Lipid biomarkers in Lake Enol (Asturias, Northern Spain): Coupled natural and human induced environmental history ☆

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A B S T R A C T

The lipid content of three cores from Lake Enol (Picos de Europa National Park, Asturias, Northern Spain) was studied. The *n*-alkane profiles indicated a major input from terrigenous plants [predominance of high molecular weight (HMW) alkanes] since ca. 1695 AD to the water body, although the uppermost cm revealed a predominance of organic matter (OM) derived from algae, as the most abundant alkane was C_{17}. Three units revealing different environmental conditions were defined. Unit A (ca. 1695–1860 AD) in the lowermost parts of ENO13-10 (< 12 cm) and ENO13-15 (< 28 cm) was identified and was characterized by higher OM input and evidence of minimal degradation (high CPI values, predominance of HMW *n*-alkanoic acids and good correspondence between the predominant *n*-alkane and *n*-alkanoic acid chains). These findings could be linked to the Little Ice Age, when cold and humid conditions may have favored an increase in total organic carbon (TOC) and *n*-alkane and *n*-alkanoic acid content (greater terrigenous OM in-wash), and may have also reduced bacterial activity. In Unit B (ca. 1860–1980 AD) the lack of correspondence between the *n*-alkane and *n*-alkanoic acid profiles of ENO13-10 (12–4 cm) and ENO13-15 (28–8 cm) suggested a certain preferential microbial synthesis of long chain saturated fatty acids from primary OM and/or bacterial activity, coinciding with a decrease in OM input, which could be linked to the global warming that started in the second half of the 19th century. In ENO13-7 the low OM input (low TOC) was accompanied by some bacterial degradation (predominance of HMW *n*-alkanoic acids but with a bimodal distribution) in the lowermost 16–5 cm. Evidence of considerable phytoplankton productivity and microbial activity was especially significant in Unit C (ca. 1980–2013 AD) identified in the uppermost part of all three cores (5 cm in ENO13-7, 4 cm in ENO13-10 and 8 cm in ENO13-15), coinciding with higher concentrations of *n*-alkanes and *n*-alkanoic acids, which were considered to be linked to warmer and drier conditions, as well as to greater anthropogenic influence in modern times.

Plant sterols, such as β-sitosterol, campesterol and stigmasterol, were significantly present in the cores. In addition, fecal stanols, such as 24-ethylcoprostanol from herbivores, were present, thereby indicating a continuous and significant pollution input derived from these animals since the 17th century, being more important in the last 20 years.

1. Introduction

Lakes are dynamic systems that hold valuable information about hydrogeological and environmental conditions. In addition, high mountain lakes, like Lake Enol, are highly sensitive to environmental change at a regional or a global scale. In this regard, the climatic conditions of such lakes change markedly with altitude, the diluted water reflects subtle changes in the lake chemistry and these water bodies are often protected from direct pollution sources. Nevertheless, despite their remote location, mountain lakes are not always free of anthropogenic influence and in this respect they can be affected by global or regional contamination sources. Their sediments are therefore useful records of paleoenvironmental change and human activity (Smol, 1995; Cohen, 2003).

The following approaches have been used to reconstruct paleoclimate from continental deposits: carbon and oxygen stable isotopes (Dansgaard, 1964; Craig, 1965; Durazzi, 1977), palynology (Follieri et al., 1988; Reille and de Beaulieu, 1990), species association (Carbonel et al., 1988; Wansard et al., 1996; Kashiwaya et al., 2003) and inorganic geochemistry (Smith and Bischoff, 1997; Vegas et al., 2010). In addition, lipid content can also contribute to climate reconstruction from continental records (Meyers and Ishiwatari, 1993; Meyers, 1997, 2003). Change in lipid composition reflects environmental change in lakes, with the variation related to oscillation in local and regional environmental conditions (Bechtel and Schubert, 2009), even during the Holocene, when relatively subtle climatic shifts occurred (cf. Nott et al., 2000; Xie et al., 2004; Zhou et al., 2005).

Interpreting organic geochemical signals is not straightforward. In most cases, OM is a mixture of components from many sources and has a variable degree of preservation. Despite diagenesis and alteration of the original OM during sinking to the lake bottom, sedimentary OM retains key information about its origin and about how it was transported and deposited (Meyers, 2003). Within molecular geochemistry proxies, *n*-alkanes are among the biomarkers most commonly used. They are relatively source-specific and offer the advantage that they are less susceptible to microbial degradation than other OM components because they lack the functional groups that confer reactivity. In addition, they show low water solubility (Prahl and Carpenter, 1984; Meyers et al., 1995). *n*-Alkanoic acids in lake sediments typically originate from multiple sources (algae, aquatic macrophytes, land plants), but are more sensitive to degradation and modification than other types of lipid biomarker (Meyers and Eadie, 1993). However, the *n*-alkanoic acid can content reveal not only the OM source but can also be used to evaluate the degree of preservation, thereby providing information about paleoenvironmental change. The sterol content is also a proxy for the source and diagenetic alteration of OM. The structural diversity of sterols and the stanols derived from them provides information about the origin and diagenetic alteration of OM in lake sediments. The relative amounts of sterols are used to identify the contributions of different types of OM (Meyers, 2003), whereas the stanol/sterol ratio can be a useful proxy for microbial reduction of OM (Wakeham, 1989). Moreover, 5β-stanols are typical of animal feces, thus allowing the identification of fecal pollution (Leeming et al., 1984, 1996; Bethell et al., 1994; Shah et al., 2007).

The northwest of the Iberian Peninsula, in the mid-latitude zone, provides climatic characteristics of interest. The oceanic influence of the area is affected by the winter/summer equilibrium of temperate and sub-tropical components, the latter being responsible for rainfall seasonality. For the purpose of this study, we selected Lake Enol, located within the Picos de Europa National Park, established in 1918. However, socioeconomic transformation during the 20th century, such as change in livestock farming related to dairy specialization, the planting of non-native trees, mining activity and the management practices of the national park, have profoundly altered the catchment and ecology of the lake. Today, the large number of tourists (> 1,500,000 each year) who visit the area also causes a considerable impact on the ecosystem.

Information about long term climate- and/or human-induced environmental changes in protected areas such as the Picos de Europa National Park is crucial for developing effective conservation strategies. As a result of human activities and natural interactions over the last few centuries, it is not easy to discriminate between the influence of climate and anthropogenic disturbance using environmental proxies in lake sediments. Therefore, identification of the main mechanisms responsible for various environmental changes is especially challenging.

Studies have indicated that Lake Enol is responsive to paleoenvironmental change. A sedimentological, geochemical and geomorphological study provided the paleoenvironmental evolution over the last 40 ka BP, with the onset of a depression that occurred during the glacial retreat from 40 to 26 ka BP, followed by deposition of glaciolacustrine sediments (26–18 ka BP), and the onset of organic-rich sedimentation ca. 18 ka ago. A multi-proxy study (sedimentology, physical properties, OC and carbonate content, mineralogy, inorganic geochemical composition, diatom and ostracod assemblages and palynology) conducted by Moreno et al. (2011) provided information about regional humidity and temperature change during the last 13,500 years. These changes included a cold and dry episode (13,500–11,600 cal. years BP), a humid and warmer period (11,600–8700 cal. years BP), a drier climate (8700–4650 cal. years BP) and a return to more humid conditions (4650–2200 cal. years BP). López-Merino et al. (2011) also inferred past climatic conditions and anthropogenic impact over the last 200 years using geochemical and biological (pollen and diatoms) variables in the lake. Ballesteros (2014) used sedimentological and geochemical proxies to study the paleoenvironmental changes in the lake's catchment during the last 400 years. Chao (2014) established the current conditions through a study of hydro-biogeochemical and limnological characteristics, and concluded that livestock was a considerable source of pollution.

Here, we sought to reconstruct the paleoenvironmental evolution of the northern part of the Iberian Peninsula by interpreting the organic geochemistry of sediments retrieved from Lake Enol. We examined three cores covering the last ca. 300 years. They were carefully sampled at high spatial and temporal resolution (1–4 cm intervals, corresponding to ca 5–20 year intervals) in order to study the C and N content of the OM and various biomarkers, mainly *n*-alkanes and *n*-alkanoic acids. In addition, using the sterol content, which complemented existing data in paleoclimate studies, we evaluated the influence of anthropogenic activity.

2. Geographical setting

Lake Enol (438160N, 48590W, 1070 m asl, above sea level) is in the eastern Cantabrian Mountain Range, Northern Spain, within the Picos de Europa National Park. It is an oligotrophic, monomictic, karstic lacustrine system (Velasco et al., 1999), located over carboniferous limestone formations. Formed ca. 38 ka ago, when the glaciers retreated (Moreno et al., 2010), the lake is fed mainly by laminar runoff and receives groundwater input. It has a surface area of 12.2 ha, a maximum depth of 22 m and a catchment of 1.5 km^2. A thick thermocline, between 4 and 10 m depth, develops during the summer: the water column remains mixed from mid-autumn until the beginning of spring. The water temperature is relatively constant (around 7–9 °C) during the entire turnover period.

The study area is characterized by a mountainous oceanic climate, with high annual precipitation (2250 mm) and a mean annual temperature of 13 °C (Meléndez Asensio et al., 2002).

3. Material and methods

A number of cores were drilled with an Uwitec® gravity corer during a sampling campaign in 2013. They were split longitudinally into two symmetrical halves, photographed and stored frozen at −4 °C until analysis. We selected three cores from different depths, in a transect from the northeast margin (ENO13-7) to the deepest part (ENO13-15). Core ENO13-7 (16 cm long) was retrieved 4.3 m below water level, in the shelf located in the northeast part of the lake, whereas ENO13-10 (28 cm) and ENO13-15

(57 cm) were drilled at depths of 12 and 20 m, respectively, both below the thermocline (Fig. 1).

The first 13 cm (17–4 cm) of ENO13-7 were characterized by laminated sediments with abundant calcite (Fig. 1). At the top of this interval there was a black thin irregular boundary with abundant plant debris. The uppermost 4 cm consisted of massive gray sandy silts with charophyte remains. The bottom of ENO13-10 (28–23 cm) consisted of brown sandy silt with dispersed bivalve shells (Fig. 1), followed by gray silt (22–16 cm) with bivalves and gray argillaceous silt with scarce plant debris (16–0 cm). The lower part (58–33 cm) of ENO13-15 was characterized by the presence of gray silt with diffuse bands, with five intervals containing bivalve

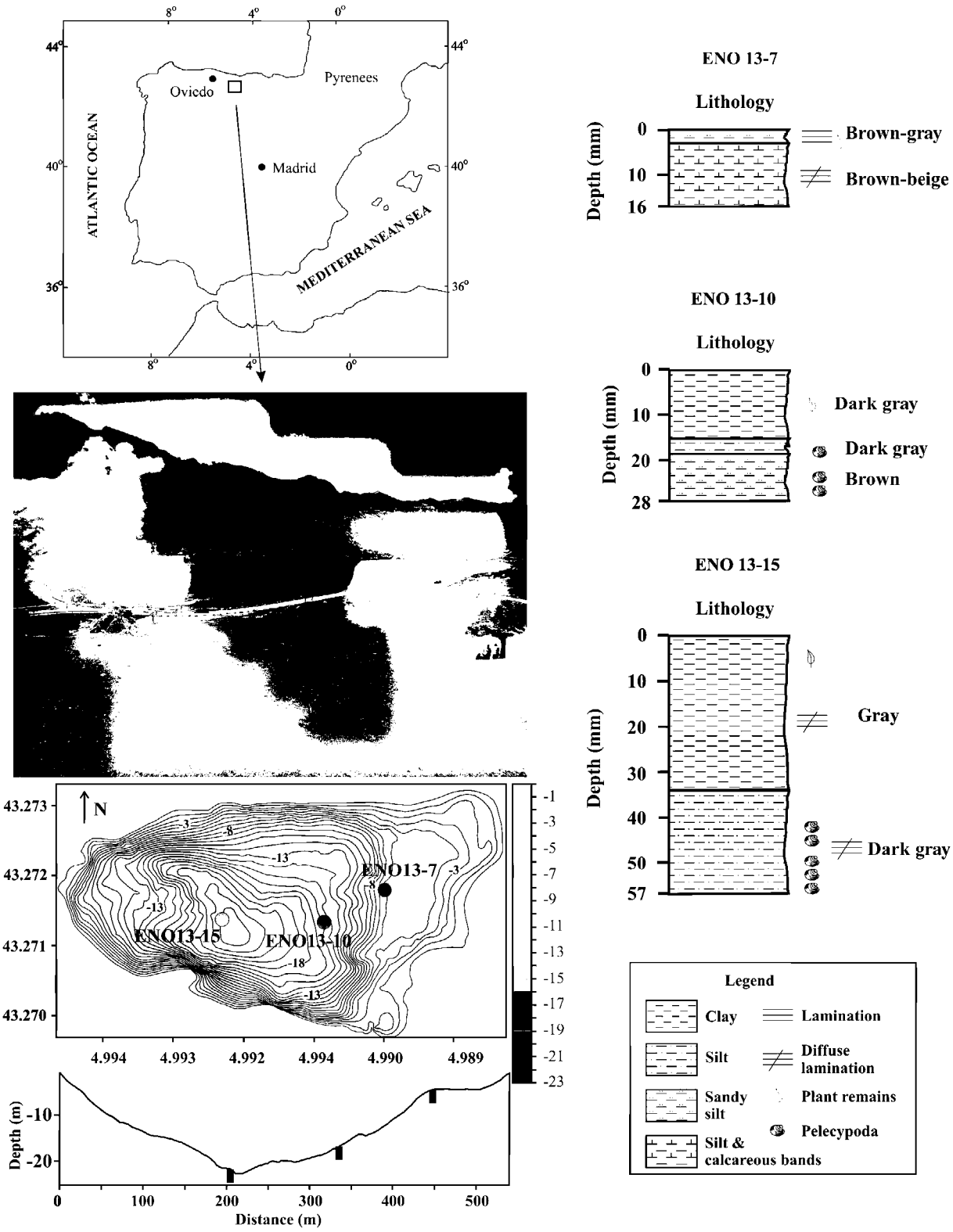

Fig. 1. Location of Lake Enol with the position of the cores, as well as stratigraphic sections of the cores.

shells. The rest of the core (33–0 cm) was made of argillaceous silt with diffuse lamination and abundant diatoms (Fig. 1).

Samples were taken for total OC (TOC) and total nitrogen (TN) content and lipid analysis. Additionally, living terrigenous plants, aquatic macrophytes and algae were selected for lipid analysis.

3.1. TOC and TN

A total of 65 samples were taken at 1 cm intervals along ENO13-7 and ENO13-10, and at 2–4 cm interval in ENO13-15. The samples were homogenized with a mortar and pestle. TOC concentration was measured using a LECO SC 144DR instrument in the Laboratories of the Instituto Pirenaico de Ecología (IPE-CSIC, Zaragoza) after removal of inorganic carbon with HCl (1:1; Morellón et al., 2008). TN was measured at the Estación Experimental de Aula Dei (EEAD-CSIC, Zaragoza) using a VARIOMAX CN system.

3.2. Lipid extraction and analysis (biomarker analysis)

A set of 65 samples was used for biomarker analysis: 17 samples from ENO13-7 taken at 1 cm intervals, 29 from ENO13-10 taken at 1 cm intervals and 22 from ENO13-15 taken at 2 cm intervals over the uppermost 26 cm and at 4 cm intervals over the lowermost part of the core (down to 57 cm).

Likewise, seven living plants from the lake and its surroundings were collected, including algae (green algae and *Chara* sp.), aquatic macrophytes (*Potamogeton* sp.), sedge (*Eriophorum* sp.) and two grasses belonging to Gramineae.

Between 0.34 and 5.01 g dried sample (50 °C, 24 h) were ground, and biomarkers were extracted with an accelerated solvent extractor (Dionex ASE 200). Free lipids were extracted with dichloromethane (DCM)/MeOH (2:1) at 1500 psi and 175 °C. The heating phase was 8 min and the static extraction time 5 min.

The extract was concentrated using a rotary evaporator. Prior to analysis using gas chromatography–mass spectrometry (GC–MS), acidic and polar fractions were methylated with trimethylsilyldiazomethane and silylated with a mixture of *N,O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) and pyridine at 70 °C for 2 h. Samples were injected into an HP 6890 gas chromatograph equipped with a selective mass detector (HP 5973) and an ATM-5 column (250 x 0.25 mm; 0.20 μm). Prior to analysis, an internal standard (decafluorobiphenyl) with a concentration of 1 μg/l was added to the extract in order to quantify the compounds. He was the carrier gas and the oven temperature was programmed from 60 to 300 °C (held 20 min) at 6 °C/min and the injector was maintained at 275 °C. Components were assigned with the Data Analysis program and the Wiley Library; *n*-alkane distributions were obtained from the *m/z* 57 chromatograms (base peak), the *n*-alkanoic acids from *m/z* 74, and sterols and stanols from *m/z* 129 and 215.

3.3. Elemental proxies

3.3.1. TOC

The concentration of TOC is a fundamental proxy for describing the abundance of OM in sediments. The proportion (%) represents OM that escaped remineralization during sedimentation. It is influenced by both the initial production of biomass and subsequent degree of degradation, and integrates various sources of OM (Meyers, 2003).

3.3.2. C/N ratio

C/N is a proxy for protein content (Müller and Mathesius, 1999). Proteins account for the greatest part of the OM in organisms, together with lipids and carbohydrates, but the proportional abundance varies. Thus, C/N provides information about the proportion of algal and land plant contribution to OM (Prahl et al., 1980; Meyers, 1994; Kaushal and Bindford, 1999). OM derived from lake algae has atomic C/N values typically between 4 and 10, whereas vascular plants usually have values of 20 and greater (Ertel and Hedges, 1985; Hedges et al., 1986; Meyers, 1994). Atomic C/N values of 12–17 suggest a mixture of algal and vascular plant input.

3.4. Biomarker proxies

3.4.1. n-Alkanes

n-Alkane profiles were used to distinguish the diverse sources of OM, namely algal, aquatic or terrigenous. Each sample can be characterized by the predominant *n*-alkane chain length. Their distribution in phytoplankton and algae is dominated by low MW *n*-alkanes, maximizing at C_{17} (Gelpi et al., 1970; Blumer et al., 1971; Cranwell et al., 1987). Submerged/floating macrophytes maximize at C_{21}, C_{23} and C_{25} (Cranwell, 1984; Ogura et al., 1990; Viso et al., 1993), while emergent macrophytes have a composition similar to that of terrestrial plants, peaking at C_{27} and C_{29} (Cranwell, 1984). Terrigenous plants contain a high proportion of HMW *n*-alkanes (C_{27}, C_{29} and C_{31}) in their epicuticular wax (Eglinton and Hamilton, 1963, 1967; Eglinton and Calvin, 1967; Cranwell et al., 1987; Rieley et al., 1991; Nott et al., 2000; Pancost et al., 2002). Deciduous trees typically maximize at C_{27}, whereas in marsh plants and possibly grasses C_{31} is dominant (Cranwell et al., 1987; Schwark et al., 2002; Ortiz et al., 2004, 2011). However, data from a broad survey of modern plants show that *n*-alkane chain length distributions are highly variable within plant groups, and chemotaxonomic distinction between grasses and woody plants is difficult, with the exception of aquatic plants and *Sphagnum* moss (Bush and McInerney, 2013). In contrast, changes in chain length distribution are likely to be a result of temperature and/or humidity conditions (Bush and McInerney, 2015).

A number of indices calculated using *n*-alkane abundance are used to discriminate the OM sources in lake sediments. Thus, the use of the average chain length (ACL; Poynter, 1989), calculated as $[(C_i \times i + C_{i+1} \times (i+1) + C_{i+2} \times (i+2) \ldots + C_n \times n))/(\Sigma\ C_{n+1} + C_{n+2} + \ldots + C_n)$, with $i = 13$, $n = 33$], is a good proxy for distinguishing between the predominance of low vs. high MW *n*-alkanes (Pancost et al., 2002; Rommerskirschen et al., 2003).

The carbon preference index (CPI; Bray and Evans, 1961), calculated as $1/2\ [(\Sigma\ C_i + C_{i+2} + \ldots C_{i+8})/(\Sigma\ C_{i-1} + C_{i+1} + \ldots + C_{i+7}) + (\Sigma\ C_i + C_{i+2} + \ldots + C_{i+8})/(\Sigma\ C_{i+1} + C_{i+3} + \ldots + C_{i+9})]$, with $i = 25$, is commonly used to discriminate between mature and immature OM in sediments, because it indicates the predominance of odd/even numbered *n*-alkanes of a certain chain length range. The index was also used by Zheng et al. (2007) to discriminate between paleoenvironmental conditions, as the *n*-alkanes from the cuticular wax of higher plants have a strong odd predominance and CPI values > 5. In contrast, *n*-alkanes from bacteria and algae have low CPI values of ca. 1 (Cranwell et al., 1987).

Silliman et al. (1996) defined the terrigenous/aquatic ratio, calculated as $(C_{31} + C_{29} + C_{27})/(C_{15} + C_{17} + C_{19})$, to distinguish between land plants and algal input.

The *Paq* index, calculated as $(C_{23} + C_{25})/(C_{23} + C_{25} + C_{29} + C_{31})$ ratio (Ficken et al., 2000), was postulated to reflect the relative contribution of emergent and submerged/floating aquatic macrophytes, which typically maximize at C_{23} and C_{25}, and that of terrigenous plants. According to Ficken et al. (2000), values < 0.1 are linked to a dominant contribution from land plants, while values between 0.1 and 0.4 reflect a significant input from emergent macrophytes. Values > 0.4 are typical in sediments with a major *n*-alkane input from submerged/floating macrophytes.

3.4.2. n-Alkanoic acids

Like aliphatic hydrocarbons, *n*-alkanoic acids in lake sediments come from OM derived from plants and micro-organisms. Long

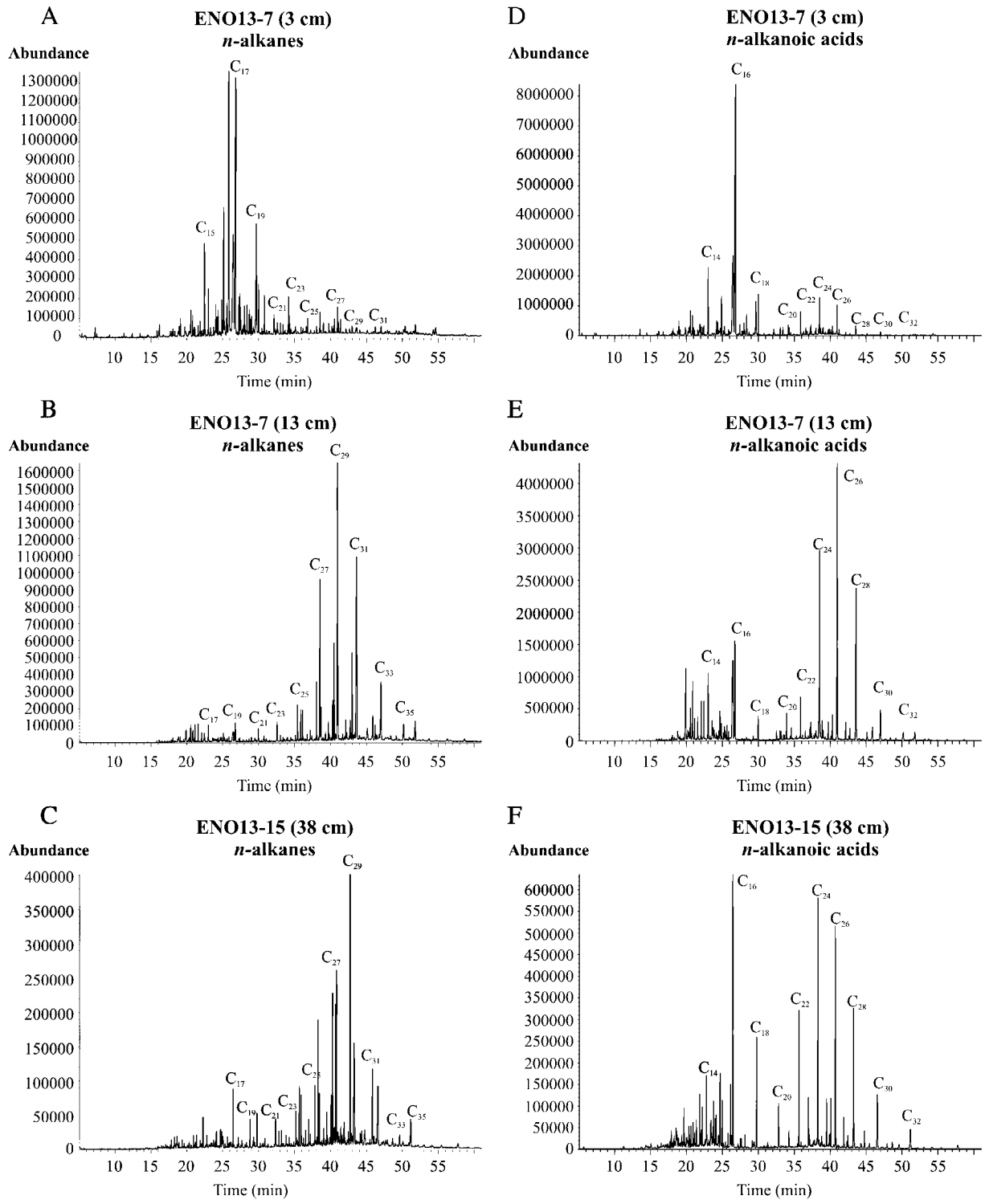

Fig. 2. Typical chromatograms of *n*-alkanes from selected samples in Lake Enol cores. Samples maximized: (A) at low MW *n*-alkanes (C_{17}); (B and C) at HMW *n*-alkanes (C_{31}); (D) at low MW *n*-alkanoic acids (C_{16}); (E) at a bimodal distribution, but maximizing at HMW *n*-alkanoic acids (C_{26}); (F) at a bimodal distribution between C_{16} and HMW *n*-alkanoic acids.

chain *n*-alkanoic acids (C_{24} to C_{30}) with a predominance of even numbers are major components of the wax of land plant leaves, flowers and pollen (Eglinton and Calvin, 1967; Rieley et al., 1991; Meyers and Ishiwatari, 1993), while algae and bacteria maximize at shorter chain length, from C_{12} to C_{18} (Eglinton and Calvin, 1967; Cranwell et al., 1987).

The ratio of terrigenous to aquatic fatty acids (TAR_{FA}; Meyers, 1997) is a measure of the sum of long chain to short chain even saturated FAs, calculated as $(C_{28} + C_{26} + C_{24})/(C_{18} + C_{16} + C_{14})$, and is used to distinguish between land plant and algal sources. However, selective degradation and diagenetic processes commonly overprint *n*-alkanoic acid distributions. Short chain acids are often

preferentially degraded by microbes during early diagenesis (Cranwell, 1974, 1976; Haddad et al., 1992; Ho and Meyers, 1994) and they produce higher TAR_{FA} values (Tenzer et al., 1999). On the other hand, the microbial synthesis of secondary FAs from primary OM produces short chain components (Kawamura et al., 1987) and can depress TAR_{FA} values.

3.4.3. Sterols and stanols

These components, especially β-sitosterol, stigmasterol and campesterol, have been reported to be good paleoclimatic indicators, as they are typical constituents of higher plants (Goad and Goodwin, 1972; Goad, 1991; Pancost et al., 2002), algae, bacteria and diatoms (Volkman, 1986). Sterols occur in animals and humans and can provide information about their influence on the environment (Leeming and Nichols, 1996; Leeming et al., 1996; Bull et al., 2002). Thus, the structural diversity of sterols, and the stanols derived from them, provides information about the sources and diagenetic alteration of OM.

5α-Stanols are produced through selective natural microbial reduction of their respective Δ^5-sterols (Wakeham, 1989; Lehtonen and Ketola, 1993; Jaffé et al., 1996; Bull et al., 1999, 2002), and only a small fraction of the latter is transformed to 5β-stanols (Gaskell and Eglinton, 1976; Grimalt et al., 1990). Thus, the 5α-stanol/sterol ratio is used as a proxy for diagenetic processes in recent marine sediments (Wakeham, 1989; Ranjan et al., 2015), lakes (Jaffé et al., 1996) and peat bogs (Andersson and Meyers, 2012; Routh et al., 2014).

5β-Stanols are commonly formed as reduction products of cholesterol and the higher MW counterparts (campesterol, sitosterol and stigmasterol) in the intestinal tracts of most mammals (Bull et al., 2002) which can accumulate in sediments. Cholesterol, a typical sterol in animals, although also found in some microalgae (Volkman et al., 1999), is converted to 5β-coprostanol in the human digestive track (Murtaugh and Bunch, 1967; Leeming et al., 1984; Bethell et al., 1994; Shah et al., 2007). However, this compound is relatively less abundant in the feces of other animals (Leeming et al., 1996; Mudge and Morrison, 2010). Small amounts of coprostanol can be generated from cholesterol in anaerobic sediments (Nishimura and Koyama, 1977; Müller et al., 1979; Mudge and Gwyn Lintern, 1999).

In contrast, herbivores tend to produce 24-ethylcoprostanol from β-sitosterol (abundant in plants). Likewise, β-sitosterol is also found in herbivore feces (Leeming et al., 1996). Thus, using the sterol content, and specifically the type of coprostanol, human input can be distinguished from that of herbivores (Leeming and Nichols, 1996; Leeming et al., 1996).

4. Results

4.1. Chronology

The chronology of ENO13-15 was obtained using the sedimentation rate of two cores drilled close to it (ENO13-9, and ENO 07-1A-1M), following Casas et al. (2015). In this approach, $^{239,240}Pu$ dating of ENO13-9, drilled in the deepest section of the lake, coincided with the chronology of ENO07-1A-1M dated through ^{210}Pb (López-Merino et al., 2011). The cores ENO13-15, ENO13-9 and ENO07-1A-1M had similar sedimentological and geochemical characteristics (TOC and TC; cf. Ballesteros, 2014; Casas et al., 2015), the bottom of ENO13-15 (57 cm) being dated at 1695 AD (with a sedimentation rate of 1.8 mm/year).

Based on the geochemical proxies presented here, ENO13-15, ENO13-10 and ENO13-7 were correlated mainly according to TOC, C/N and concentration of *n*-alkane and *n*-alkanoic acid profiles (Fig. 3–5), i.e. higher values of the above indices between 57 and 28 cm in ENO13-15 were correlated with those from 28 to 12 cm in ENO13-10, whereas low values in 28–8 cm in ENO13-15 were comparable with 12–4 cm in ENO13-10 and 17–4 cm in ENO13-7. Of note, the values of the indices showed noticeable increases in the uppermost cm of the cores. The sedimentological characteristics of ENO13-15 and ENO13-10 were similar. In contrast, the sedimentology of ENO13-7 differed from that of the other two cores, showing laminated sediments with abundant calcite between 17 and 4 cm. This difference was attributed to local environmental differences, ENO13-7 being above the thermocline in a platform 4.3 m below the water level, and ENO13-15 and ENO13-10 below it.

4.2. TOC

TOC values < 3.0% were found in sediments from 16 to 5 cm of ENO13-7, in which laminated sediments with calcite were dominant. In contrast, higher values (up to 4.4%) were found in the silt of the upper 4 cm of this core. TOC values in ENO13-10 were > 4.0% from 28 to 12 cm and in the uppermost 4 cm but less than this between 12 and 4 cm. The maximum value was ca. 7.3% at 17 cm and the minimum 3.2% at 8 cm. In ENO13-15, TOC was ca 8.0% in the lower 57–40 cm, with a continuous decrease to 3.0% until the uppermost 6 cm, in which values tended to increase up to ca. 6.5%.

4.3. C/N

Atomic C/N values oscillated between 16.9 and 12.8 in the lower interval of ENO13-7 (16–5 cm). In contrast, they decreased to 5.8 in the uppermost 4 cm. In ENO13-10, values varied between 15.22 (top) and 10.11 (10 cm), with higher values from 28 to 12 cm. ENO13-15 registered values > 10.0 between 57 and 40 cm, whereas in the upper 40 cm they decreased, varying between 8.5 and 10.5.

4.4. Lipid biomarkers in plants

In order to discern the origin of *n*-alkanes in the sediments, we analyzed the lipid content of living plants in the surroundings and from the lake. Green algae and *Chara* sp. both maximized at C_{17} and the aquatic macrophyte *Potamogeton* sp. at C_{25}. C_{29} and C_{31} were predominant in Gramineae and *Eriophorum* sp. (Table 1).

4.5. Lipid biomarkers in cores

4.5.1. n-Alkanes

Typical chromatograms of *n*-alkanes from selected samples are shown in Fig. 2. All the samples from the three cores showed an odd carbon number predominance, with a chain length distribution ranging mainly from C_{15} or C_{17} to C_{31} or C_{33}, maximizing either at low MW (C_{17} or C_{19}; Fig. 2A) or at C_{29} and C_{31} (Fig. 2B and C), the former group being clearly predominant in the uppermost 4 cm of ENO13-7 (Fig. 3). A bimodal distribution was detected in samples from the uppermost cm of the cores.

The profiles of the various indexes related to the *n*-alkane content, namely *n*-alkane predominant chain, ACL, CPI, terrigenous/aquatic ratio of hydrocarbons (TAR_{HC}) and the aquatic macrophyte proxy (*Paq*), are shown in Figs. 3–5.

4.5.1.1. Core ENO13-7. The predominant *n*-alkane chain varied between C_{17} and C_{29} or C_{31}. It was possible to identify two phases (Fig. 3), namely a period with a clear C_{31} dominance from the bottom (16 cm) to 5 cm, with only two samples (14 and 5 cm) in which C_{29} was the most abundant *n*-alkane, and C_{17} dominance from 4 cm to the top, although with a bimodal distribution of *n*-alkanes. The ACL also showed two marked intervals (Fig. 4), with

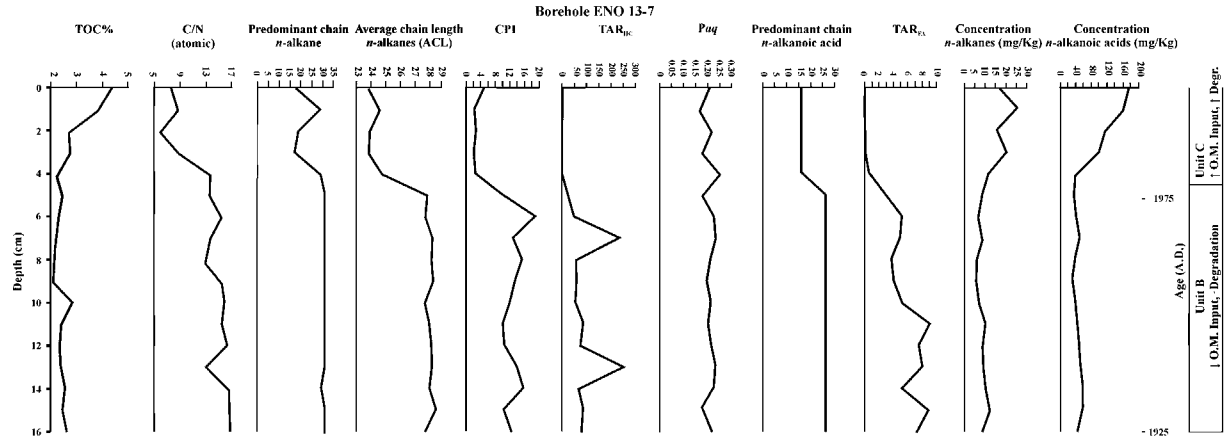

Fig. 3. Profiles of TOC (%), C/N (atomic), predominant *n*-alkane chain, ACL, TAR_{HC}, *Paq* index, predominant *n*-alkanoic acid chain, TAR_{FA} and concentration of *n*-alkanes and *n*-alkanoic acids in core ENO13-7. A tentatively chronological scale was included based on the correlation of geochemical proxies between ENO13-7 and ENO13-15.

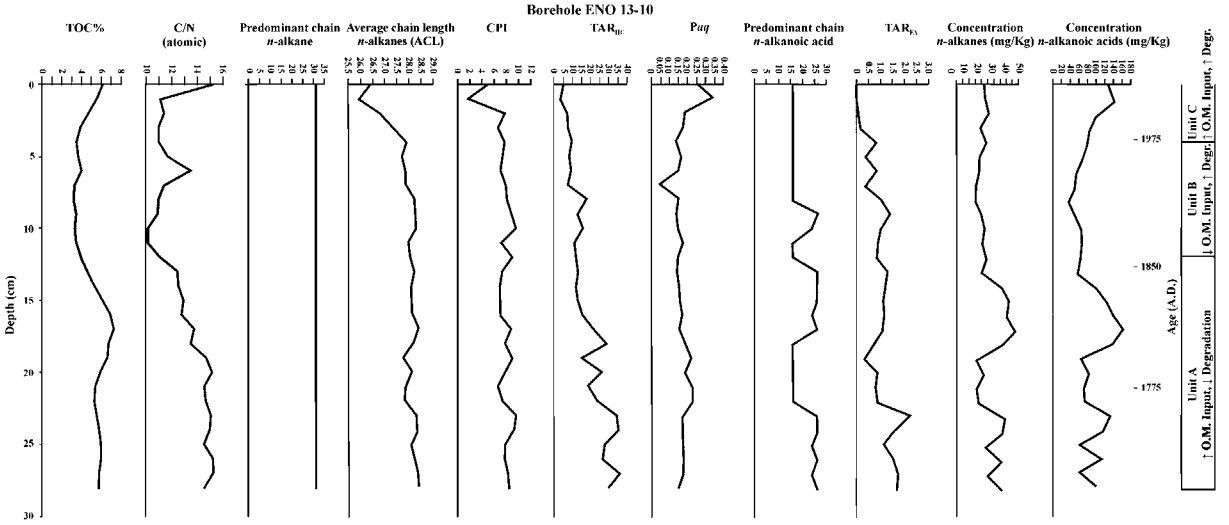

Fig. 4. Profiles of TOC (%), C/N (atomic), predominant *n*-alkane chain, ACL, TAR_{HC}, *Paq* index, predominant *n*-alkanoic acid chain, TAR_{FA} and concentration of *n*-alkanes and *n*-alkanoic acids in core ENO13-10. A tentative chronological scale is included based on the correlation of geochemical proxies between ENO13-7 and ENO13-15.

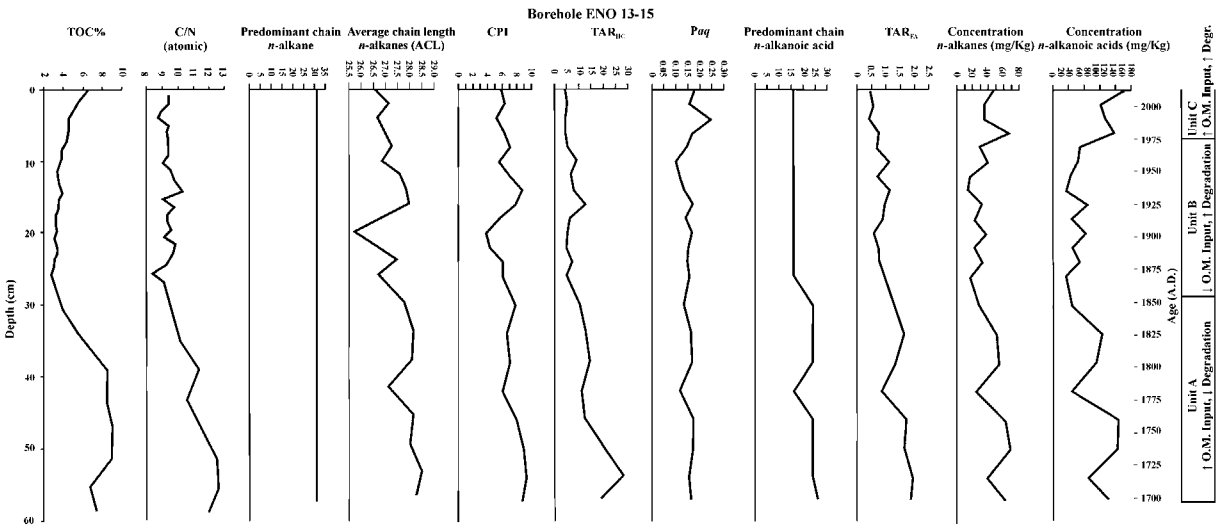

Fig. 5. Profiles of TOC (%), C/N (atomic), predominant *n*-alkane chain, ACL, TAR_{HC}, *Paq* index, predominant *n*-alkanoic acid chain, TAR_{FA}, and concentration of *n*-alkanes and *n*-alkanoic acids in core ENO13-15.

Table 1
Concentration (n.d., not detected) of *n*-alkanes in plants from Lake Enol (highest values in bold).

Species	Alkane C_{no} (μg/g dry plant matter)														
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Green alga	**2.41**	0.37	0.18	n.d.	n.d.	0.24	0.16	0.07	0.32	0.10	0.40	0.20	0.53	0.09	0.48
Chara sp.	1.25	0.34	**2.74**	n.d.	n.d.	0.43	0.45	0.28	1.75	0.39	1.52	0.68	1.00	0.39	1.19
Potamogeton sp.	1.09	n.d.	3.68	n.d.	n.d.	0.99	6.78	2.32	**12.41**	2.32	8.34	1.59	12.02	1.45	6.37
Juncus effusus	0.10	0.12	1.09	n.d.	n.d.	0.28	2.18	0.39	2.58	0.31	4.20	0.70	**6.24**	0.38	2.72
Carex sp.	n.d.	n.d.	5.96	n.d.	n.d.	1.65	3.69	1.76	15.12	1.12	15.61	2.63	**16.25**	1.51	8.50
Gramineae 1	n.d.	n.d.	0.67	n.d.	n.d.	0.06	0.51	0.13	1.07	0.12	1.89	0.25	**2.41**	0.18	1.17
Gramineae 2	n.d.	n.d.	1.35	n.d.	n.d.	0.43	7.83	0.85	9.24	0.99	17.92	5.39	**65.14**	3.04	14.48

values varying between ca. 28.0 in the lowermost 16–5 cm (coinciding with a dominance of long chain *n*-alkanes) and ca. 24.5 in the uppermost 4 cm (together with a predominance of short chain *n*-alkanes).

CPI values along the ENO13-7 sequence showed oscillations, with values > 10 in the lower part of the record (16–5 cm). In contrast, the values were around 2.0 in the uppermost 4 cm. TAR_{HC} values were > 5 in the lowermost interval, with maxima of 254 and 239 at 13 and 7 cm, respectively, whereas values were around 1.0 in the uppermost 5 cm. In contrast to the previous indices, *Paq* values were virtually unchanged and remained between 0.15 and 0.25.

The sum of the concentration of *n*-alkanes ranged between 5 and 12 mg/kg (Fig. 3), with the exception of the uppermost 4 cm, which showed higher values, reaching 25 mg/kg at 1 cm.

4.5.1.2. Core ENO13-10. C_{31} *n*-alkane was predominant along the whole record in ENO13-10 (Fig. 4) and the ACL index did not show significant oscillation, with values ranging between 27.3 and 28.4, although in the uppermost 2 cm they were slightly lower (26.8–25.9).

CPI values were > 6, with a maximum of 9.6 (at 23 and 10 cm), except in the uppermost 2 cm, which registered lower values. *Paq* values varied between 0.04 (7 cm) and 0.33 (1 cm), with the highest ones in the uppermost 2 cm. TAR_{HC} values showed a decrease from the bottom to top of the core, but were always > 3.7. The *n*-alkane concentration showed some oscillation, with intervals with higher values at 28–23 and 18–14 cm (36–47 mg/kg), alternating with others with a concentration between 15 and 24 mg/kg.

4.5.1.3. Core ENO13-15. The predominant *n*-alkane was C_{31} along the whole record (Fig. 5). The ACL index showed slight oscillation between 25.7 (20 cm) and 28.5 (54 cm), with a slightly decreasing trend from the bottom to the top of the core. *Paq* values were also homogenous, varying only between 0.09 (10 cm) and 0.24 (4 cm). TAR_{HC} values were > 4, but showed a decreasing trend from bottom to top.

Two intervals with higher *n*-alkane concentration were identified between 57 and 34 cm, and from 6 cm to the top, reaching a maximum value of 67 mg/kg (50 cm) and alternating with samples with values ranging from 39 to 13 mg/kg (Fig. 2).

4.5.2. n-Alkanoic acids

All samples showed a strong predominance of even numbered acids, ranging from C_{12} to C_{32}, and maximizing at C_{16} (Fig. 2D), C_{24} and C_{26} (Fig. 2E), sometimes with a bimodal distribution (Fig. 2E and F). The profiles of the various indexes related to the *n*-alkane content in the three cores, namely the predominant *n*-alkanoic chain and the TAR_{FA}, are shown in Figs. 3–5.

4.5.2.1. Core ENO13-7. Coinciding with the two intervals identified from the *n*-alkane indexes, the predominant *n*-alkanoic acid was C_{26}, found from 16 to 5 cm, while C_{16} was the most abundant homolog in the uppermost 4 cm (Fig. 3). Likewise, TAR_{FA} values were > 3 in the lowermost 16–5 cm, falling to < 0.5 from 4 cm to the top.

The *n*-alkanoic acid concentration was higher than that of *n*-alkanes, with values between 31 and 59 mg/kg, reaching the highest concentration in the uppermost 4 cm (Fig. 3).

4.5.2.2. Core ENO13-10. Some oscillation was observed in the predominant *n*-alkanoic acids (Fig. 4): three intervals with a clear abundance of C_{24}–C_{26} between 28–23, 17–13 and 10–9 cm, alternating with three periods (22–18, 12–11 and 8–0 cm) in which C_{16} was dominant. However, a bimodal distribution was observed in almost all samples, with the exception of those in the uppermost 4 cm. In fact, TAR_{FA} values oscillated around 1 or 1.5, and only between 4 cm and the top were values < 0.5.

The concentration of *n*-alkanoic acids was higher than that of *n*-alkanes. Coinciding with the intervals identified, the highest values occurred at 28–23 and 18–14 cm (> 100 mg/kg), and also in the uppermost 2 cm, with a maximum of 169 mg/kg (17 cm). The rest of the record showed values ranging between 42 and 74 mg/kg (Fig. 3).

4.5.2.3. Core ENO13-15. A bimodal distribution was observed in almost all samples (Fig. 5), maximizing either at C_{24} or C_{26} (57–46, 38–28 cm) or at C_{16} (42 cm and uppermost 26 cm). Moreover, TAR_{FA} did not show important oscillation, the values falling between 1.9 and 0.43.

As for the other two cores, the concentration of *n*-alkanoic acids was higher than that of *n*-alkanes. The highest concentration occurred between 57 and 34 cm and in the uppermost 6 cm, with 231 mg/kg registered at the top. The values for the rest of the samples varied between 40 and 107 mg/kg (Fig. 3).

4.5.3. Sterols and stanols

The major sterols and stanols in the plants taken are shown in Table 2, 24-ethylcholest-5-en-3β-ol (β-sitosterol), 24-ethylcholesta-5,22-dien-3β-ol (stigmasterol) and 24-methylcholest-5α-en-3β-ol (campesterol) being the most abundant. The presence of cholest-5-en-3β-ol (cholesterol) in most species should be highlighted.

The following major sterols were identified in the cores (Fig. 6): β-sitosterol, stigmasterol, campesterol and cholesterol, coinciding with those observed in the living plants. However the concentration was much lower in the sediments. The main stanols included 24-ethyl-5β-cholestan-3β-ol (24-ethylcoprostanol), 5β-cholestan-3β-ol (coprostanol), 5α-cholestan-3β-ol (cholestanol) and 24-ethyl-5α-choles-22E-en-3β-ol (5α-stigmastanol) among others. In some levels we also found 5β-cholestan-3α-ol (epicoprostanol) and 24-ethyl-5β-cholestan-3α-ol (24-ethylepicoprostanol) in low amount. ENO13-10 and ENO13-15 showed greater concentrations of sterols and stanols than ENO13-7 (Fig. 6).

Of note, the uppermost levels of the three cores showed the highest sterol and stanol abundance, decreasing after the first cm; 24-ethylcoprostanol and coprostanol were found in all three cores.

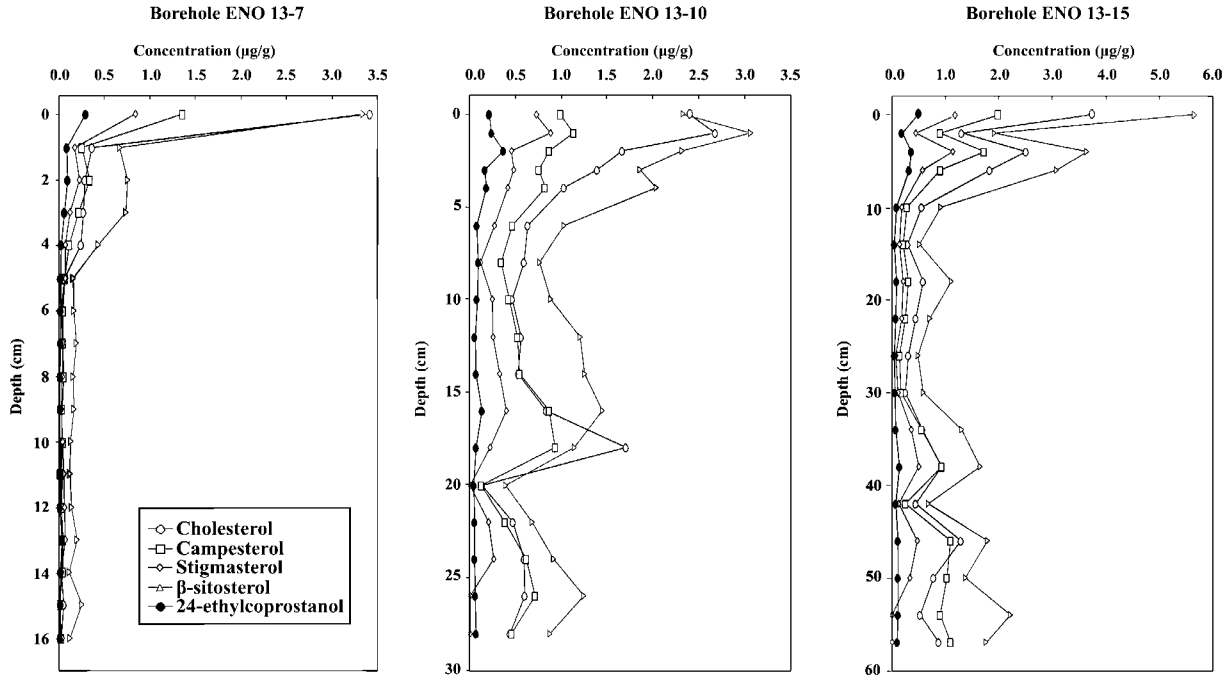

Fig. 6. Profiles of sterols and stanols content (mg/kg) in cores ENO13-7, ENO13-10 and ENO13-15.

5. Discussion

5.1. Origin of OM-paleoenvironmental implications

We used TOC content, C/N and *n*-alkane profiles to distinguish the diverse sources of OM in the three cores. The predominant *n*-alkane chain can provide information about the input of OM from algae (C_{17} max.), aquatic macrophytes (maximizing at C_{21}, C_{23} and C_{25}) and terrigenous plants (maximizing at C_{27}, C_{29} and C_{31}). In fact, living plants sampled from Lake Enol showed similar results (Table 1).

There was a general predominance of C_{31} and C_{29} alkanes in the cores, indicating a major input from terrigenous plants, with some exceptions. There was generally good correspondence between TAR_{HC}, predominant *n*-alkane chain, CPI and ACL in the three cores (Figs. 3–5). Indeed, the correlation coefficients between ACL, CPI and TAR_{HC} were high and significant for all three (Tables 3–5). However, there was a lack of correlation or a certain degree of inverse covariation between *Paq* and other indices, thereby indicating that aquatic macrophytes did not make a significant contribution to the OM, in spite of the fact that living specimens of *Potamogeton* sp. were observed. In fact, the pollen of aquatic macrophytes is scarce in the lake record (Moreno et al., 2010; López-Merino et al., 2011).

In spite of the considerable content of the alga *Botryococcus* in the record (Moreno et al., 2010; López-Merino et al., 2011), we did not detect any trace of botryococcane. We did not find any biomarker linked to diatoms, and the diatom content of sediments was low throughout most of the cores, with few exceptions, especially at the top. According to López-Merino (2001), these changes in the diatom community, with the presence of *Naviculadicta vitabunda*, *Cavinula scutelloides*, *Cyclotella radiosa* at the top, are probably linked to eutrophication and human disturbance, such as tourism, which is concentrated in the lake area, or the recent increase in livestock in the catchment.

The *n*-alkanoic acid profiles were used to confirm the information obtained from the above proxies, and also to identify the microbial degradation of OM. Based on these indices we identified three geochemical units, namely A, B and C (Figs. 3–5).

Table 2
Concentration (n.d., not detected) of sterols in living plants from Lake Enol.

	Campesterol	Stigmasterol	β-Sitosterol	Cholesterol
Green alga	0.283	0.384	2.218	2.167
Chara sp.	1.012	2.018	38.113	5.005
Potamogeton sp.	0.171	0.660	0.167	n.d.
Juncus effusus	1.129	0.442	2.927	0.291
Carex sp.	6.536	5.499	25.170	0.741
Gramineae 1	0.000	5.764	23.679	0.864
Gramineae 2	11.013	3.980	50.533	2.469

Table 3
Correlation coefficients and significant levels (correlations statistically significant at the level of $p < 0.001$ are in bold) for ACL, TAR_{HC}, *Paq* and TAR_{FA} indexes for ENO13-7.

	CPI	*Paq*	TAR_{HC}	TAR_{FA}
ACL	**0.8673**	0.1096	**0.6059**	**0.8511**
CPI		0.2912	**0.5200**	**0.6730**
Paq			0.3597	0.1134
TAR_{HC}				**0.6300**

Table 4
Correlation coefficients and significant levels (correlations statistically significant at the level of $p < 0.001$ are in bold) for ACL, TAR_{HC}, *Paq* and TAR_{FA} indexes for ENO13-10.

	CPI	*Paq*	TAR_{HC}	TAR_{FA}
ACL	**0.7902**	**−0.6417**	**0.6357**	**0.7837**
CPI		**−0.6466**	**0.5230**	**0.5545**
Paq			−0.0413	−0.3350
TAR_{HC}				**0.7471**

5.1.1. Unit A

This unit was identified in the lowermost part of ENO13-10 (28–12 cm; Fig. 4) and ENO13-15 (57–28 cm; Fig. 5). According to the chronology of ENO13-15, it covered ca. 1695–1860 AD.

C/N showed values (8.4–15.2) typical of a mixed source derived from vascular plants and algae (Hedges et al., 1986; Meyers, 1994). The predominant *n*-alkane chain (C_{31}), the high ACL (28.4–26.7), CPI (> 6) and TAR_{HC} (5.4–36.0) values, and the homogenous *Paq* index profile (< 0.17) indicated a continuous and considerable

Table 5
Correlation coefficients and significant levels (correlations statistically significant at the level of $p < 0.001$ are in bold) for ACL, TAR_{HC}, *Paq* and TAR_{FA} indexes for ENO13-15.

	CPI	*Paq*	TAR_{HC}	TAR_{FA}
ACL	**0.8887**	−0.0956	**0.7714**	**0.8127**
CPI		−0.0913	**0.7317**	**0.7355**
Paq			−0.0214	−0.1074
TAR_{HC}				**0.8688**

input of OM from land plants and a small input from aquatic macrophytes.

The predominant *n*-alkanoic acid, maximizing at C_{24}–C_{26} (with the exception of some levels: 42 cm in ENO13-15; 22–18 cm in ENO13-10) in which C_{16} was predominant), the higher TAR_{FA} values than in the other units, and high CPI pointed to minimal microbial degradation of OM.

This observation was accompanied by evidence of substantial OM input in this unit, as the TOC and *n*-alkane and *n*-alkanoic acid contents were higher than in other parts of the records. These findings could be linked to the climatic conditions that occurred during the Little Ice Age (LIA), which is widely believed to have ended around the second half of the 19th century (Jones et al., 2001, 2009), when humidity increased (Moreno et al., 2008, 2010; Martín-Puertas et al., 2008, 2010; Morellón et al., 2009, 2012; Nieto-Moreno et al., 2011), producing greater runoff and therefore an increase in OM input. Moreover, cold conditions could have inhibited bacterial activity and diagenesis of OM (cf. Zheng et al., 2007). We consider that reduced mixing of water would have been common during this period.

5.1.2. Unit B

This unit was defined in the lower part of ENO13-7 (16–4 cm; Fig. 3), and intermediate parts of ENO13-10 (12–4 cm; Fig. 4) and ENO13-5-15 (28–8 cm; Fig. 5), covering ca. 1860–1980 AD.

The atomic C/N values, which were lower than in the previous unit, indicated a mixed input of terrigenous plants, with a greater influence of OM derived from algae than in unit A.

The predominant *n*-alkane chain (C_{31}), ACL (28.3–26.8), CPI (> 5.5) and TAR_{HC} (15.7–6.2) values reflected the presence of OM derived mainly from terrigenous plants. However, ACL and TAR_{HC} values were lower than in unit A, indicating that other kinds of material (probably algae) contributed to the OM. The lack of marked variation in the *Paq* index ruled out a significant contribution from aquatic macrophytes. Of note, in ENO13-7, there was a greater input of land plants than in the other two cores, as reflected in significantly greater TAR_{HC} values in the former.

The low TAR_{FA} values, between 1.1 and 0.6, and the bimodal distribution of *n*-alkanoic acids, maximizing mainly at C_{16}, and the most abundant alkane being C_{31}, pointed to the occurrence of microbial degradation of HMW alkanoic acids from primary OM, a process that produces short chain homologs (cf. Kawamura et al., 1987). This process was probably linked to global warming that began at the end of 19th century and continued during the 20th century. However, in ENO13-7 there was predominance of long chain *n*-alkanoic acids from 16 to 5 cm, although a bimodal distribution indicated some diagenesis of OM.

Low TOC values and *n*-alkane and *n*-alkanoic acid contents revealed less OM input and/or greater degradation of organic compounds during ca. 1860–1980 AD than in the LIA. In fact, coinciding with the presence of calcite bands in ENO13-7, there was a decrease in OM content, which may indicate warmer conditions (Fig. 3). However, anthropogenic influence cannot be ruled out as the exploitation of the Buferrera mine, which began in the 1870s (Rodríguez Terente et al., 2006) used water from the lake to produce power. Likewise, according to López-Merino et al. (2011), mining activity might have forced some groundwater into the lake, thereby affecting the local phreatic level and groundwater flow. Moreover, the construction of a dyke for a road in 1890 increased the water level of the lake and the likelihood of thermal stratification (Vegas et al., 2015). Also, changes in livestock management were considerable during the 20th century—a period marked by a decline in sheep and goats. Furthermore, cattle, which were used mainly for meat, were replaced by milk-producing breeds, which spent more time in the valleys rather than in the mountains (Suárez Antuña et al., 2005).

5.1.3. Unit C

This unit was identified in the uppermost part of ENO13-7 (4–0 cm; Fig. 3), ENO13-10 (4–0 cm; Fig. 4) and ENO13-15 (8–0 cm; Fig. 5), representing the last ca. 40 years (1970–2013 AD).

The low atomic C/N values, mainly in ENO13-7 (9.0–5.8), indicated a substantial algal source. There was also good correspondence with the interpretation derived from the predominant *n*-alkane chain and ACL profiles. The decreasing trend from the bottom to the top of the cores observed in ACL indicated that other sources (algae) contributed to the OM. This was especially noticeable in ENO13-7, in which short chain homologs (C_{17}) were dominant and low ACL values (23.8–24.7) occurred. Nevertheless, most of the samples showed a bimodal distribution of *n*-alkanes, thereby pointing to a mixed OM input. In fact, ACL values in ENO13-15 varied between 27.0 and 26.0.

Although CPI ranged between 6.0 and 8.0 in some samples of ENO13-15 and ENO13-10, CPI values (4.7–2.0) confirmed that terrigenous plants were less significant than in other units (cf. Hedges and Prahl, 1993). However, these values may also be attributed to a certain degree of ongoing microbial degradation in the cores. Moreover, low TAR_{HC} values (being < 1 in ENO13-7), were linked to a significant algal input or bacterial activity. As in the other units, *Paq* values ranged between 0.1 and 0.2, suggesting some input from aquatic macrophytes, although much smaller than that of OM from land plants and algae.

Coinciding with the interpretation derived from the *n*-alkane indices, low TAR_{FA} values (< 0.5.) and predominance of short chain *n*-alkanoic acids indicated a greater algal source. However, we cannot rule out bacterial activity, which may have led to the production of low MW *n*-alkanoic acids at the expense of long chain homologs (cf. Kawamura et al., 1987). In fact, Chao (2014) reported considerable activity of bacterial colonies (iron bacteria and sulfate reducing bacteria) in the uppermost 4 cm of ENO13-7 and 8 cm of ENO13-15.

It is worth noting that the total sum of *n*-alkanes and *n*-alkanoic acids was significantly higher than in other units, coinciding with an increase in TOC (Figs. 3–5), and was accompanied by evidence of considerable algal input (high content of low MW *n*-alkanes) and some microbial degradation, as there was a predominance of low MW *n*-alkanoic acids. This observation can be partly interpreted in terms of climatic change as, according to meteorological data, the regional precipitation apparently decreased and the temperature increased since 1973 AD (López-Merino et al., 2011); these changes have thus affected the catchment hydrology of the lake, with less land plant input at the expense of more phytoplankton production. Furthermore, this may be also linked to anthropogenic influence, as livestock farming increased within the catchment in the last decades (Rodríguez Castañón, 1996) and tourism brings around 1.8 million visitors per year to the National Park (http://www.mma.es), that are concentrated mainly around the lake. Similarly, López-Merino et al. (2011) interpreted lower runoff, more intense mixing of the lake water, more input of nutrients and increasing lake productivity with the presence of diatoms characteristic of mesotrophic and eutrophic waters since 1970.

5.2. Pollution

The wide variety of sterols and their derivatives are key paleoenvironmental indicators. In living plants from Lake Enol and surroundings, the major sterol was β-sitosterol, followed by stigmasterol and campesterol, coinciding with observations by Goad and Goodwin (1972), Nishimura and Koyama (1977), Nishimura (1977), Volkman (1986), Gülz et al. (1987), Goad (1991) and Laureillard and Saliot (1993) for vascular plants. We also found that β-sitosterol was abundant and predominant in the green alga, *Chara* sp., and the aquatic macrophyte, *Potamogeton* sp., stigmasterol and campesterol being detected in smaller amount. Similarly, β-sitosterol occurs in blue-green algae from saline lakes (Matsumoto et al., 1982) and in chlorophyceae algae and cyanobacteria (Volkman, 1986). Therefore, the presence of these sterols along the three cores is not unusual. However, the concentration of sterols in sediments was much lower than in living plants (cf. Table 2), being higher in the uppermost levels of the cores and decreasing sharply below ca 3–5 cm—an observation attributed to microbial degradation. The following processes may be responsible for the loss of sterols: (i) mineralization, (ii) conversion to modified sterols and (iii) condensation of steroid moieties to form non-volatile constituents (van Bergen et al., 1997). However, sterols are relatively more resistant to degradation than other lipids, and we did not find altered compounds derived from them, such as stanols or keto- or hydroxylated products. In contrast, bacterial mats were identified at the top of cores (Chao, 2014) and can be incorporated into sediment macromolecules (cf. Michaelis et al., 1989). Likewise, according to Bull et al. (2000), sitosterol rapidly diminishes in soil, possibly as a result of assimilation by soil-dwelling invertebrates (cf. Svoboda and Thompson, 1985; Nes et al., 1997) and rapid oxidative degradation. In fact, we found evidence of the degradation of *n*-alkanoic acids and the presence of bacterial activity in the three cores.

Cholesterol was another constituent in some of the living plants (Table 2). It was traditionally considered to be virtually absent from plants and present only in animals; however, its presence is now also largely accepted in higher plants (Noda et al., 1988; Al Easa et al., 1995; Shukla et al., 2002). Furthermore, it is the dominant sterol in algae from Lake Suwa (Japan; Nishimura, 1977; Nishimura and Koyama, 1977) and has been reported in diatoms and cyanobacteria and in some algae (Volkman, 1986; Volkman et al., 1999). Thus, its presence in lacustrine sediments cannot be attributed solely to an animal source. Therefore, the main sterols could not be used to discriminate the source of OM, as they may have been present in the original input of plant material.

The presence of 24-ethylcoprostanol must be highlighted. We also detected coprostanol, considered a biomarker of human fecal pollution.

Bethell et al. (1994) and Evershed and Bethell (1996) proposed the use of the coprostanol/24-ethylcoprostanol ratio as proxy for distinguishing human vs. herbivore fecal input, with values > 1.5 considered indicative of human pollution and < 0.25 when herbivore feces predominates. An extension of this approach to estimate the relative contribution of different fecal sources was proposed by Leeming et al. (1997), the relative human and herbivore inputs being assessed using the index $100 \times [(\text{coprostanol})/(\text{coprostanol} + \text{24-ethylcoprostanol})]$ where values > 73% represented solely human contamination and values < 38% solely that from herbivores. Here, we used the ratio proposed by Bull et al. (2002) (coprostanol + epicoprostanol)/(24-ethylcoprostanol + 24-ethylepicoprostanol) in order to distinguish between human (> 1) and ruminant species (< 1). However, the epimers from both stanols were weakly present, and we therefore calculated the ratio principally with the main stanols (Evershed and Bethell, 1996). Values for the three cores were < 1 (Fig. 7), indicating a continuous pollution source derived mainly from cattle. In the uppermost levels of the cores (last 20–30 years), the concentration of 24-ethylcoprostanol

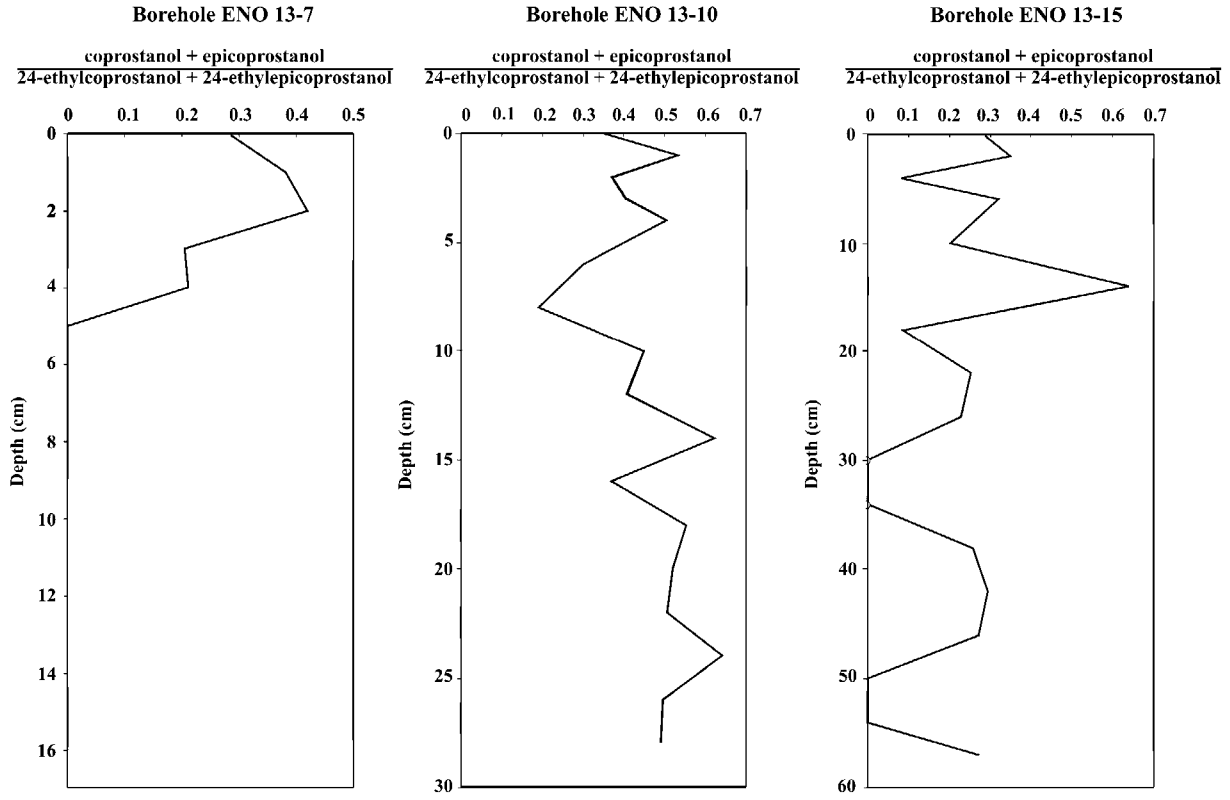

Fig. 7. Profiles of (coprostanol + epicoprostanol)/(24-ethylcoprostanol + 24-ethylepicoprostanol) in cores ENO13-7, ENO13-10 and ENO13-15.

was higher than in the rest of the record (Fig. 6). In fact, Chao (2014) reported coliform bacteria in the water column of the same lake, especially in the upper part. This was attributed to cattle depositing feces on the edge of the lake, the feces reaching the water body through surficial runoff. Meléndez-Asensio et al. (1997) also determined microbiological pollution in the aquifer caused by livestock activity.

According to López-Merino et al. (2011), the largest change in the pollen record of the lake in the last 200 years is related to pastoral activity, as shepherding was, and continues to be, a major economic activity in the Cantabrian region (Domínguez Martín and Puente Fernández, 1995; Mayor López, 2002). Coincidentally, coprophilous fungi were found to be abundant, especially during the 19th century, although they decreased in the 20th century (López-Merino et al., 2011).

However, the replacement of native cattle (used mainly for meat) that grazed mainly on the mountains by breeds (used for milk production) that spent long periods in the valleys (Rodríguez Castañón, 1996; Suárez Antuña et al., 2005), together with the decline in sheep and goats, did not lead to a decrease in the 24-ethylcoprostanol concentration in the sediments, as occurred with the reduction of coprophilous fungi at the expense of the Atlantic bushes Erica and Cytisus/Ulex (López-Merino et al., 2011). Moreover, our results show that the contamination linked to herbivores began in the 17th century, perhaps even earlier.

6. Conclusions

The *n*-alkane, *n*-alkanoic acid and sterol content in the Lake Enol record allowed us to reconstruct the paleoenvironmental evolution during the last ca. 320 years. In this regard, we identified three units with distinct environmental conditions. In all three cores there was good correspondence between the predominant *n*-alkane chain, ACL, TAR_{HC} index and C/N values. The record showed dominant OM input from land plants (Unit A—ca. 1695–1860 AD and Unit B—ca. 1860–1980 AD) as the predominant *n*-alkane was C_{31}, whereas aquatic macrophytes did not contribute significantly, and only the uppermost levels (4–8 cm) of the cores were characterized by considerable algal input (Unit C, ca. 1980–2013 AD). The terrestrial influence was more distinct in the upper core (ENO-13-7) than in ENO13-10 and in the latter more than in ENO13-15.

The lack of correspondence between the *n*-alkane and *n*-alkanoic acid indices in some intervals of the cores (Units B and C) was interpreted as microbial synthesis of fatty acids from primary OM, the process being especially significant in the uppermost part of the cores (Unit C).

The OM content varied. The lowermost part of ENO13-10 and ENO13-15 (Unit A) showed a higher concentration than in the other intervals. In our view, this could be linked to the paleoenvironmental conditions, as during the LIA the increase in rainfall favored the contribution of terrigenous OM to the lake via runoff. This OM was subject to less degradation because of colder conditions, which inhibited microbial action, and probably had some influence in more prolonged episodes of stratification of the water column. Warmer conditions at the end of the 19th century and during the 20th century, together with anthropogenic influence (increased presence of livestock in the catchment and the lake shores, afforestation, mining activity) seemed to have produced a decrease in the input of OM to the lake and favored microbial activity (Unit B).

The last ca. 40 years (uppermost 4–6 cm of the 3 cores, Unit C) were characterized by considerable phytoplankton productivity (predominance of C_{17} *n*-alkane) and microbial degradation (predominance of C_{16} *n*-alkanoic acid), accompanied by an increase in OM, the latter possibly linked to increased human activity (change of land use and lake management) and a warmer and drier climate.

The sterol content showed continuous pollution derived from livestock and wild herbivores since the 17th century, as revealed by the ubiquitous presence of 24-ethylcoprostanol, a compound linked to animal feces.

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