolla showed an age of 9152 cal. yr BP (2σ : 7356-7047 BC) at a depth of 43-44 cm. Below this sample, at a depth of 49-50 and 52-53 cm the age of the sediment was very close but slightly younger (8086 and 8994 cal. yr BP, respectively). The scattering in the ages of sediments with a low C content ($\sim 2\%$) can be interpreted as evidence of material reworking before the conditions for significant accumulation of organic matter were established. The age of the samples at depths 12, 23, 32 and 43 cm fits in well with a linear trend indicating an accumulation rate for the peat of around 0.05 mm/yr. The sample at 17 cm depth could have contained roots from younger plants because its age appears younger than that of sample 12 (3103 cal. yr BP). Considering that the upper 8-cm-thick layer clearly shows signs of living vegetation, peatification might have ceased at this site around 2800 years ago.

The four samples dated in Buelna also adjust well to a linear trend and the deepest sample dated (65-66 cm) yielded an age of 9483 cal. yr BP (2σ : 7613-7453 cal. yr BC), similar to that reported at the bottom of La Borbolla. The carbon content of this sample is below 7% and can be considered bottom sediment, although below this point there is a 20-cm-thick silt layer very lean in organic matter. From the trend shown in Figure 3, the first peat sample below living vegetation at a depth of 16 cm depth would have an age of 2044 cal. yr BP, a similar age to that observed for the end of peat growth in La Borbolla. The accumulation rate in Buelna profile was slightly higher (0.07 mm/yr) than in La Borbolla (0.05 mm/yr). In both profiles a similar time interval is represented allowing comparison of the various proxies in two independent settings.

4.3. Bulk composition of the peat

The ash yield, carbon content and some other relevant parameters for finding out about organic matter input and alteration in the profiles are shown in Figure 4. The parameters are plotted versus age that was calculated using the depth-age models of Figure 3. The ash yield in the raised bog is lower than 10% with rather small variations in the uppermost 26 cm (last 5500 cal. yr BP). In a 10 cm interval (from a depth of 26 to 36 cm) the ash yield increases sharply from 10 to 84% and from this point down it slowly increases up to 96%. The variation of the ash yield in the profile indicates that the establishment of bog conditions occurs in an age interval comprised between 5500 and 7500 cal. yr BP. In the Buelna profile, the ash yield in the uppermost 20 cm (less than 2500 cal. yr. BP) is around 10-15%, close to that observed in top of La Borbolla raised bog. The following 20 cm (2500-6000 cal. yr. BP) are characterised by an ash yield of around 30% that decreases downwards, staying at around 20% in the interval 44-60 cm (6000-8500 cal. yr. BP). In the interval 60-68 cm (8500-9000 cal. yr. BP) the ash yield sharply increases (from 20 to 95%), remaining close to 95% until the bottom of the profile. As can be observed in Figure 4 the ombrotrophic conditions in Buelna were established 3000 years earlier than in La Borbolla. The variation of carbon content is the reverse of that of ash yield in both profiles while the N content follows a similar trend to that of C with some differences. In the 2000-3000 cal. yr BP interval a slight increase in N is observed in both profiles that could be interpreted as an acceleration of the destruction of organic matter based on the assumption that the alteration of organic matter results in a relative enrichment of N vs. C (Sternberg et al., 2007). The C/N atomic ratio decreases in this interval (particularly in the raised bog), then increases again for samples younger than 2000 cal. yr BP. Between 3000 and 7000 cal. yr BP the C/N atomic ratios in both profiles were close to each other, though slightly higher in La Borbolla raised bog. The values are typical for ombrotrophic bogs in the region

(Pontevedra-Pombal et al., 2006; Ortiz et al., 2010, 2016; Lopez-Dias et al., 2013). The H/C atomic ratio reflects differences in the nature of the organic matter accumulated. The organic matter in blanket bog profile is richer in H compared to that of the raised bog and this coincides with the generally lower extraction yields (Fig. 4). At the top of the profiles (last 2000 years) the extraction yields approach one another.

4.4. Humification and level of tissue preservation

Huminite reflectance would provide an indication of the degree of humification, although it should be taken into account that a significant part of lignocellulose tissues did not undergo even biochemical gelification and therefore reflectance values were taken on the lighter reflecting components. The number of readings varied between few points and around forty; measurements were taken on telohuminite, detrohuminite and corpohuminite (Fig. 5). The reflectance values were in the range 0.25-0.12% without significant differences between telohuminite and detrohuminite in both profiles. The number of readings increased towards the top in both profiles indicating a higher amount of gelified macerals, which was accompanied by slightly higher values in the topmost 20 cm of both profiles. This could be interpreted as an intensification of the humification processes as consequence of the stopping of peat growth and the seasonal dryness in the acrotelm increasing the amount of oxygen available. The lowermost part of the Buelna profile yielded few particles over 0.3% reflectance, measured on oxidized particles associated to high mineral matter levels. Corpohuminite generally showed higher reflectance values than both detrohuminite and telohuminite and the differences were larger in La Borbolla than in Buelna profile.

As shown in the maceral analysis (Table 2), huminite macerals are dominant, with inertinite ranging between 10 and 25% in the major part of the profile and liptinite being below 15%. Among the huminite macerals, textinite was the dominant maceral and a totally transparent variety with intense green fluorescence was separately recorded as textinite 0 (Fig. 6). This component was abundant in the topmost samples and sharply decreased with increasing depth. Comparatively the amount of ulminite, indicating a higher degree of gelification, was rather low. Among the structureless huminite macerals attrinite was dominant and only densinite reached a relevant proportion in the topmost part of BO profile. A Tissue Preservation Index for huminite macerals (TPI_H) has been calculated to account for the structured huminite vs total huminite macerals and is plot in Figure 7, along with the maceral group concentrations in both profiles. TPI_H decreased with age in both profiles, but was significantly higher in the raised bog (BO) profile than in the blanket bog profile (BU). Even not considering the oldest two points in BU profile, which correspond to samples with over 90% mineral matter, which are subjected to large calculation errors when percentages are expressed on mineral-free basis, a significant consistency in the variation of the maceral content with age is observed suggesting that this variation is palaeoenvironmentally driven. A peak in liptinite content is observed between 3500 and 4500 cal. yr BP, coinciding with a drop in inertinite content. The major liptinite maceral is sporinite, cutinite ranges from 0.5 and 3% and the highest contents are in the upper part. Liptodetrinite has been restricted to unassignable fragments of very high fluorescence intensity (as those observed in pollen) since most variably fluorescing debris are likely to derive from lignocellulose tissues. Liptodetrinite is generally scarce and only increases coinciding with the liptinite peak in BO profile. In both profiles inertinite is peaking between 5000 and 5500 cal. yr BP and the maximum occurs earlier in the blanket bog profile (BU) situated at lower

altitude. An additional maximum is observed in BU profile at 3000 cal. yr BP, which likely represents a more local phenomenon. Within the inertinite macerals, funginite is present throughout the profiles in amounts below 3% and reaches higher values in BU profile, whereas semifusinite is only present in the upper part of La Borbolla profile and coincides with lower inertodetrinite amounts. Fusinite and inertodetrinite are the most abundant inertinite macerals. The drop in inertinite occurring in the upper part of both profiles is mainly due to the lower inertodetrinite content in these intervals, whereas the abundance of fusinite is more regular throughout the profiles (Table 2). An Inertodetrinite Index (Idt index) reflects the relative proportion of inertodetrinite vs. inertinite (Fig. 7), which is higher in BU profile. It continuously decreases towards the top in La Borbolla profile suggesting a decrease in the level of inertinite fragmentation. In both cases the uppermost 10 cm of peat contain low inertinite proportion.

4.5. Biomarkers

The main families of compounds identifiable under the chromatographic conditions used in this study are marked in Figure 8 in which samples of similar age (ca. 3200 and 7100 cal. yr BP) are shown for both peat profiles. The chromatograms show peaks corresponding to linear lipids of different molecular weight. The high molecular weight (HMW) homologues are thought to derive from plant wax and the medium molecular weight (MMW) ones from bacterial lipids (Eglinton and Hamilton, 1963). A more clear distribution of linear lipids is observed in the m/z 57-59 chromatographic traces (Fig. 9) which show predominant odd n -alkanes in the C_{15} - C_{35} range with major peaks at C_{27} or C_{31} depending on the sample and odd n -alkane-2-ones (K_{13} - K_{35}) with major C_{27} or C_{19} members. Other linear lipids are even n -aldehyde with major peaks at

C₂₂ and C₁₈ in the raised bog and at C₂₂-C₂₆ in the blanket bog. These are thought to act as intermediates in the biosynthesis of *n*-alcohols from *n*-acids in higher land plants and can accumulate as components of cuticular wax (Prahl and Pinto, 2007). They are readily altered during diagenesis (Lehtonen and Ketola, 1990) and are preserved only under favourable conditions. Methyl esters with an even carbon number, formed by the methylation of fatty acids during the extraction process in the presence of MeOH, whose dominant counterparts are either C₂₀ or C₂₆ depending on the sample, are also found in the extracts.

Among the triterpenoids both the bacterial-derived ones with a hopane structure (Ourisson and Rohmer, 1992) and vascular plant-derived ones with ursane, oleanane and lupane skeletons (Simoneit, 1986) are the dominant compounds. Sitosterol and stigmasterol derivatives, the most common steroids in the epicuticular waxes of higher plants (Baker, 1982; Bianchi, 1995), are the dominant steroids in the profiles. Prominent peaks in some samples correspond to alkyl benzene derivatives (Fig. 8), whose dominant representative is a methyl-dodecyl-benzene. Alkyl-benzenes have been reported to be present in coal wax and are thought to derive from the direct cyclization of straight-chain fatty acids or alcohols via a clay-catalyzing mechanism (Dong et al., 1993), although they could also have an anthropogenic origin (Eganhouse et al., 1983).

4.5.1. Linear Lipids

Linear lipids such as *n*-alkane and *n*-ketone have a high stability and potential for self-preservation and their chain lengths can be associated to different sources (Bray and Evans, 1961), even within strictly terrestrial environments such as those of bogs. Peat-forming grasses tend to peak at *n*-C₃₁ (Bi et al., 2005; Seki et al., 2010) and trees

and shrubs at $n\text{-C}_{27}$ (Rieley et al., 1991). A high abundance of $n\text{-C}_{31}$ is not only found in higher plants but also in *Sphagnum magellanicum* and *S. capillifolium* (Nichols et al. 2006), although most *Sphagnum* species display maximum at $n\text{-C}_{23}$, high amounts of $n\text{-C}_{25}$ and low concentrations of $n\text{-C}_{29}$ (Baas et al., 2000; Bingham et al., 2010). In general the *Sphagnum* species occupying the relatively drier hummock habitat have lower values of $n\text{-C}_{23}$ and are dominated by the C_{25} and/or C_{31} homologues (Bingham et al., 2010). Based on these findings the $n\text{-C}_{23}/n\text{-C}_{25}$ ratio can be used as humidity proxy (Bingham et al., 2010). Other ratios which contain information about the hydrological regime are $n\text{-C}_{23}/n\text{-C}_{29}$, $n\text{-C}_{25}/n\text{-C}_{29}$, Paq ($\sum[\text{C}_{23},\text{C}_{25}]/\sum[\text{C}_{23},\text{C}_{25},\text{C}_{29},\text{C}_{31}]$), which can be used to estimate the bryophytic vs. vascular plant input and ratios such as $n\text{-C}_{27}/n\text{-C}_{31}$ for woody vs. herbaceous input (Ficken et al., 2000; Nott et al., 2000; Pancost et al., 2002; Nichols et al., 2006; Schellekens et al., 2011). The distribution of odd n -alkane in the studied profiles is shown in Figure 10. In both profiles a minimum concentration of n -alkanes is observed at around 5000 ca. yr BP. The HMW homologues predominate over the MMW compounds and the most recent samples have a high concentration of $n\text{-C}_{33}$ and $n\text{-C}_{31}$, also $n\text{-C}_{23}$ in the raised bog. The concentrations of n -alkanes throughout the profiles are very different. La Borbolla shows a higher concentration of n -alkanes than Buelna in the first 5000 cal. yr BP. A peak in the concentration of n -alkane in the blanket bog profile coincides with the commencement of organic matter accumulation. In the last 5000 cal. yr BP, the concentration of n -alkane in the raised bog is rather constant whereas a maximum occurs in the blanket bog profile in $n\text{-C}_{29}$ concentration at 3000-5000 cal. yr BP interval coinciding with higher mineral matter contents. The concentration of $n\text{-C}_{33}$ in the upper part of both profiles increases reaching values close to those of $n\text{-C}_{31}$ in La Borbolla. A higher concentration of $n\text{-C}_{33}$ than of $n\text{-C}_{31}$ has been reported for different parts of *Calluna vulgaris* and *Erica tetralix* (Pancost et al., 2002)

and *Juncus*, all of which have been collected in the vicinity (Ortiz et al., 2011). In general longer chain lengths have been associated to drier conditions in regional studies (Feakins and Sessions, 2010). The Average Chain Length calculated multiplying the amount of an *n*-alkane by its number of carbons over a given range referred to the total *n*-alkane concentrations allows comparing with a single parameter the variation of change length over the profile. In this study it has been calculated in the range C₁₉-C₃₃ and only for odd *n*-alkanes (ACL_{19-33odd} in Fig. 11). ACL_{19-33odd} keeps values close to 29 for the most part of Buelna profile increasing only at the bottom and in the last 3000 years. The ACL_{19-33odd} in La Borbolla is very similar before the raised bog period starts and then increases to later decrease in the last two millennia (coinciding with high Paq periods). The differences in Paq and ACL_{19-33odd} trends are essentially due to the strong effect of *n*-C₃₃ in ACL_{19-33odd}, a compound found in high concentrations in the upper part of both profiles.

HMW methyl-ketones have often been reported to occur in peat (Lehtonen and Ketola, 1990; Xie et al., 2004; Nichols and Huang, 2007; Zheng et al., 2007 and Ortiz et al., 2010) and recent organic-rich sediments (Wenchuan et al., 1999; Hernandez et al., 2001; Xie et al., 2003; Xu et al., 2007; Zeng et al., 2011). Four possible sources of these compounds are currently under discussion: (i) direct input from peat-forming plants: K₂₅ and K₂₇ are considered biomarkers of *Sphagnum* (Nichols and Huang, 2007), high concentrations of K₂₅ have been found in seagrasses (Hernandez et al., 2001), K₂₉ and K₁₇ have been associated to blue-green algae and submergent plants respectively in the Taihu Basin in China (Wenchuan et al., 1999) and some mosses enriched in K₁₉ have been recently reported in Penido Vello, N Spain (Schellekens et al., 2015). The concentration of methyl-ketones in living plants has been shown to be significantly lower than in peat (Lehtonen and Ketola, 1990; Nichols and Huang, 2007; Ortiz et al.,

2011); (ii) microbial oxidation of the related *n*-alkanes (van Bergen et al., 1998), the most widely accepted source of *n*-alkane in sediments (Jansen et al., 2008); (iii) oxidative decarboxylation of fatty acids (Volkman et al., 1981), proposed as the main origin of these compounds in the Zoigê-Hongyuan peat in West China (Zheng et al., 2007) and in the Chinese Loess Plateau (Bai et al., 2009); (iv) a microbial source associated to temperate raised bogs based on the higher isotopic signal of *n*-alkane-2-ones compared to the *n*-alkanes (López-Días et al., 2013a).

The concentrations of methyl-ketones are lower than those of *n*-alkane in Buelna and have maxima at similar intervals (Fig. 10), but the carbon number of the major compounds is not always coincident. Those with 23, 29, 31 and 33 carbons have relatively high correlation coefficients ($R^2=0.73-0.98$), which might support a microbial oxidation path. Concentrations of K_{25} and K_{27} are higher than those of the corresponding *n*-alkanes and require an additional input, probably of *Sphagnum* spp. (Nichols and Huang, 2007). The R^2 of *n*-C₂₃, K_{25} and K_{27} , all compounds associated with *Sphagnum* (Nichols and Huang, 2007; Bingham et al., 2010) are very high (Table 3). The concentration of K_{19} methyl ketone is significantly higher than that of *n*-C₁₉, requiring an additional input for K_{19} , a characteristic frequently observed in the bogs of North Spain (Ortiz et al., 2010, López-Días et al., 2010b, 2013a, Schellekens et al., 2011). In addition, generally high R^2 coefficients are obtained among the ketone homologues, except for K_{19} , whereas the R^2 among *n*-alkanes are significantly lower.

The correlations are very different in La Borbolla raised bog. The different ketones tend to concentrate at similar intervals which do not necessarily coincide with those of *n*-alkane enrichment, as can be seen from the poor correlations between *n*-alkanes and *n*-ketones with similar carbon numbers (Table 3). In the C₁₉-C₂₉ range ketones predominate over *n*-alkanes, whereas the concentrations of *n*-C₃₁ and *n*-C₃₃ are

higher than those of the corresponding ketones (K_{31} and K_{33}). The K_{19} methyl-ketone increases towards the bottom, a characteristic that has also been observed in a Swedish peat bog (Lehtonen and Ketola, 1990). This was not the case in Roñanzas or Las Conchas profiles, located only a few km away, where high concentrations of K_{19} extended throughout the whole three-meter profiles (Ortiz et al., 2010, 2016; López-Días et al., 2013a). Methyl-ketones in bogs from North Spain have been recently attributed to a microbial source on the basis of the isotopic values, most probably associated with *Sphagnum* rich in n - C_{25} (López-Días et al., 2013a). Nevertheless, the correlation coefficient between K_{19} and n - C_{25} in La Borbolla is rather low ($R^2=0.61$).

The compound ratios allow different studies to be compared. Figure 11 shows the variation of some ratios that are of palaeohydrological significance. Paq (Ficken et al., 2000) while having similar values in the lower part of both profiles, indicate wetter conditions in the raised bog in the last 6000 years. This is reflected not only in the values of Paq, but also in those of n - C_{23}/n - C_{29} and n - C_{23}/n - C_{25} ratios. In addition the n - C_{27}/n - C_{29} ratio indicates an enrichment in leaf waxes in 5500-8000 cal. yr BP. This is evident in both profiles, although it has greater significance in the raised bog. Some ratios involving n -ketones have also been suggested as proxies for palaeohydrological conditions in the Hongyuan peat in China (Zheng et al., 2011). One of the proposed ratios estimates the relative contribution of MMW to HMW ketones ($(\sum[C_{23},C_{25}]/\sum[C_{27},C_{29},C_{31}]-KET)$) in a profile with a relatively high contribution of K_{23} . In the present case, the ratio has been adapted to include the concentrations of K_{19} in the numerator, which is characteristic of these peats. Figure 11 shows that except for a short period (6000-7000 cal. yr BP), the ratio is higher in the raised bog and this interval coincides with the high values of n - C_{27}/n - C_{29} and with the fen period that led to the installation of bog conditions in the peat (Fig. 4).

4.5.2. Hopanoids

Hopanes are pentacyclic triterpanes ubiquitously found in sediments and petroleum and are readily identified in the m/z 191 mass chromatogram. The biohopanoids, although detected in several higher plants, ferns, mosses, fungi and protists, are particularly abundant in the membranes of cyanobacteria and bacteria living in oxygenated media and are generally considered as biomarkers for these organisms (Ourisson et al., 1987). Regular hopanes with different configurations at C-17 and C-21 are present in the profiles with a carbon number from C_{27} to C_{31} (there being no C_{28} member). The most abundant is 17α , 21β -homohopane (with an unstable R-configuration), whose formation is favoured in the acidic environment typical for peatlands (van Dorselaer et al., 1977). The presence of homologues with an unstable $\beta\beta$ configuration confirms the low maturity of peat. The concentration of regular hopanes, which is generally higher in the upper part of the profile, peaks at intervals with higher $n-C_{23}/n-C_{29}$ and $n-C_{23}/n-C_{25}$ ratios (Figs. 11 and 12). The 22,29,30-trisnorhopan-21-one is also a prominent peak among the hopanoids and is present throughout the profile, maximizing in its upper half, but following a distribution that is not exactly coincident with that of the $C_{31}\alpha\beta$ -homohopane.

Other hopanoids in the samples have a diplotene (C_{30} hop-22(29)-ene) configuration, a compound which is considered to be directly inherited from living or recently decayed microorganisms (Ries-Kautt and Albrecht, 1989). This compound tends to transform into hop-21(22)-ene and hop-17(21)-ene in acid catalyzed reactions (Ageta et al., 1987). Of these compounds, the C_{27} and C_{30} homologues with a double bond in the 17-21 position were identified in the peat profiles in small amounts and the

presence of their acetyl and ketone derivatives, functionalized at the C-3 position, is restricted to the upper part of both profiles (Fig. 12). The Hop-22(29)-en-3-one and its acetate are also present in the upper part of the profile. Hopanoids with oxygen functionalities at the C-3 position and no extended side chain, are considered to derive from oxidosqualene rather than squalene, and are associated to higher plants (Kanneberg and Poralla, 1999). The 17(21)- and 22(29)-hopen-3-ones follow a very similar distribution in both profiles with the former predominating over the latter and maximizing at a depth of 10-20 cm in the profile. The maxima do not coincide with those of regular hopane concentrations (Fig 9). This discrepancy, together with the fact that the hopen-3-ones are more abundant than the regular hopanes, suggests a different source for both classes of compounds. The period of high concentration of hopen-3-ones coincides with the last period of regular peat accumulation.

4.5.3. Higher-plant triterpenoids

Among the triterpenoids associated with the higher plants input, derivatives with oleanane (I), ursane (II), friedelane (III), bauernane (IV), simiarenane (V) and lupane (VI) skeletons, whose structures are shown in Figure 13, have been identified. They elute in the high boiling point region of the chromatograms (Fig. 8) and generally have ketone or acetyl functionality at carbon C-3. The amounts of the different compounds are shown in Figure 14. Overall higher plant-derived triterpenones concentrate in the upper part of the La Borbolla profile and follow distributions that peak at similar intervals, with friedelin folding at least ten times the concentration of other triterpenoid ketones, and lupanone and bauerenone following a different concentration pattern. The concentration of α -amyrenone (urs-12-en-3-one), β -amyrenone (olean-12-en-3-one) and

simiarenone (D:B-Friedo-B':A'-neogammacer-5-en-3-ona) peaked at around 2400 and 4000 cal. yr BP and the friedelin concentration (DA-friedooleanan-3-one) has an additional peak at 5500 cal. yr BP. Lupan-3-one in La Borbolla profile could only be quantified separately in a couple of samples, and has comparatively high values at 4800 cal. yr BP, an interval in which the concentration of other triterpenoid ketones is low. The concentration bauerenone (D:C-Friedours-7-en-3-one) has higher values at around 3000, 6000 and 8000 cal. yr BP. Concentration maxima are observed in the acetyl-derivatives at similar intervals to those of the ketones for the 2400 and 5500 cal. yr BP intervals, and slightly earlier in the case of the 4700 cal. yr BP maxima (ketones peak at 4000 cal. yr BP), a phenomenon which is particularly important for the 3β -lup-20(29)-en-3-yl acetate. As in the case of bauerenone, bauerenyl acetate had a different distribution to the other triterpenyl acetates, peaking at the same intervals as ketone, but with a wider distribution and in greater abundance (Fig. 14).

In the blanket bog profile, the concentration of triterpenoid derivatives is lower than in the raised bog, except for bauerenyl acetate. The concentrations of all the ketones parallel each other, with different compounds predominating at different intervals. In the lower part of the profile lupanone and bauerenone have the highest concentrations whereas in the upper part, bauerenone decreases significantly and both friedelin and lupanone are the dominant ketones with their highest values at 5500 and 4000 cal. yr BP. The concentration of acetyl derivatives is lower in this profile except in the case of the bauer-7-en- 3β -yl acetate, which has very high concentrations in the lower part of the profile (Fig. 14).

Triterpenoids with lupane, ursane, oleanane and friedelane skeletons have a typically low specificity and are considered as markers of angiosperms (Cranwell, 1984; Simoneit 1986). Friedelane and ursane derivatives have been reported in high amounts

in the roots of Ericaceae (Pancost et al., 2002), typical peat-forming plants in the area (Mary et al., 1973; Ortiz et al., 2011). The distribution of the bauerane derivatives requires an additional source. These compounds have been associated with the input of Asteraceae reported in open grasslands (Lavrieux et al., 2011). Bauerane derivatives are mainly associated with the period with high mineral matter content in La Borbolla and with the initial period of bog conditions in Buelna. The occurrence of other triterpenyl acetates with similar distribution to that of the corresponding ketones could be associated with a postdepositional reaction caused by the persistence of acetate in the profile. Thus the formation of either ketones or acetates would depend on the availability of acetate and the relative stability of both molecules. Friedelin is much more abundant than its acetate, whereas bauerenyl acetate is more abundant than its ketone in both profiles. More similar concentrations are observed for the ursane, oleanane and lupane derivatives (Fig. 14).

4.5.4. Steroids

The chromatogram of the total extract also showed steroid alcohols and ketones (Fig. 8), probably derived from higher plants (Hinrichs and Rullkötter, 1997). The most abundant are Stigmast-4-en-3-ona and 5α -Stigmastanona, which have been identified in Ericaceae remains (Pancost et al., 2002). In La Borbolla profile the sterones are more abundant than in Buelna and have a similar distribution to that of the bauerane derivatives. This is not the case in Buelna, where the sterones show a higher concentration in the upper part of the profile. Overall both hopanoids and higher plant triterpenoids are comparatively more abundant than steroids in Buelna profile compared with La Borbolla.

5. Discussion

The study of two neighbouring relatively short peat profiles, representing a long period of time, developed on siliceous substrate at very low and similar (0.05-0.07 mm/yr) growth rate allows the establishment of similarities and differences between the two peatlands. La Borbolla mire with a slightly domed surface can be considered a raised bog not only because of the geomorphology but also for the low mineral matter content (<10%). The establishment of the bog conditions lasted 2000 yr and dated back to 5500-7500 cal. yr BP (Middle Holocene), during which a pool infilling likely occurred. The blanket bog mire initiated on a smooth slope 3000 years earlier than the raised bog and developed relatively fast (500 years). The first part of the Middle Holocene (from 8000-7500 to 5500 cal. yr BP) reflect enrichment in woody plants in both Buelna and La Borbolla profiles as determined *n*-alkane ratios. Bauerenyl derivatives, attributed to Asteraceae are the only higher plant triterpenoid found in significant amounts in this period, and the presence of plants of this family is confirmed by the petrographic studies (Fig. 15). The nearby Roñanzas peat profile also records a vegetation change from vascular plant-rich (Middle Holocene) to *Sphagnum*-dominated peat (Late Holocene) as indicated by the higher-plants triterpenoids (López-Días et al., 2013b) and the transition occurs later (at around 4000 cal. yr BP) than observed in La Borbolla.

The last part of the Middle Holocene appears to have been more humid in La Borbolla (6000-4000 cal. yr BP), and is followed by drier conditions at the beginning of the Late Holocene, which turns more humid in the last 2500 cal. yr BP. The Buelna profile (blanket mire) appears to be dominated by a more herbaceous vegetation as

shown by high $n\text{-C}_{31}$, $n\text{-C}_{33}$, and occasionally $n\text{-C}_{27}$, relatively low but rather constant presence of higher plant triterpenoids (dominated by friedelin and lupanone) and low values of ratios reflecting moss input (Paq, $n\text{-C}_{23}/n\text{-C}_{25}$ and $n\text{-C}_{23}/n\text{-C}_{29}$). The Buelna floral association appears to be less sensitive to climate variation than the *Sphagnum*-rich vegetation.

The humid/arid intervals recorded in La Borbolla are also represented as pulsating intervals in the nearby Roñanzas profile, which showed a higher growth rate (Ortiz et al., 2010; López-Días et al., 2013b). Other studies of peat profiles in the geographical area (Las Conchas) indicated that local hydrology and drainage from the nearby Cuera Range ensured humid conditions in this record over the entire Holocene (Ortiz et al., 2016) masking the changes attributable to climate variation.

Studies based on palynology of a 13000 years old glacial lake sequence in the region (Lake Enol) and of other peat profiles (Mary et al., 1973; Fábregas Valcarce et al., 2003; Muñoz Sobrino et al., 2004; Magny et al., 2007; López-Merino et al., 2010; Moreno et al., 2011; Rubiales et al., 2012) indicate a warm and dry Middle Holocene followed by a wet and cold Late Holocene. The present study reflect little oscillations of climate in the blanket bog compared with the raised bog and overall more humid conditions in the raised bog, in particular in the last couple of millennia.

The petrographic composition of the peat profiles indicates a high level of lignocellulose tissue preservation (TPI_H) in agreement with the high C/N ratios typical for mostly ombrotrophic records. The higher TPI_H is recorded in the raised bog, associated to higher moss contributions, and would indicate that these tissues are more resistant to alteration. The record of passed fire events is essentially represented by inertodetrinite, which is the most abundant inertinite component. The fact that most of the charred material is formed by small tissue fragments suggests their derivation from

unlignified tissues as could be expected from moss and herbaceous plant tissues. The amount of inertinite is relatively high and rather similar for both peat profiles indicating that this characteristic is probably related to the low growth rate of both peats, giving time for tissue mouldering and desiccation. The upper levels have lower inertinite contents. Peaking at around 5000-5500 cal. yr BP there is an increase in inertinite recorded in both profiles, whereas a peak at 2500-3000 cal. yr BP is only registered in the blanquet bog and could be related with a local episode.

Peat accumulation ceased 2500 years ago in the Buelna and La Borbolla sites and the uppermost peat has developed under more humid conditions and maintains a higher index of tissue preservation. This level coincides with the acrotelm and the huminite reflectance indicates a higher level of humification to which likely contributed oxy-bacteria as indicated by regular hopanoid concentration.

6. Conclusions

Two condensed peat profiles from a raised bog and a blanket bog within a distance of 3.5 km, covering the Middle and the transition-to-Late Holocene in the North of Spain have been studied for organic petrology and geochemistry with the focus being placed on their ability to record environmental changes. Both have a similarly slow accumulation rate and cover a similar time span, stopping around 2500 years ago which facilitates the comparison, although peat began accumulating later in the raised bog. Some typical characteristics of Asturian peats such as a high amount of nonadecan-2-one (K_{19}) and high concentrations of triterpenyl acetates have been observed in these profiles indicating that the specific conditions, probably associated to wet temperate climate, were widespread throughout the region.

The two peat profiles have some specific characteristic that differentiate each other: The raised bog is more bryophytic has lower mineral matter content and higher C/N ratio and extraction yields compared to the blanket bog; the latter resulting in a generally higher concentration of biomarkers. It has a relatively high concentration of *n*-alkane-2-ones compared to *n*-alkane of similar carbon number, suggesting an additional source for the *n*-ketones and the proxies for humidity indicate that developed under more humid conditions. The bryophytic peat has a higher Tissue Preservation Index for huminite macerals (TPI_H) indicating a higher resistance to decay of bryophytic vegetation. The blanket bog is dominated by herbaceous vegetation, has a more regular, although lower, concentration of higher plant and hopanoid triterpenoids throughout the profile and the high correlation coefficients between concentration of *n*-alkane and *n*-ketone suggest a related origin for both compounds classes. A lower variability of humidity proxies is recorded in the blanket bog compared to the raised bog.

Both profiles have a significant amount of inertinite, predominantly inertodetrinite, a characteristic which is probably related with the very low growth rate. The inertodetrinite index peaks in both profiles at a level probably associated to wild fires (5000-6000 cal. yr BP). Both profiles can be considered analogues for inertinite-rich coals since the amounts in a sediment which has not been yet compacted and dehydrated is between 10 and 25%.

The huminite reflectance appears to be related at this mild maturation stage more with differences in the oxidative conditions than with variation of peat maturity. It is higher in the levels with high mineral matter content at the beginning of organic matter accumulation and also in the upper part of the profile exposed to seasonal desiccation. The concentration of hopanoids derived from bacteria in the upper part of the profile

(acrotelm) coincides with a larger amount of material reflecting biochemical gelification.

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References

- Ageta, H., Shiojima, K., Arai, Y., 1987. Acid-induced rearrangement of triterpenoid hydrocarbons belonging to the hopane and migrated hopane series. *Chemical and Pharmaceutical Bulletin* 35, 2705-2716.
- Bai, Y., Fang, X., Nie, J.; Wang, Y., Wu, F., 2009. A preliminary reconstruction of the paleoecological and paleoclimatic history of the Chinese Loess Plateau from the application of biomarkers. *Palaeogeography Palaeoclimatology Palaeoecology* 271, 161–169
- Baker, E.A. , 1982. Chemistry and morphology of plant epicuticular waxes. In: Cutler DF, Alvin KL, Price CE, (Eds.), *The plant cuticle*. London: Academic Press, pp. 139–165.
- Barber, K.E., Chambers, F.M., Maddy, D., 2003. Holocene palaeoclimates from peat stratigraphy: macrofossil proxy climate records from three oceanic raised bogs in England and Ireland. *Quaternary Science Reviews* 22, 521–539.
- Barber, K.E., Chambers, F.M., Maddy, D., Stoneman, R., Brew, J.S., 1994. A sensitive high resolution record of late Holocene climatic change from a raised bog in northern England. *The Holocene* 4, 198–205.
- Barber, K.E., Langdon, P.G., 2007. What drives the peat-based palaeoclimate record?. A critical test using multi-proxy climate records from northern Britain. *Quaternary Science Reviews* 26, 3318–3327.

- Bass, M., Pancost, R., van Geel, B., Sinninghe-Damsté, J., 2000. A comparative study of lipids in *Sphagnum* species. *Organic Geochemistry* 31, 535–541.
- Bi, X., Sheng, G., Liu, X., Li, C., Fu, J., 2005. Molecular and carbon and hydrogen isotopic composition of *n*-alkanes in plant leaf waxes. *Organic Geochemistry* 36, 1405–1417.
- Bianchi, G., 1995. Plant waxes. In: Hamilton, R.J. (Ed.), *Waxes: Chemistry, Molecular Biology and Functions*. The Oily Press, Dundee, pp. 175–222.
- Bindler, R., 2006. Mired in the past-looking to the future: Geochemistry of peat and the analysis of past environmental changes. *Global and Planetary Change* 53, 209–221.
- Bingham, E.M., McClymont, E.L., Väliranta, M., Mauquoy, D., Roberts, Z., Chambers, F.M., Pancost, R.D., Evershed, R.P., 2010. Conservative composition of *n*-alkane biomarkers in *Sphagnum* species: Implications for palaeoclimate reconstruction in ombrotrophic peat bogs. *Organic Geochemistry* 41, 214–220.
- Blackford, J., 2000. Palaeoclimatic records from peat bogs. *Trends in Ecology and Evolution* 15, 193–198.
- Blackford, J.J. and Chambers, F.M. 1991. Proxy records of climate from blanket mires: evidence for a Dark-Age (1400 BP) climatic deterioration in the British Isles. *Holocene* 1, 63–7.
- Blytt A. 1876. *Essay on the Immigration of the Norwegian Flora During Alternating Rainy and Dry Periods*. Cammermey: Christiania.
- Bray, E.E., Evans, E.D., 1961. Distribution of *n*-parafins as a clue to recognition of source beds. *Geochimica et Cosmochimica Acta* 22, 2–15.
- Chambers, F.M., Barber, K.E., Maddy, D., Brew, J., 1997. A 5500-year proxy-climate and vegetation record from blanket mire at Talla Moss, Borders, Scotland. *Holocene* 7, 391–399
- Chambers, F.M., Daniell J.R.G., ACCROTELM members, 2010. Peatland archives of late-Holocene climate change in northern Europe. *PAGES news* 18, 4–6.
- Charman, D.J., 2002. *Peatlands and Environmental Change*, John Wiley and Sons, West Sussex.
- Charman, D.J., Barber, K.E., Blaauw, M., Langdon, P.G., Mauquoy, D., Daley, T.J., Hughes, P.D.M., Karofeld, E. 2009. Climate drivers for peatland palaeoclimate records *Quaternary Science Reviews* 28, 1811–1819
- Cohen, A.D., 1982. Obtaining more precise description of peat by use of oriented microtome sections. In: Jarrett, P. (Ed.), *Testing of Peats and Organic Soils: American Society for testing and materials, STP, 820*, pp. 21–36.
- Cohen, A.D., Spackman, W., 1972. Methods in peat petrology and their application to reconstruction of paleoenvironments. *Geological Society American Bulletin* 83, 129–142.
- Cranwell, P.A., 1984. Organic geochemistry of lacustrine sediments: triterpenoids of higher plants origin reflecting post-glacial vegetational succession. In Haworth, E.Y., Lund, J.W.G. (Eds), *Lakes Sediments and Environmental History*. University Press, Leicester, pp. 69–92.
- Dehmer, J., 1993. Petrology and organic geochemistry of peat samples from a raised bog in Kalimantan (Borneo). *Organic Geochemistry* 20, 349–362.
- Dong, J., Vorkink, W. P. and Lee, M. L., 1993. Origin of long-chain alkylcyclohexanes and alkylbenzenes in a coal-bed wax. *Geochimica et Cosmochimica Acta* 57, 837–849.
- Eganhouse, R.P., Blumfield, D.L., Kaplan, I.R., 1983. Long-chain alkylbenzenes as molecular tracers of domestic wastes in the marine environment. *Environment Science and Technology* 17, 523–53

- Eglinton, G., Hamilton, R.J., 1963. The distribution of *n*-alkanes, in: Swan, T. (Ed.), Chemical plant taxonomy. Academic Press, London, pp. 187-217.
- Esterle, J., 1994. Spatial variability in modern tropical peat deposits from Sarawak, Malaysia and Sumatra, Indonesia: analogues for coal. *International Journal of Coal Geology* 25, 1–42.
- Ellis, C.J., Tallis, J.H., 2000. Climatic control of blanket mire development at Kentra Moss, north-west Scotland. *Journal of Ecology* 88, 869–889.
- Fábregas Valcarce, R., Martínez Cortizas, A., Blanco Chao, R., Chesworth W., 2003. Environmental change and social dynamics in the 2nd–3rd millennium BC in NW Iberia. *Journal of Archaeological Sciences*, 30 859–871.
- Feakins, S.J., Sessions, A.L., 2010. Crassulacean acid metabolism influences D/H ratio of leaf wax in succulent plants. *Organic Geochemistry* 41, 1269-1276.
- Fernández Prieto, J.A., Fernández Ordóñez, M.C., Collado Prieto, M.A., 1987. Datos sobre la vegetación de las «turberas de esfagnos» galaico-asturianas y orocantábricas. *Lazaroa* 7, 443-47
- Ficken, K.J., Li, B., Swain, D.L., Eglinton, G., 2000. An *n*-alkane proxy for the sedimentary inputs of submerged/floating freshwater aquatic macrophytes. *Organic Geochemistry* 31, 745–749.
- Galka, M., Miotk-Szpigianowicz, G., Goslar, T., Ješko, M., van der Knaap, W.O., Lamentowicz, M. 2013. Palaeohydrology, fires and vegetation succession in the southern Baltic during the last 7500 years reconstructed from a raised bog based on multi-proxy data. *Palaeogeography, Palaeoclimatology, Palaeoecology* 370, 209-221
- Glasspool, I.J., Scott, A.C., 2013. Identifying Past Fire Events. In: Belcher, C.M. (Ed.), *Fire phenomena in the Earth System – An Interdisciplinary Approach to Fire Science*. Wiley-Blackwell, Chichester, pp. 179–206.
- Hagemann, H.W., Dehmer, J., 1991. Spectrophotometric lightness and chromaticity measurements on absorbing peat and brown coal surfaces: Part I — method and evaluation. *International Journal of Coal Geology* 19, 163–183.
- Hernandez, M.E., Mead, R., Peralba, M.C., Jaffé, R., 2001. Origin and transport of *n*-alkane-2-ones in a subtropical estuary: potential biomarkers for seagrass-derived organic matter. *Organic Geochemistry* 32,21-32.
- Hinrichs K.-U., Rullkötter J., 1997. Terrigenous and marine lipids in Amazon fan sediments: Implications for sedimentological reconstructions. In: Flood R.D., Piper D.J.W., Klaus A. et al. (eds.), *Proc. ODP, Sci. Res.* 155, 539-553. Ocean Drilling Program, College Station (TX).
- Hugues, P.D.M., Barber, K.E., 2004. Contrasting pathways to ombrotrophy in three raised bogs from Ireland and Cumbria, England. *The Holocene* 14 (1), 65–77.
- International Committee for Coal and Organic Petrology (ICCP), 2001. The new inertinite classification (ICCP System 1994). *Fuel* 80, 459–471.
- ISO 7404-2 (2009). Methods for the petrographic analysis of bituminous coal and anthracite-Part 2: Method of preparing coal samples. International Organization for Standardization, Geneva, Switzerland, 8pp.
- ISO 7404-3 (2009). Methods for the petrographic analysis of bituminous coal and anthracite-Part 3: Method of determining maceral group composition. International Organization for Standardization, Geneva, Switzerland, 6pp.
- ISO 7404-5 (2009). Methods for the petrographic analysis of bituminous coal and anthracite-Part 5: Method of determining microscopically the reflectance of vitrinite. International Organization for Standardization, Geneva, Switzerland, 14pp.

- Jansen, B., Haussmann, N.S., Tonneijck, F.H., Verstraten, J.M., de Voogt, P., 2008 Characteristic straight-chain lipid ratios as a quick method to assess past forest-paramo transitions in the Ecuadorian Andes. *Palaeogeography, Palaeoclimatology, Palaeoecology* 262, 129-139
- Kannenbergh, E.L. and Poralla, K., 1999. Hopanoid biosynthesis and function in bacteria. *Naturwissenschaften* 86, 168-176.
- Koch, J., 1969. Mikropetrographische Untersuchungen an einigen Organischen Komponenten jungpleistozäner um holozäner Torfe Süddeutschlands und der Schweiz. *Geologisches Jahrbuch* 87, 333–360.
- Lavrieux, M., Jacob, J., LeMilbeau, C., Zocatelli, R., Masuda, K., Bréheret, J.G., Disnar, J.R., 2011. Occurrence of triterpenyl acetates in soil and their potential as chemotaxonomical markers of Asteraceae. *Organic Geochemistry* 42, 1315-1323.
- Lehtonen, K., Ketola, M., 1990. Occurrence of long-chain acyclic methyl ketones in *Sphagnum* and *Carex* peats of various degrees of humification. *Organic Geochemistry* 15, 275-280.
- Loisel, J., Garneau, M. 2010. Late Holocene paleoecohydrology and carbon accumulation estimates from two boreal peat bogs in eastern Canada: Potential and limits of multi-proxy archives. *Palaeogeography, Palaeoclimatology, Palaeoecology* 291, 493–533
- López-Días, V., Borrego, Á.G., Blanco, C.G., Arboleya, M., López-Sáez, J.A., López-Merino, L., 2010a. Biomarkers in a peat deposit in Northern Spain (Huelga de Bayas, Asturias) as proxy for climate variation. *Journal of Chromatography A* 1217, 3538-3546.
- López-Días, V., Blanco, C.G., Bechtel, A., W., Püttmann, W., Borrego, A.G., 2013a. Different source of *n*-alkanes and *n*-alkane-2-ones in a 6000 cal. yr BP *Sphagnum*-rich temperate peat bog (Roñanzas, N Spain). *Organic Geochemistry*, 57, 7-10.
- López-Días, V., Borrego, A.G., Blanco, C.G., 2010b. Vertical evolution of petrographic and organic geochemical parameters in Las Dueñas mire (Cantabrian Coast, North Spain). *International Journal of Coal Geology* 84, 179-189.
- López-Días, V., Urbanczyk J., Blanco, C.G., Borrego, A.G., 2013b Biomarkers as palaeoclimate proxies in peatlands from coastal high plains in Asturias, N Spain. *International Journal of Coal Geology* 116–117, 270–280
- López-Merino, L., Martínez Cortizas, A., López Sáez, J.A., 2010. Early agriculture and palaeoenvironmental history in the North of the Iberian Peninsula: a multi-proxy analysis of the Monte Areo mire (Asturias, Spain). – *Journal of Archaeological Science* 37, 11pp.
- Magny, M., de Beaulieu, J.L., Drescher-Schneider, R., Vannièrè, B., Walter-Simonnet, A.V., Miras, Y., Millet, L., Bossuet, G., Peyron, O., Brugiapaglia, E., Leroux, A., 2007. Holocene climate changes in the central Mediterranean as recorded by lakelevel fluctuations at Lake Accesa (Tuscany, Italy). *Quaternary Science Reviews* 26, 1736–1758
- Mary, G., 1983. Evolución del margen costero de la Cordillera Cantábrica en Asturias desde el Mioceno. *Trabajos de Geología*, 13, 3-35. Univ. de Oviedo
- Mary, G., Beaulieu, J.L. de, Medus, J., 1973. Un diagramme sporopollinique et des datations 14C pour la tourbière du Llano Ronanzas (Asturies-Espagne). *Bull. Soc. Geol. France* 7, 37-38.
- Mauquoy, D., Barber, K.E., 1999. Evidence for climatic deteriorations associated with the decline of *Sphagnum imbricatum* Hornsch. Ex Russ. in six ombrotrophic mires from Northern England and the Scottish Borders . *The Holocene* 9, 423–437.

- Moreno A, López-Merino L., Leira M., Marco-Barba J., González-Sampériz P., Valero-Garcés B.L., López-Sáez J.A., Santos L., Mata P., Ito E., 2011. Revealing the last 13500 years of environmental history from the multiproxy record of a mountain lake (Lago Enol, northern Iberian Peninsula). *Journal of Paleolimnology* 46, 327-349.
- Muñoz-Sobrino C, Ramil-Rego P, Gómez-Orellana L. 2004. Vegetation of the Pleistocene: a palaeoecological reconstruction on the basis of two new pollen sequences. *Vegetation History and Archaeobotany* 13, 1-22
- Nichols, J.E., Booth, R.K., Jackson, S.T., Pendall, E.G., Huang, Y., 2006. Paleohydrologic reconstruction based on *n*-alkane distributions in ombrotrophic peat. *Organic Geochemistry* 37, 1505–1513.
- Nichols, J.E., Huang, Y., 2007. C₂₃–C₃₁ *n*-alkan-2-ones are biomarkers for the genus *Sphagnum* in freshwater peatlands. *Organic Geochemistry* 38, 1972-76.
- Nott, C.J., Xie, S., Avsejs, L.A., Maddy, D., Chambers, F.M., Evershed, R.P., 2000. *n*-Alkane distributions in ombrotrophic mires as indicators of vegetation change related to climatic variation. *Organic Geochemistry* 31, 231-235.
- Ortiz, J.E., Díaz-Bautista, A., Aldasoro, J.J., Torres, T., Gallego, J.L.R, Moreno, L., Estébanez, B., 2011. *n*-Alkan-2-ones in peat-forming plants from the Roñanzas ombrotrophic bog (Asturias, northern Spain). *Organic Geochemistry* 42, 586-592.
- Ortiz, J.E., Gallego, J.L.R., Torres, T., Díaz-Bautista, A., Sierra, C., 2010. Palaeoenvironmental reconstruction of Northern Spain during the last 8000 cal yr BP based on biomarker content of the Roñanzas peat bog (Asturias). *Organic Geochemistry* 41, 454-466.
- Ortiz, J.E., Borrego, A.G., Gallego, J.R.L., Sánchez-Palencia Y., Urbanczyk J., Torres T., Domingo L., Estébanez B., 2016. Biomarkers and inorganic proxies in the paleoenvironmental reconstruction of mires: The importance of landscape in Las Conchas (Asturias, Northern Spain). *Organic Geochemistry* 95, 41–54
- Ourisson, G., Rohmer, M., Poralla, K., 1987. Prokaryotic hopanoids and other polyterpenoid sterol surrogates. *Annual Review of Microbiology* 62, 301-333.
- Ourrison, G. and Rohmer, M., 1992. Hopanoids. 2. Biohopanoids: a novel class of bacterial lipids. *Accounts of Chemical Research* 25, 403-408.
- Pancost, R.D., Baas, M., van Geel, B., Sinninghe Damsté, J.S., 2002. Biomarkers as proxies for plant inputs to peats: an example from a sub-boreal ombrotrophic bog. *Organic Geochemistry* 33, 675–690.
- Peters, K.E., Walters, C.C., Moldowan, J.M., 2005. The biomarker guide. Volume 1: Biomarkers and isotopes in the environment and human history, second ed. Cambridge University Press, Cambridge.
- Philp, R.P., 1985. Fossil fuel biomarkers. Applications and spectra. *Geochemistry and Geophysics*, 23. Elsevier, Amsterdam.
- Pontevedra-Pombal, X., Novóa Muñoz, J.C., García-Rodeja, E., Martínez-Cortizas, A., 2006. Mountain mires from Galicia (NW Spain), in: Martini, I.P., Martínez-Cortizas, A., Chesworth, W. (Eds.), *Peatlands. Evolution and records of environmental and climate changes: Developments in Earth and Surface Processes* 9. Elsevier, Amsterdam, pp. 85-109.
- Prahl, F.G., Pinto, L.A., 2007. A geochemical study of long- chain *n*-aldehydes in Washington coastal sediments. *Geochimica et Cosmochimica Acta* 51, 1573-1582.
- Reimer, P.J., Baillie, M.G.L., Bard, E., Bayliss, A., Beck, J.W., Blackwell, P.G., Bronk Ramsey, C., Buck, C.E., Burr, G.S., Edwards, R.L., Friedrich, M., Grootes, P.M., Guilderson, T.P., Hajdas, I., Heaton, T.J., Hogg, A.G., Hughen, K.A., Kaiser, K.F.,

- Kromer, B., McCormac, F.G., Manning, S.W., Reimer, R.W., Richards, D.A., Southon, J.R., Talamo, S., Turney, C.S.M., van der Plicht, J., & Weyhenmeyer, C.E., 2009. IntCal09 and Marine09 radiocarbon age calibration curves, 0-50,000 years cal BP. *Radiocarbon* 51(4), 1111-1150.
- Rieley, G., Collier, R.J., Jones, D.M., Eglinton, G., Eakin, P.A., Fallick, A.E., 1991. Sources of sedimentary lipids deduced from stable carbon-isotope analyses of individual compounds. *Nature* 352, 425-427
- Ries-Kautt, M., Albrecht, P., 1989. Hopane derived triterpenoids in soils. *Chemical Geology* 76, 143-151.
- Rubiales, J.M., Ezquerro, J., Muñoz Sobrino, C., Génova, M.M., Gil, L., Ramil-Rego P., Gómez Manzanque, F., 2012 Holocene distribution of woody taxa at the westernmost limit of the Circumboreal/Mediterranean boundary: Evidence from wood remains. *Quaternary Science Reviews* 33, 74-86.
- Schellekens, J., Buurman, P., Fraga, I., Martínez-Cortizas, A., 2011. Holocene vegetation and hydrologic changes inferred from molecular vegetation markers in peat, Penido Vello (Galicia, Spain). *Palaeogeography Palaeoclimatology Palaeoecology* 299, 56-69.
- Schellekens, J., Bradley, J., Abbott, G.D., Fraga, I., Buurman, P., Pontevedra-Pombal, X., Vidal-Torrado, P., 2015c. The use of plant-specific pyrolysis products as biomarkers in peat deposits. *Quaternary Science Reviews* 123, 254-264.
- Seki, O., Nakatsuka, T., Shibata, H., Kawamura, K., 2010. A compound-specific n -alkane $\delta^{13}\text{C}$ and δD approach for assessing source and delivery processes of terrestrial organic matter within a forested watershed in northern Japan. *Geochimica et Cosmochimica Acta* 74, 599-613.
- Sernander R. 1909. De scanodaniska torfmossarnas stratigrafi. *Geologiska Föreningens i Stockholm Förhandlingar* 31, 423-448.
- Simoneit, B.R.T., 1986. Cyclic terpenoids of the geosphere, in: Johns R.B (Ed.). *Biological markers in the sedimentary record.* (ed.). Elsevier, Amsterdam pp. 175-221.
- Sternberg, L.S.L., Pinzon, M.C., Vendramini, P.F., Anderson, W.T., Hope Jahren, A., Beuning, K., 2007. Oxygen isotope ratios of cellulose-derived phenylglucosazone: an improved paleoclimate indicator of environmental water and relative humidity. *Geochimica et Cosmochimica Acta* 71, 2463-2473.
- Stuiver, M., Reimer, P.J., Reimer, R., 2005. CALIB Radiocarbon Calibration <http://radiocarbon.pa.qub.ac.uk/calib>. CALIB 6.0. Available online at <http://calib.qub.ac.uk/calib> (accessed January 17, 2012).
- Sýkorová, I., Pickel, W., Christanis, K., Wolf, M., Taylor, G.H. Flores, D., 2005. Classification of huminite- ICCP System 1994. *International Journal of Coal Geology* 62, 85-106.
- Van Bergen, P.F., Nott, C.J., Bull, I.D., Poulton, P.R., Evershed, R.P., 1998. Organic Geochemical studies of soils from the Rothamsted Classical Experiments – IV. Preliminary results from a study of the effect of soil pH on the organic matter decay. *Organic Geochemistry* 29, 1779-1795
- Van Dorsselaer, A., Albrecht, P., Connan, J., 1977. Changes in composition of polycyclic alkanes by thermal maturation (Yallourn Lignite, Australia), in: Campos, R., Goñi, J. (Eds.), *Advances in Organic Geochemistry 1975*. Enadimsa, Madrid, pp.53-59.
- Van Geel, B., 1978. A palaeoecological study of Holocene peat bog sections in Germany and the Netherlands., *Review of Palaeobotany and Palynology* 25, 1-120

- Volkman, J.K., Johns, R.B., Gillan, F.T., Perry, G.J., Bavor, Jr, H.J., 1981. Microbial lipids of an interstitial sediment-I. Fatty acids and hydrocarbons. *Geochimica et Cosmochimica Acta* 44, 1133–1143.
- Wenchuan, Q., Dickman, M., Sumin, W., Ruijin, W., Pingzhong, Z., Jianfa, C., 1999. Evidence for an aquatic plant origin of ketones found in Taihu Lake sediments. *Hydrobiologia* 397, 149-154.
- Xie, S., Chen, F., Wang, Z., Wang, H., Gua, Y., Huang, Y., 2003. Lipid distributions in loess-paleosol sequences from northwest China. *Organic Geochemistry* 34, 1071-1079
- Xie, S., Nott, C.J., Avejs, L.A., Maddy, D., Chambers, F.M., Evershed, R.P., 2004. Molecular and isotopic stratigraphy in an ombrotrophic mire for paleoclimate reconstruction. *Geochimica et Cosmochimica Acta* 68, 2849-2862.
- Xu, Y., Holmes, C.W., Jaffé, R., 2007. Estuarine paleoenvironmental assessment of recent environmental changes in Florida Bay, USA: A biomarker based study. *Coastal and Shelf Science* 73, 201-210
- Zeng, F., Xiang, S., Zhang, K., Lu, Y., 2011. Environmental evolution recorded by lipid biomarkers from the Tawan loess–paleosol sequences on the west Chinese Loess Plateau during the late Pleistocene. *Environmental Earth Science* 64, 1951–1963
- Zheng, Y., Zhou, W., Meyers, P.A., Xie, S., 2007. Lipid biomarkers in the Zoigê-Hongyuan peat deposit: Indicators of Holocene climate changes in West China. *Organic Geochemistry* 38, 1927–1940.
- Zheng, Y.J., Zhou, W.J., Liu, X., Zhang, C.L., 2011. *n*-Alkan-2-one distribution in a northeastern China peat core spanning the last 16 kyr. *Organic Geochemistry* 42, 25-30

Figure Captions

Fig.1. General view of the raised bog (left: La Borbolla) and blanket bog (right: Buelna) studied.

Fig. 2. Location of the study sites and lithological description of the profiles.

Fig. 3. Age-depth models for La Borbolla and Buelna profile.

Fig. 4. Variation of some bulk chemical parameters along both peat profiles. Solid symbols=La Borbolla, voids=Buelna

Fig. 5. Reflectance of huminite (HRr) sub-macerals measured in the two peat profiles; ch=corpohuminite, dh=detrohuminite, th=telohuminite

Fig. 6. Photomicrographs of the most common macerals in the peat profiles (oil immersion, left: under blue-violet light excitation, right: under white reflected light)

Fig. 7. Variation of maceral group composition and petrographic indices with age. TPI_H =Tissue Preservation Index calculated for huminite macerals Idt Index=relative proportion of inertodetrinite to inertinite.

Fig. 8. Total Ion Current (TIC) Chromatograms of the total extracts showing the variation of biomarkers at different depths in the two profiles. The subscripts indicate the number of carbons in the corresponding compound (C_n , K_n , E_n).

Fig. 9. Chromatograms of the ion m/z 57-59 of the extracts showing n -alkane (C_n), aliphatic esters (E_n), aliphatic aldehydes (A_n) and methyl ketones (K_n) at different depths. The subscripts indicate the number of carbons in the corresponding compound.

Fig.10. Concentration of odd n -alkanes and methyl-ketones in La Borbolla and Buelna profiles.

Fig. 11. Ratios based on linear lipid concentrations in La Borbolla and Buelna profiles. Solid symbols=La Borbolla, voids=Buelna. ACL=Average Chain Length calculated for odd n -alkanes in the interval C19-C33; $ACL = \frac{\sum(n-C_{19} * 19 + \dots + n-C_{33} * 33)}{(n-C_{19} + \dots + n-C_{33})}$. MMW and HMW=Medium and High Molecular Weight, Paq after Ficken et al., 2000. See text for explanation. Shadow areas=Humid periods.

Fig.12. Concentration of hopanoids derivatives in the peat profiles. Solid symbols=La Borbolla, voids=Buelna

Fig.13. Structures of higher plant triterpenoids mentioned in the text.

Fig.14. Concentration of higher plant triterpenoids in the peat profiles. The arrow after the compound indicates that concentrations for these compounds should be read in the bottom abscise axis.

Fig.15. Some examples of Asteraceae pollen in the peat profiles. Blue-violet light excitation.

La Borbolla



Buelna



Fig. 1

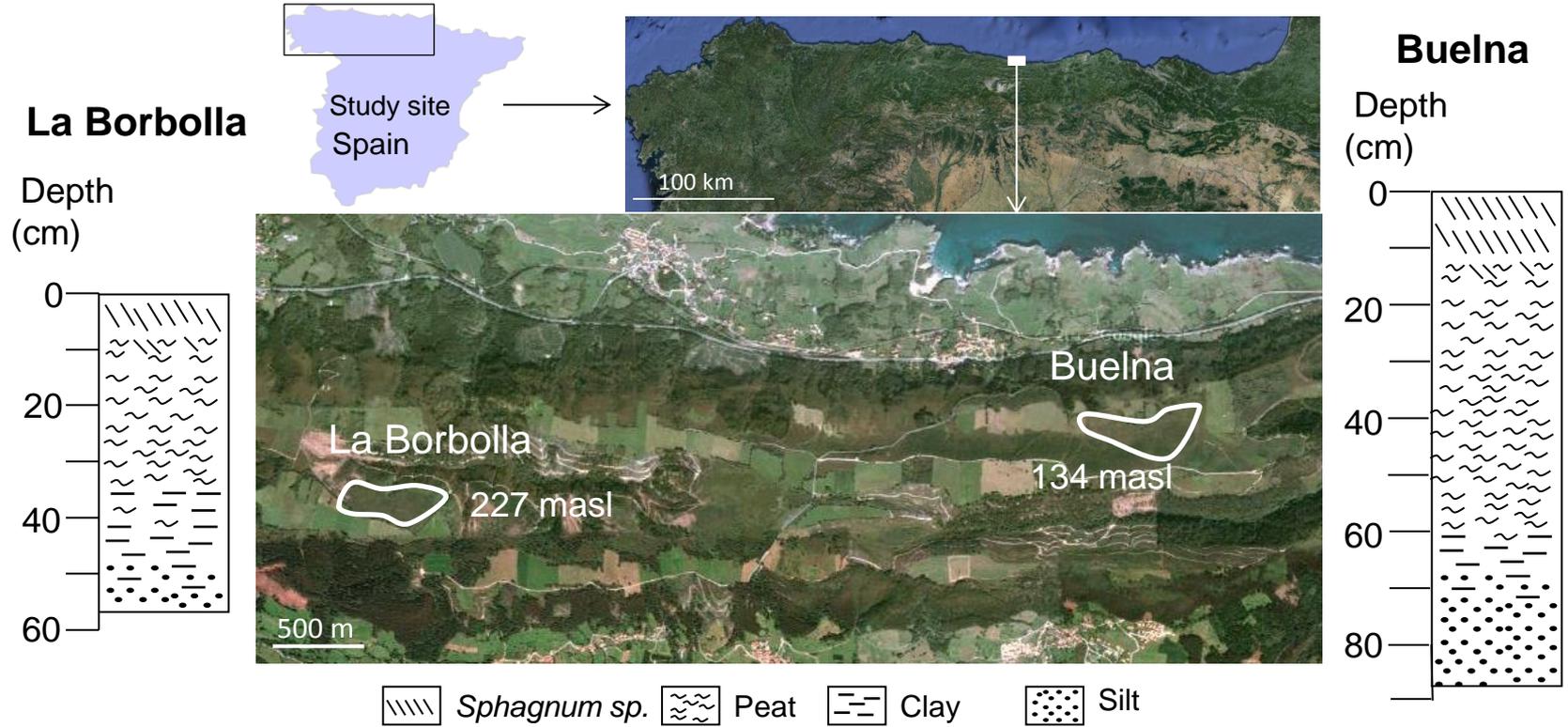


Fig. 2

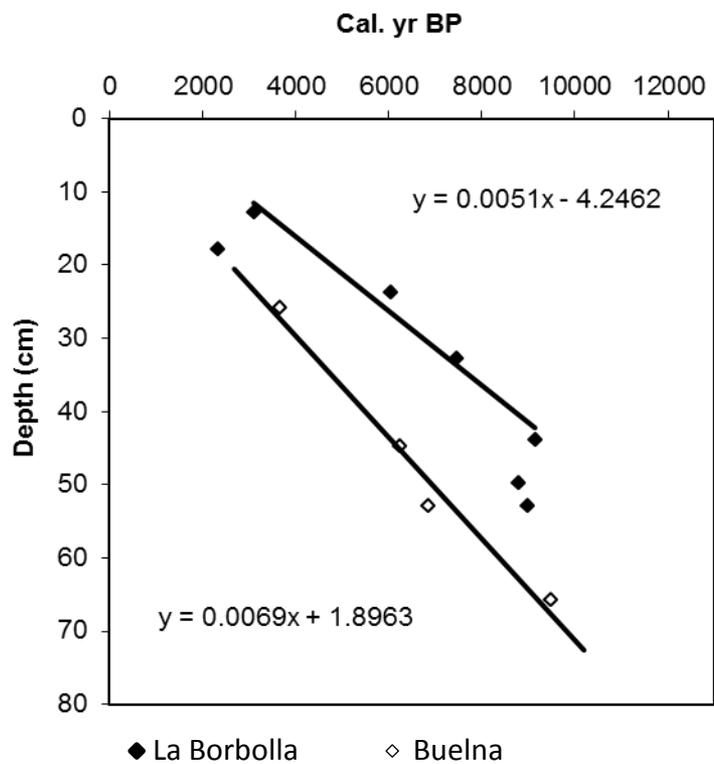


Fig. 3

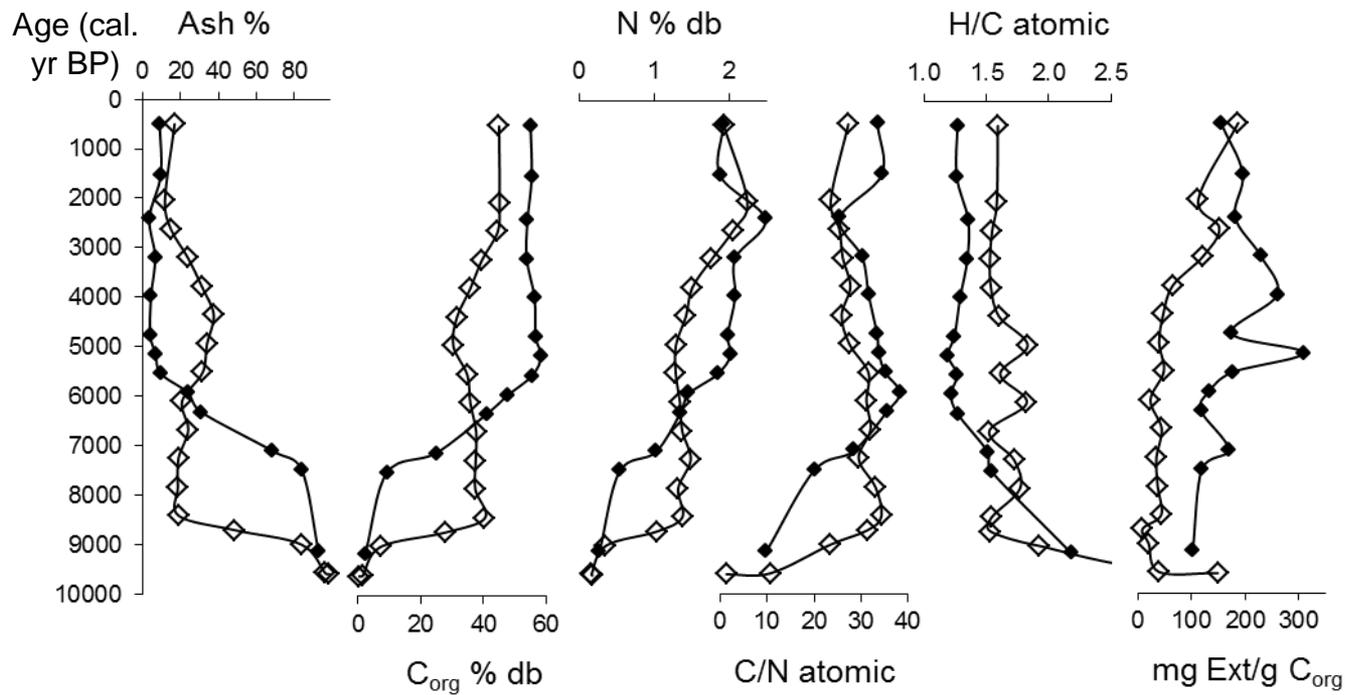


Fig. 4

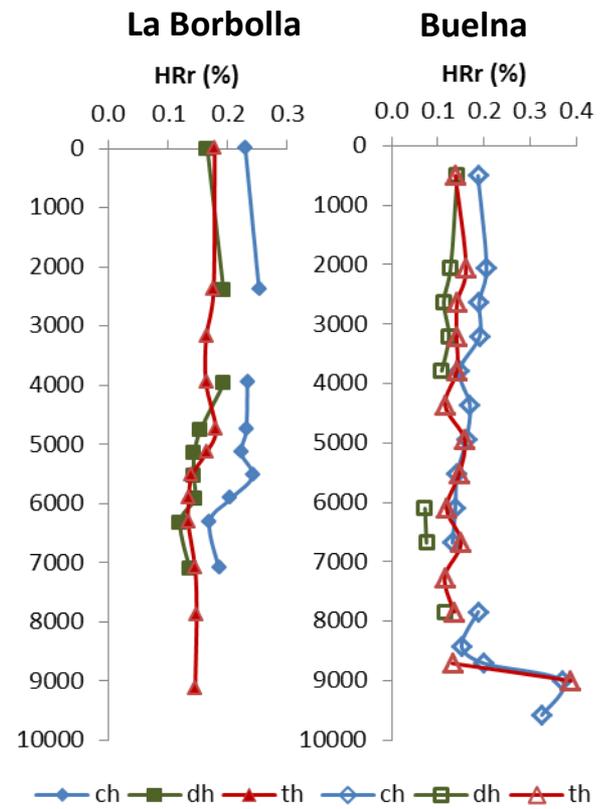


Fig. 5

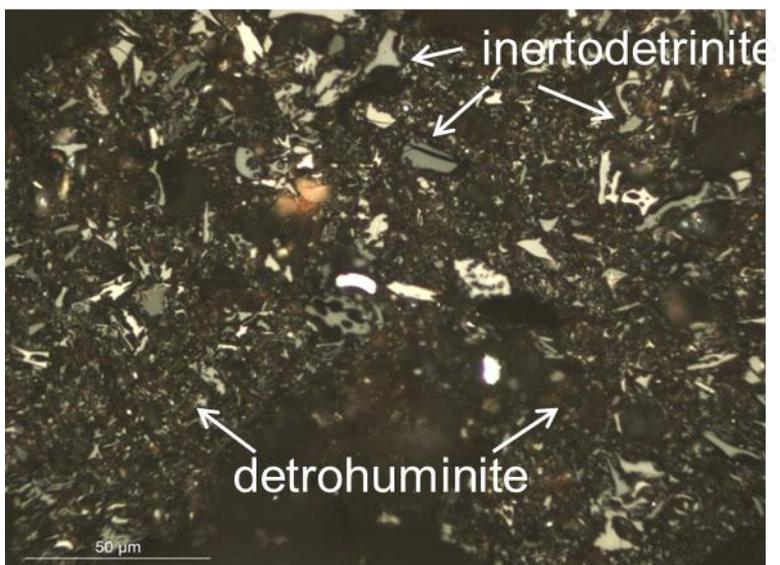
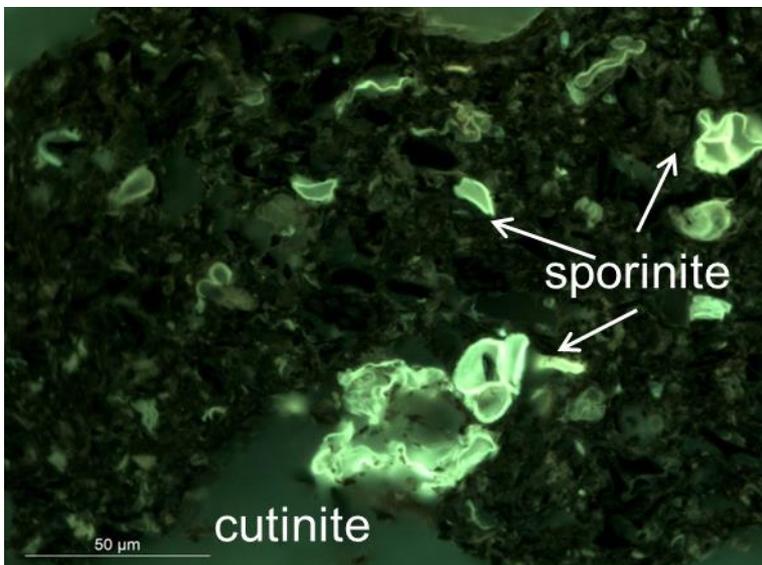
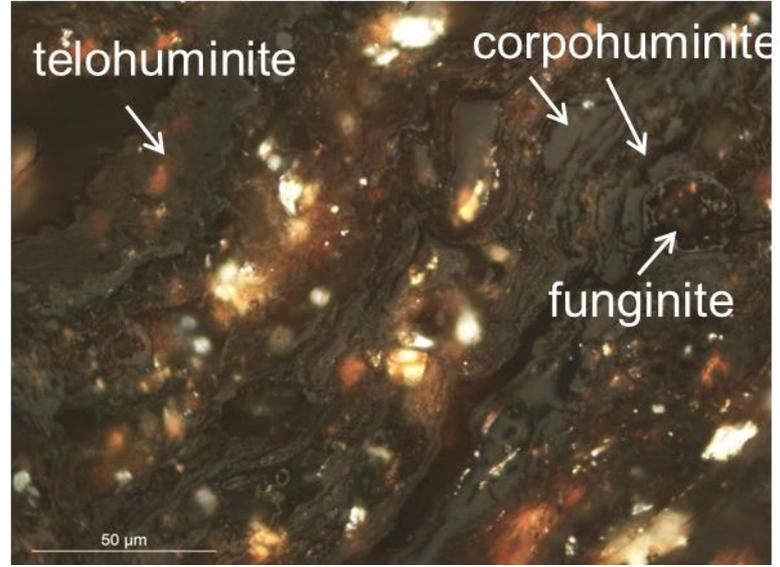


Fig. 6

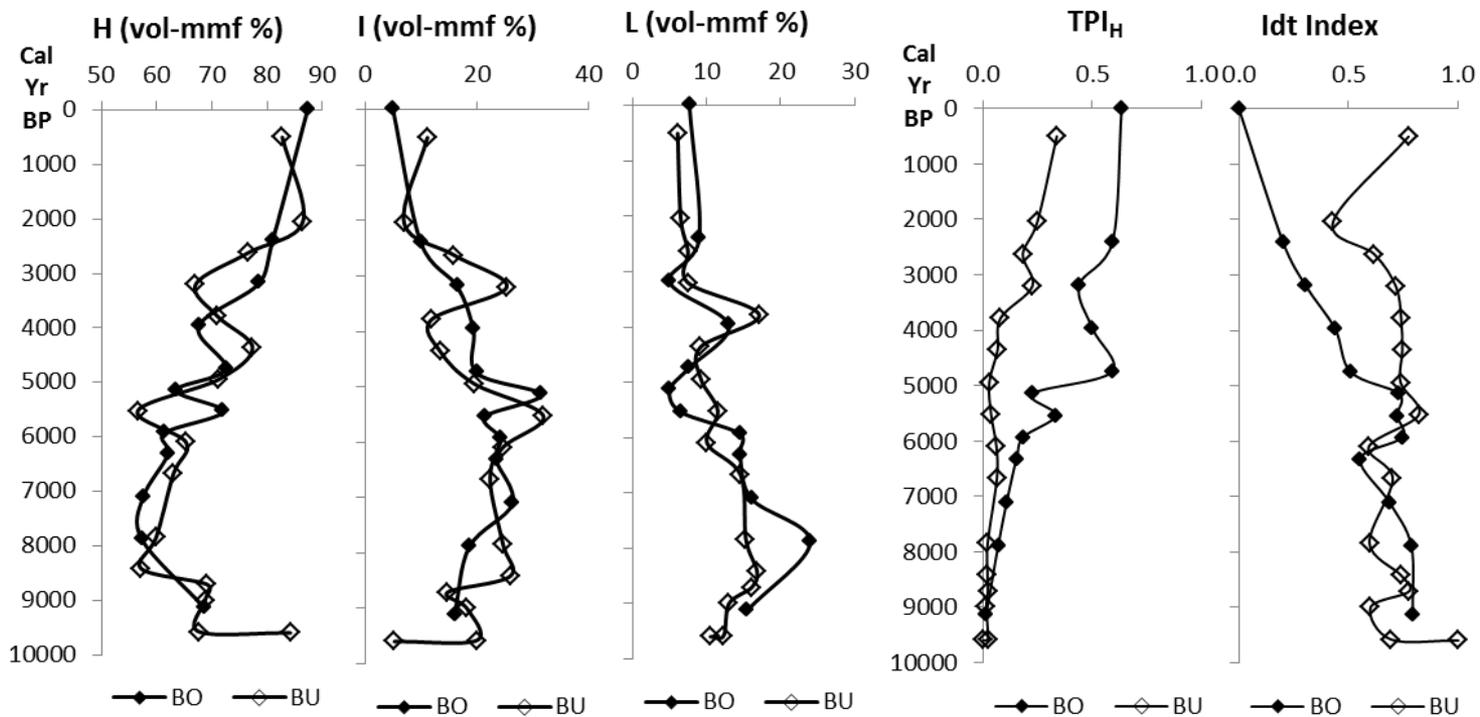


Fig. 7

TIC

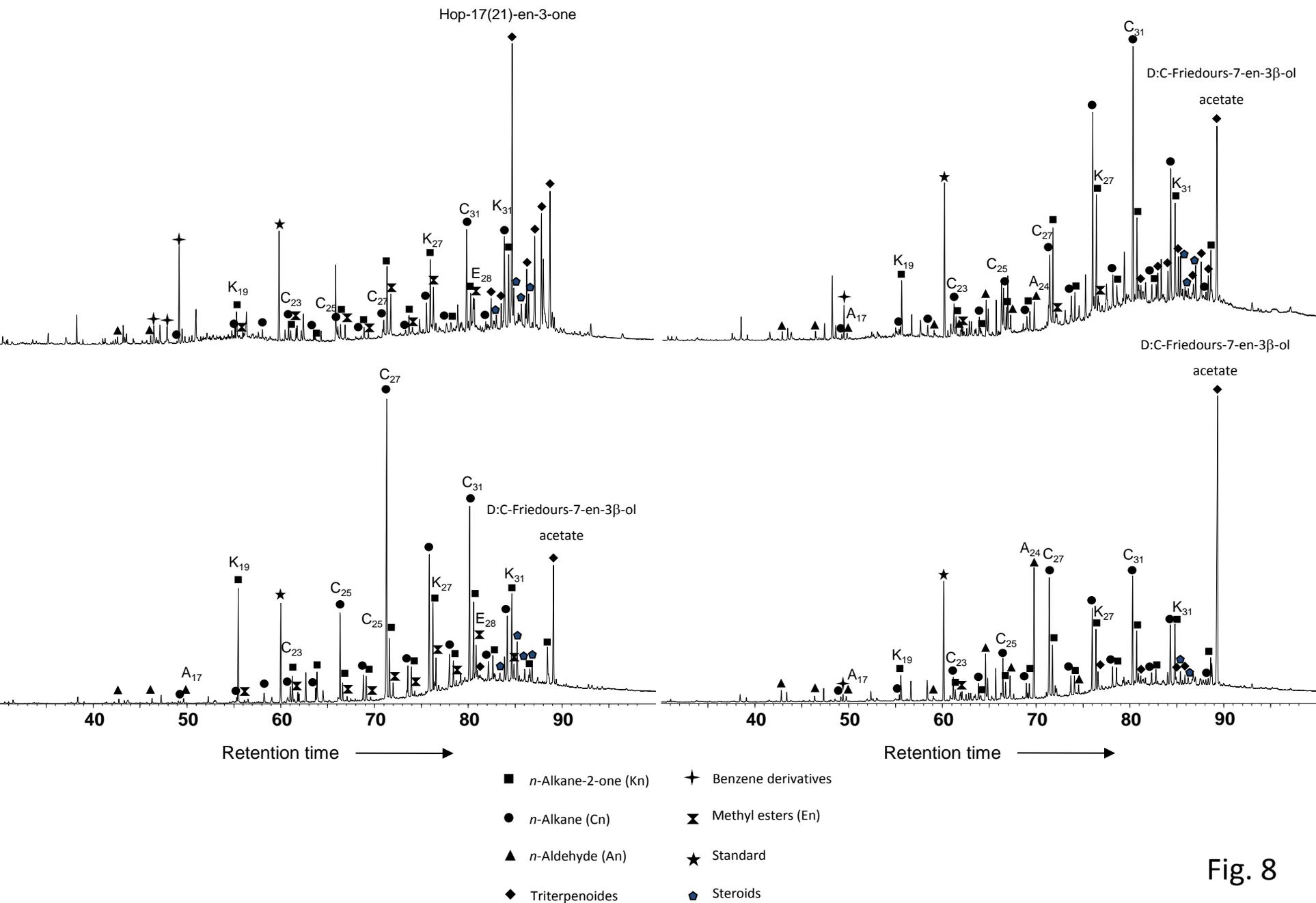
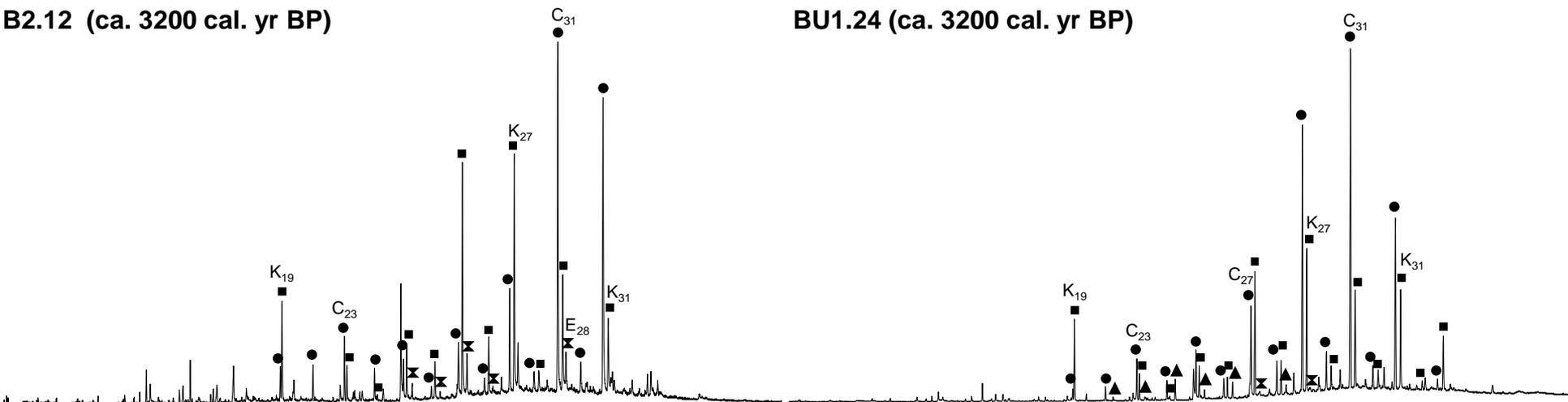


Fig. 8

m/z 57-59

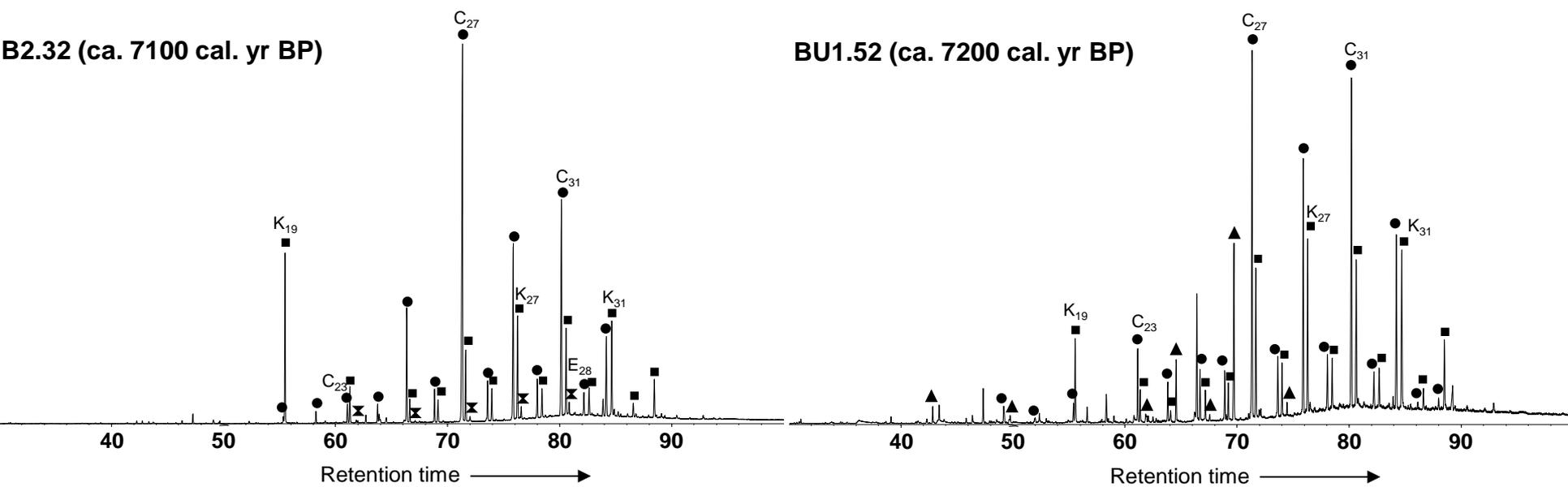
B2.12 (ca. 3200 cal. yr BP)

BU1.24 (ca. 3200 cal. yr BP)



B2.32 (ca. 7100 cal. yr BP)

BU1.52 (ca. 7200 cal. yr BP)



- *n*-Alkane-2-one (Kn)
- ▲ *n*-Aldehyde (An)
- *n*-Alkane (Cn)
- ⌘ Methyl esters (En)

Fig.9

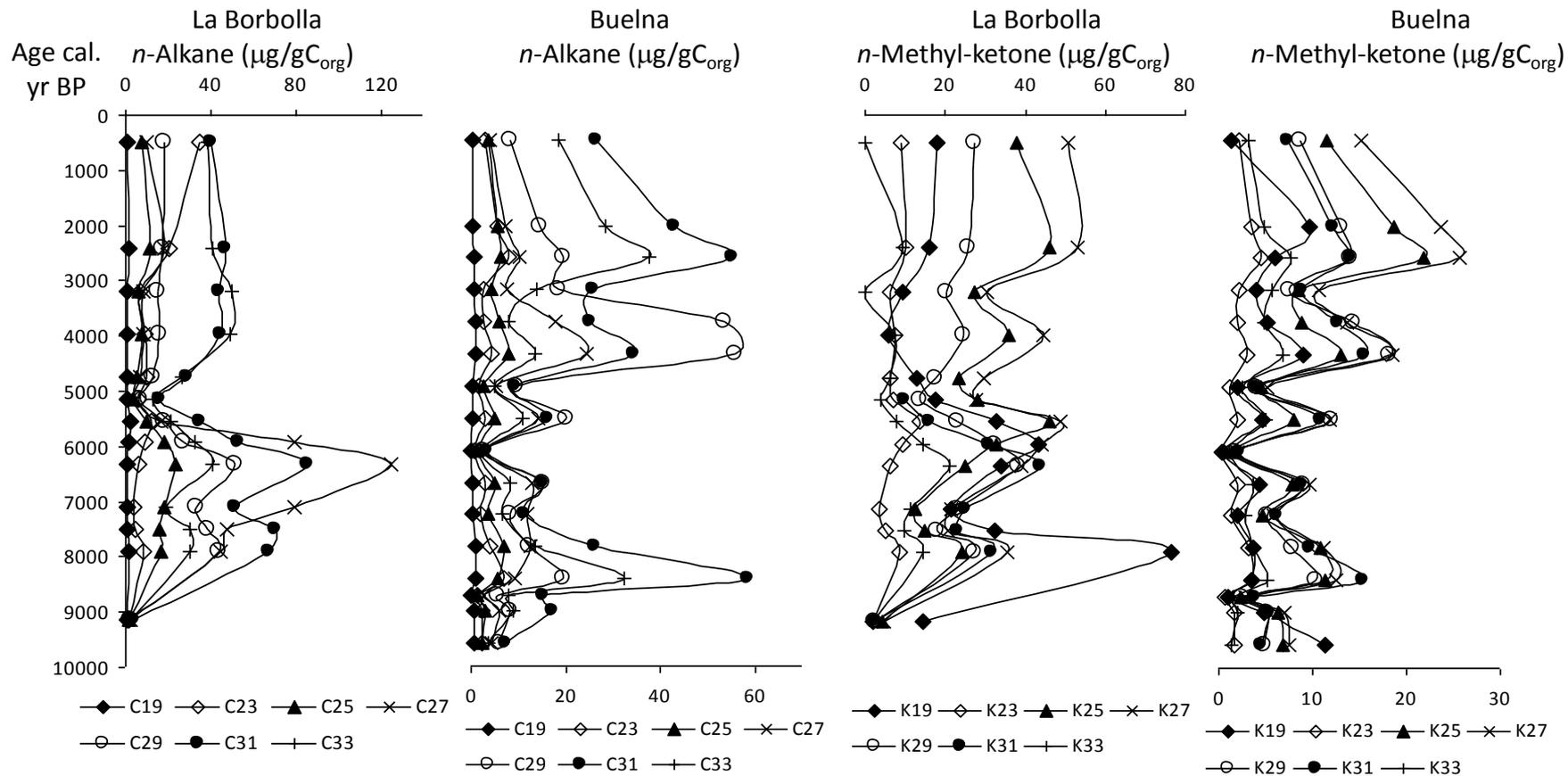


Fig.10

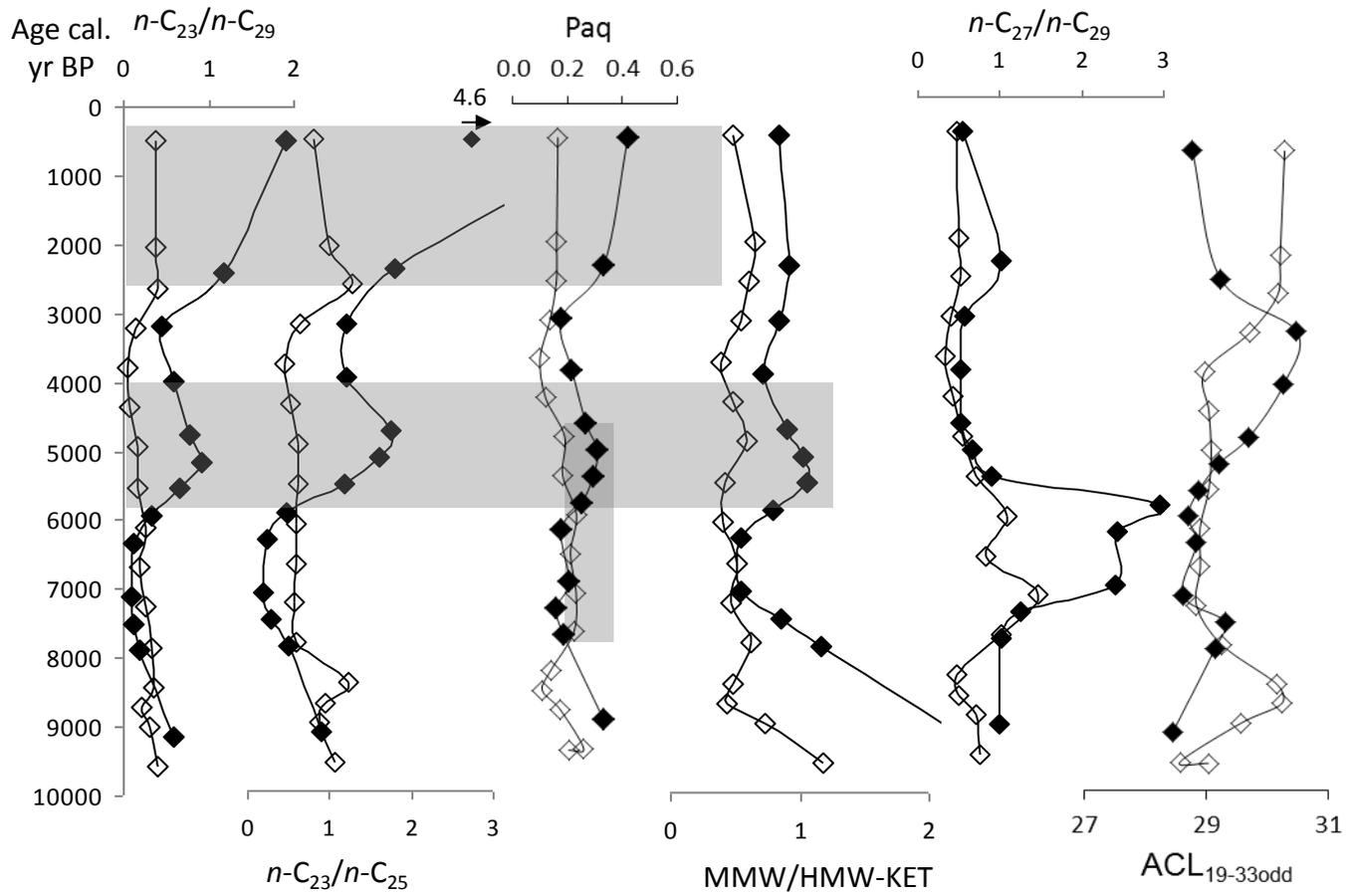


Fig. 11

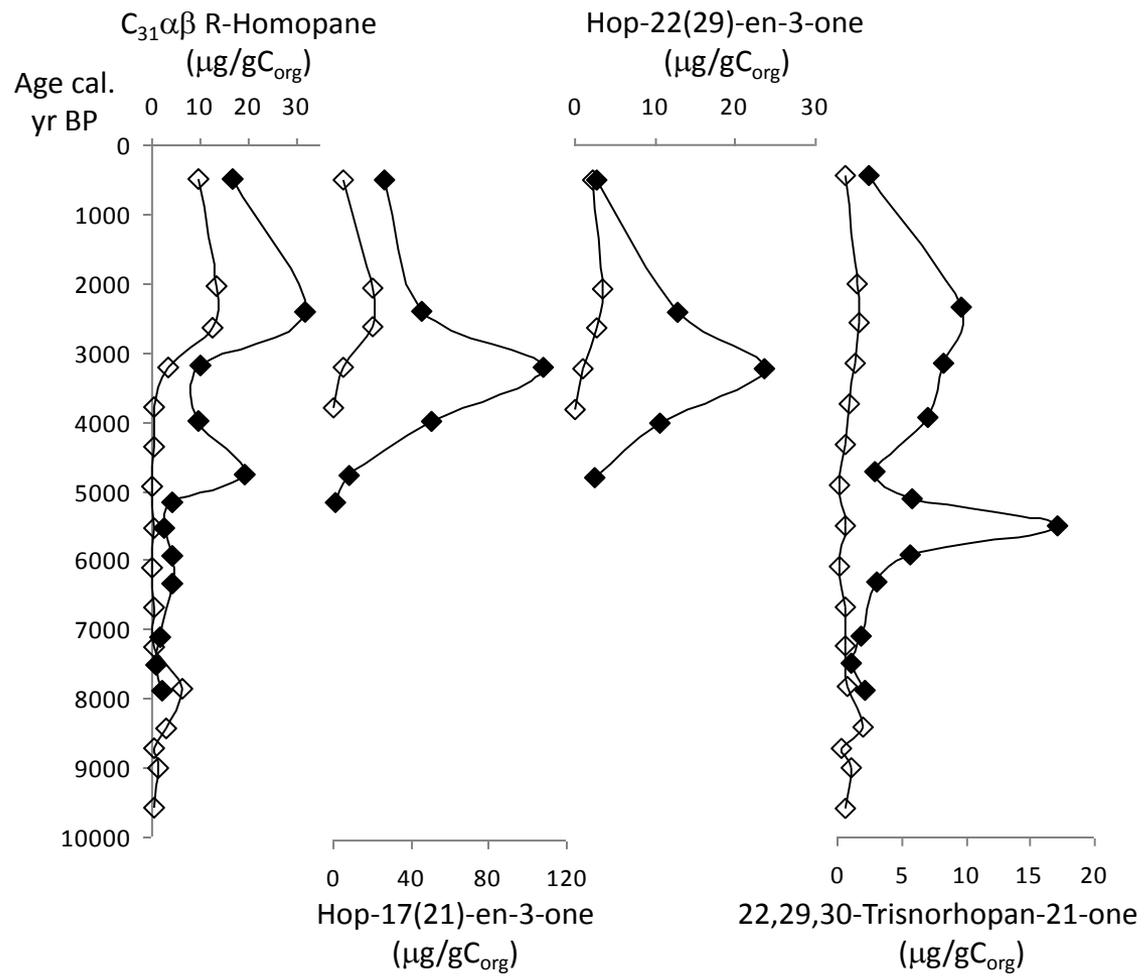
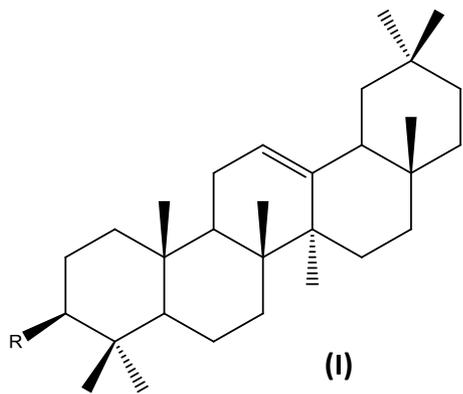
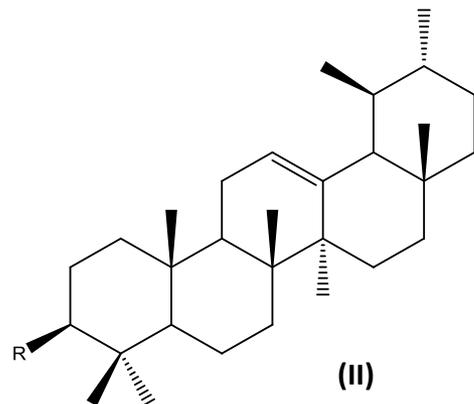


Fig.12



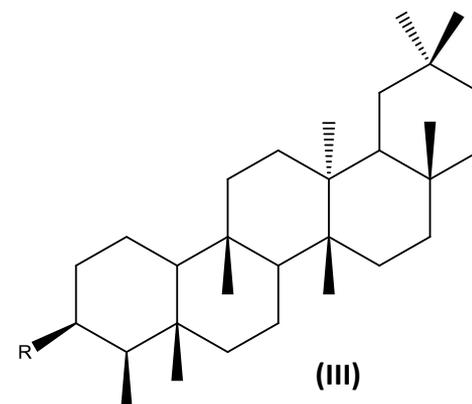
R = :O → β -Amyrenone

R = :OCOCH₃ → β -Amyrin acetate



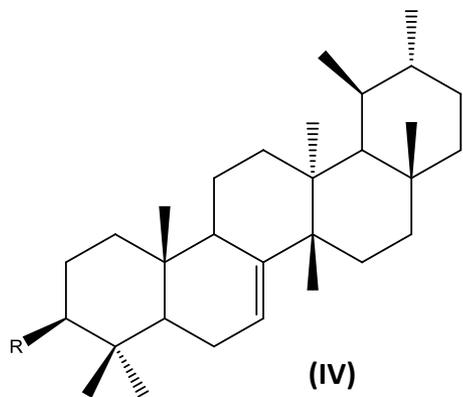
R = :O → α -Amyrenone

R = :OCOCH₃ → α -Amyrin acetate



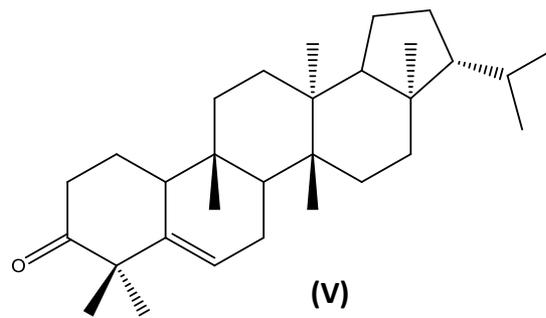
R = :O → Friedelin

R = :OCOCH₃ → Friedelinol acetate

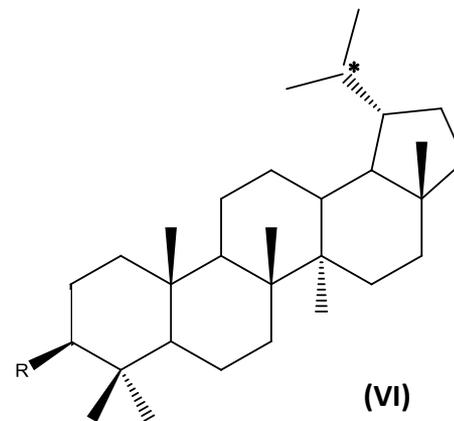


R = :O → Bauerenone

R = :OCOCH₃ → Bauerenyl acetate



Simiarenone



R = :O → Lupanone

R = :OCOCH₃; * 20(29)-en → Lupeyl acetate

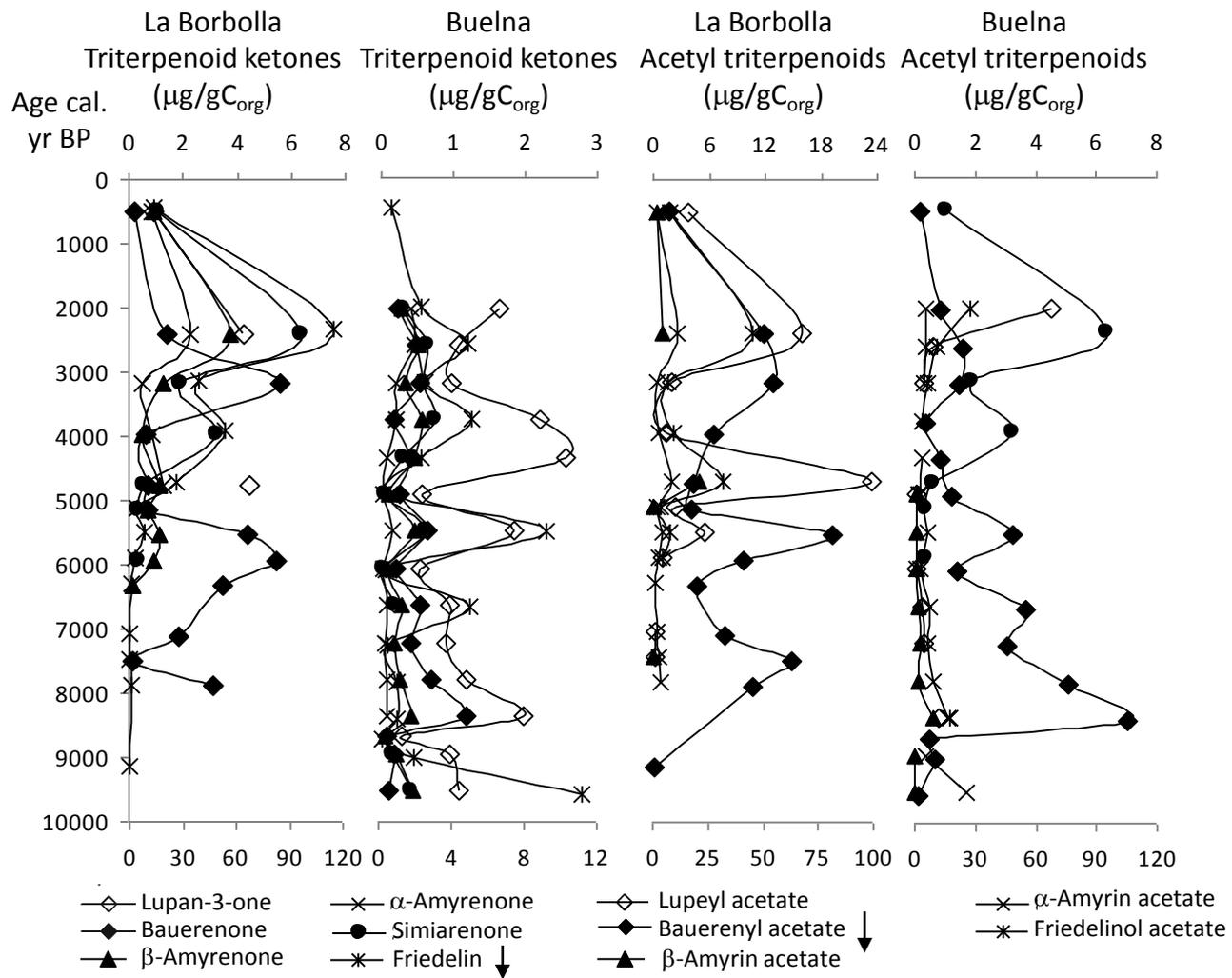


Fig.14

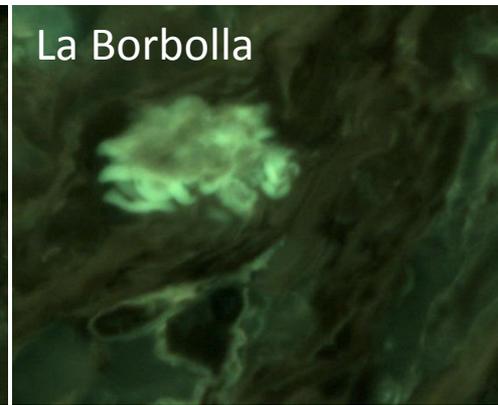
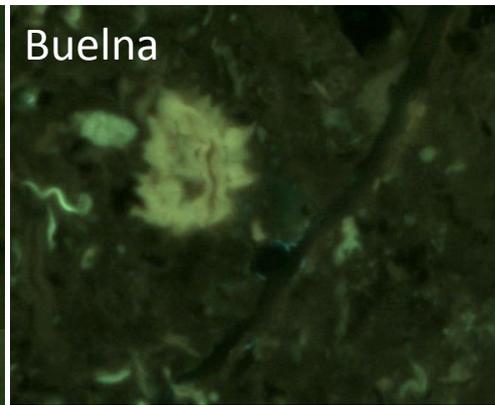
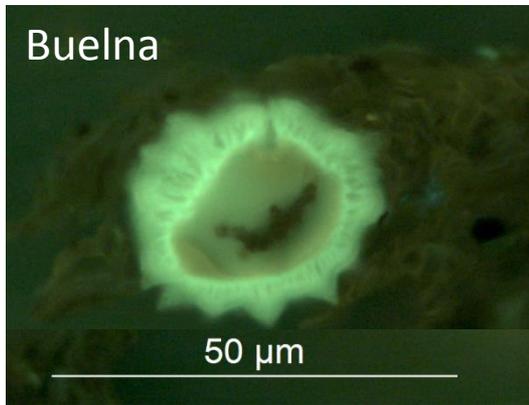


Fig. 15