

1 **Evidence for humid conditions during the last glacial from leaf**
2 **wax patterns in the loess-paleosol sequence El Paraíso, Central**
3 **Spain**

4 Imke K. Schäfer^{a,*}, Marcel Bliedtner^a, Daniel Wolf^b, Dominik Faust^b, Roland Zech^a

5
6 ^a *Institute of Geography and Oeschger Centre for Climate Change Research, University of*
7 *Bern, Hallerstrasse 12, CH-3012 Bern, Switzerland*

8 ^b *Department of Geography, Technical University of Dresden, Helmholtzstraße 10, DE-01069*
9 *Dresden, Germany*

10 * Corresponding author: imke.schaefer@giub.unibe.ch (I.K. Schäfer).

11
12 **Abstract**

13 The Mediterranean region is affected by the first consequences of anthropogenic
14 climate change and suffers from aridization and drought periods. Reconstructing
15 past climate and environmental changes might help to put those consequences into
16 context, identify underlying mechanisms and improve predictions. Here we present
17 leaf wax analyses for the loess-paleosol sequence (LPS) from El Paraíso, located in
18 Central Spain and a selection of plants growing there today. The long-chain *n*-
19 alkanes in almost the whole LPS are characterized by the dominance of C₂₉, C₃₁ and
20 C₃₃, indicating the presence of grasses and drought-adapted tree species, such as
21 *Juniperus* and *Olea*. However, samples correlated with marine isotope stage (MIS) 2
22 (~29 to 14 ka) have higher abundances of C₂₅, C₂₇ and C₂₉, which may signal the
23 presence of less drought-adapted deciduous trees and more humid conditions. *n*-

24 Alkanoic acid patterns can tentatively be interpreted to confirm these results, but
25 are less robust, because more plant species are needed for comparison. Our findings
26 and interpretation are in line with climate modelling studies that suggest a
27 southward shift of the westerlies and storm tracks during MIS 2, with fluvial and
28 lacustrine records, and with glacial refugia for temperate trees in southern Europe.
29 Compound-specific isotope analyses will hopefully soon provide additional
30 information about paleoclimatic and –hydrologic changes and help establish a more
31 precise and robust age control.

32

33 **Keywords**

34 Climate reconstruction; leaf wax biomarkers; *n*-alkanes; *n*-alkanoic acids; Loess-
35 paleosol sequences; Iberia

36

37 **1. Introduction**

38 Today’s climate change is of great importance. Hardly a day goes by without reports
39 about extreme weather phenomena like hurricanes, droughts and floods in some
40 regions of the earth. To better understand the mechanisms and environmental
41 consequences of recent anthropogenic climate change, it is indispensable to
42 reconstruct past climate and environmental changes, especially in regions that
43 already suffer today. More and more attention is being paid, for example, to the
44 Mediterranean region, which is prone to droughts and affected by aridization (Giorgi
45 and Lionello, 2008; Seager et al., 2014). Paleoclimatically, the Iberian Peninsula is a

46 very interesting location, because its climate is influenced by the polar front, the
47 storm tracks from the North Atlantic, the Mediterranean Sea, and by its proximity
48 to Africa and Africa's monsoons (Bout-Roumzeilles et al., 2007; Lewis et al., 2009;
49 Beghin et al., 2015).

50
51 Information from Iberia about past climate and environmental changes is sparse
52 and contended. One controversy arose from findings that glaciers in some parts of
53 Iberia may have already reached the maximum extent before the global Last Glacial
54 Maximum (LGM: ~25 to 19 ka, Clark et al. 2009; e.g. Pallàs et al., 2006 and
55 references therein; Lewis et al., 2009). Another long-standing controversy concerns
56 the hydrological conditions during the LGM. Pollen records are traditionally
57 interpreted as indicating more arid conditions, whereas lacustrine and fluvial
58 records, as well as climate models, all suggest more precipitation (Prentice et al.,
59 1992; recent reviews of Moreno et al., 2012; Moreno et al., 2014; Beghin et al., 2015
60 and references therein).

61
62 Increasingly, new and interesting insights may soon come from biomarker analysis
63 in LPS. Compared to pollen records, which are mostly established from lake
64 sediments and peat bogs (i.e. from specific topographical and microclimatological
65 settings), biomarker records can provide valuable complementary
66 paleoenvironmental information. This is because LPS occur on plateaus and in
67 protected slope positions. Although pollen is often not preserved in LPS, some
68 biomarkers are (Zhang et al., 2006; Zech et al., 2011). Loess is often interpreted as

69 documenting glacial, cold and arid conditions, and paleosols as documenting more
70 humid conditions during interglacials and interstadials (Garcia Giménez et al.,
71 2012; Zech et al., 2013). However, variable dust accumulation rates need to be taken
72 into account, which is why proxies independent of dust accumulation rate, such as
73 biomarkers, are particularly valuable.

74
75 Long chain *n*-alkanes and *n*-alkanoic acids are essential constituents of epicuticula
76 leaf waxes that are produced by all types of plants to protect them from water stress
77 and microbial attack (Eglinton and Hamilton, 1967; Gülz, 1994; Eglinton and
78 Eglinton, 2008). Because of this protective function, leaf waxes can be highly inert;
79 hence, they can be preserved in soils and sediments over geological timescales and
80 serve as biomarkers. Numerous studies have investigated the chemotaxonomic
81 potential of leaf waxes (Cranwell, 1973; Ficken et al., 1998; Maffei et al., 2004;
82 Rommerskirchen et al., 2006; Zech et al., 2009; Zocatelli et al., 2012). Higher
83 abundances of the *n*-C₂₇ and *n*-C₂₉ alkanes have been found to be characteristic for
84 deciduous trees and shrubs, while the longer alkane homologues *n*-C₃₁ and *n*-C₃₃ are
85 more abundant in grasses and herbs. We recently conducted a transect study in
86 Central Europe and were able to confirm this pattern (Schäfer et al., submitted).
87 Moreover, we found the *n*-C₂₄ alkanolic acid to be particularly abundant under
88 coniferous trees, the *n*-C₂₈ alkanolic acid under deciduous trees, and grasslands to
89 have relatively high amounts of *n*-C₃₂ and *n*-C₃₄ (Schäfer et al. submitted). Leaf wax
90 production and homologue patterns can be highly variable, even within plants
91 belonging to the same vegetation type (Diefendorf et al., 2011; Bush and McInerney,

92 2013; Schäfer et al., submitted). Therefore, vegetation reconstructions (i) should be
93 evaluated against leaf wax patterns at a regional scale, and (ii) might be biased or
94 even flawed due to specific species. Climate conditions, such as changes in
95 temperature, precipitation and relative humidity, may influence the homologue
96 patterns (e.g. Poynter, 1989; Sachse et al., 2006; Tipple and Pagani, 2013; Bush and
97 McInerney, 2015). Nevertheless, leaf waxes have been successfully applied in many
98 paleostudies (e.g. Brincat et al., 2000; Schwark et al., 2002; Zhang et al., 2006; Zech
99 et al., 2010; Tarasov et al., 2013).

100 For this study we analysed *n*-alkanes and *n*-alkanoic acids in the LPS El Paraíso, as
101 well as in some plants growing at and near that site. The objectives of our study
102 were (i) to test whether or not leaf wax patterns of local common plants are in line
103 with homologue patterns typical for vegetation types in Central Europe; and (ii) to
104 use the leaf wax patterns in the LPS El Paraíso to infer changes in
105 paleoenvironmental conditions during the Late Quaternary in Central Spain.

106

107 **2. Regional setting, climate and vegetation**

108 **2.1 Regional setting**

109 The LPS El Paraíso (560 m a.s.l., N 40° 01.855' W 03° 28.031') is located east of
110 Aranjuez (Fig 1a) on north facing slopes that mediate between the Mesa de Ocaña
111 and the incised river valleys of the Tagus River. The Tagus is the longest river of
112 the Iberian Peninsula and it has the third largest catchment area (Benito et al.,
113 2003). The Tagus Basin evolved during the Tertiary with thick successions of

114 alluvial fan material and lacustrine deposits (Calvo et al. 1996). These sediments
115 consist of evaporites that are assigned to the Miocene Lower Unit and the Miocene
116 Intermediate Unit. The sediments of the Miocene Upper Unit are transformed into
117 conglomerates and lacustrine limestones (Garcia Giménez et al., 2012 and references
118 therein). After intense dissection of these basin deposits, Pleistocene and Holocene
119 deposits have accumulated above the Miocene marls (Roquero et al., 2015; Wolf and
120 Faust 2015). Today, these Quaternary sediments are mainly exposed along the valley
121 floor and the remains of river terraces where also the main loess outcrops occur (Fig. 1b;
122 Garcia Giménez et al., 2012).

123 **2.2 Climate and vegetation**

124 Today's climate in Central Spain is Mediterranean, with warm arid summers and
125 more humid mild winters. While the summer regime is controlled by the subtropical
126 anticyclone of the Azores, the conditions during winter are strongly affected by the
127 westerlies (Benito et al., 2003, Beghin et al., 2015). Most of the annual precipitation
128 occurs during winter when cold air masses from the North Atlantic are mixed with
129 warmer and more humid ones leading to precipitation. Annual rainfalls are
130 moderate (400-500 mm, García et al., 2011), and mean annual temperatures are
131 between 11-14°C (Roquero et al., 2015).

132
133 The modern vegetation has adapted to these particular conditions. Large parts of
134 the region are inhabited by *Quercus ilex*. Other typical tree species in this region are
135 *Pinus nigra*, *Quercus lusitanica*, *Juniperus sabina*, *Pinus maritime* and *Olea*
136 *europaea*. The lower plant layers are composed of *Juniperus* and *Quercus* species,

137 *Thymus vulgaris*, *Asparagus acutifolius*, *Salvia lavandulaefolia* and *Rosmarinus*
138 *officinalis*. Xerophytic and halophytic grasses, and herbs like *Stipa tenacissima*,
139 build up the herb layer. However, the potential natural vegetation has been strongly
140 reduced due to human intervention, thus the modern vegetation cover surrounding the
141 LPS El Paraíso mainly consists of almond trees (*Prunus dulcis*) and grass species, e.g.
142 *Stipa tenacissima* and *Cupressus sempervirens*.

143

144 **3. Material and Methods**

145 **3.1 Sampling and sample material**

146 The LPS El Paraíso is 8 m high and consists of alternating units of reddish ochre
147 loess and fossil soils (Fig. 1c). The upper part (<4 m depth) is characterised by low
148 conductivity (<800 μScm^{-1}) and the absence of calcareous concretions. Intercalated
149 soil horizons show various but mainly low degrees of weathering. The sediments of
150 the lower part (>4 m depth) consist of partly reworked material. They are
151 characterised by an increase in clay content (>15%) and calcareous concretions of
152 various sizes. Here, soil horizons appear much stronger developed indicating more
153 intense weathering processes. A detailed sedimentological and pedological
154 description is provided by Wolf et al. (in preparation). They investigated several LPS
155 along the Tagus River. Magnetic susceptibility and further parameters made it
156 possible to correlate the sequences and revealed ages of ~25 ka and ~35 ka for the
157 paleosols at ~2 and ~4.5 m depth in the LPS El Paraíso, dates which were based on
158 luminescence ages in the LPS Fuentidueña (not shown). Preliminary luminescence

159 ages from the LPS El Paraíso suggest that the paleosol at ~2 m depth might actually
160 be slightly older, and first compound-specific ¹⁴C analyses identify the soil at ~1 to
161 1.4 m depth as Holocene soil and the overlying sediments as colluvium. The loess
162 sediments from ~1.0 to 1.9 m depth were thus most likely deposited during MIS 2
163 and overprinted in the upper part by Holocene pedogenesis.

164
165 Soil and sediment samples were taken during a field trip in March 2014. At least
166 one sample per paleosol/loess horizon was taken, and 31 samples in all were
167 available for biomarker analyses. The samples were dried immediately after
168 sampling. In September 2014, ten vegetation samples were collected from living
169 plants, which are common in the study area. The samples are comprised of leaves
170 from broadleaf evergreen *Quercus coccifera*, *Quercus ilex* and *Olea europea*, as well
171 as needles from *Juniperus phoenicea*, *Juniperus thurifera*, *Pinus halepensis* and
172 *Pinus nigra*. In addition, a herbaceous plant (*Thymus vulgaris*) and a
173 Mediterranean grass species (*Stipa tenacissima*) were sampled. These samples were
174 also dried immediately after sampling.

175 **3.2 Biomarker analyses**

176 1 g fresh plant material was extracted by using microwave assisted solvent
177 extraction with 15 ml dichloromethane (DCM)/methanol (MeOH) (9:1) at 100°C for 1
178 h. For the paleosols, ~40 g sediment were extracted with an Accelerating Solvent
179 Extractor (Dionex 200, two extraction cycles, 6.9 MPa, 100°C), and by using the
180 same solvent mixture as for the plant samples. Total lipid extracts were passed over
181 pipette columns filled with aminopropyl silica gel (Supelco, 45 µm). The aliphatic

182 fraction (incl. *n*-alkanes) was eluted with hexane, more polar compounds (e.g.
183 alcohols) with DCM/MeOH (1:1), and acids (incl. *n*-alkanoic acids) with 5% acetic
184 acid in diethylether. The *n*-alkanoic acids were converted to fatty acid methyl esters
185 (FAMEs) by using MeOH/HCl (95/5; 70°C, overnight). The FAMEs were recovered
186 by using liquid-liquid extraction with hexane. The aliphatic fraction and the acid
187 fraction were spiked with a known amount of an internal standard (5 α -
188 androstanone) and analyzed by gas chromatography-mass spectrometry (GC-MS).
189 The analysis was carried out by using an Agilent MS 5975 (EI) interfaced with an
190 Agilent 7890 GC equipped with a 30 m fused silica capillary column (HP5-MS 0.25
191 mm i.d., 0.25 μ m film thickness), and a split/splitless injector operating in splitless
192 mode at 350°C. Carrier gas was helium and the temperature program was 1 min at
193 50°C, from 50 to 200°C at 30°Cmin⁻¹, from 200 to 320°C at 7°Cmin⁻¹, 5 min at 320°C.
194 Data recording comprised the Total Ion Current (TIC, scan mode from *m/z* 40 to *m/z*
195 600) and SIM (Single Ion monitoring) scan (*m/z* 57, 71, 85 and 99). Concentrations
196 were calculated relative to the internal standard and to an external standard (*n*-C₂₀
197 to *n*-C₄₀ alkane mixture, Supelco), injected in different concentrations (40 ng/ μ l, 4
198 ng/ μ l, 1 ng/ μ l, 0.4 ng/ μ l).

199 **3.3 Data analyses**

200 Total *n*-alkane and *n*-alkanoic acid concentrations (c_{tot}) are calculated as the sums of
201 C₂₅ to C₃₅ and C₂₀ to C₃₄, respectively. The average chain length (ACL) describes
202 variations in chain lengths of *n*-alkanes (Poynter et al., 1989).

222 4. Results and Discussion

223 4.1 *n*-Alkane patterns in plants

224 C_{tot} in the sampled plants range from 3.9 $\mu\text{g/g}$ to 391 $\mu\text{g/g}$ dry weight (Tab. 1). *Pinus*
225 *h.* and *Pinus n.* have very low *n*-alkane concentrations, which is typical for conifers
226 (e.g. Diefendorf et al., 2011; Zech et al., 2012; Bush and McInerney, 2013). However,
227 *Juniperus p.* and *Juniperus t.* have high concentrations. This is unusual for conifers,
228 but has been reported previously (Tarasov et al., 2013). While the needles of the two
229 *Juniperus* species and *Pinus n.* are dominated by the *n*- C_{33} alkane, the needles of
230 *Pinus h.* show a dominance of the C_{29} and C_{31} *n*-alkanes, although not a pronounced.
231 C_{tot} in *Quercus c.* and *Quercus i.* is high (Tab. 1). Both those species show a strong
232 dominance of the *n*- C_{29} alkane, which is consistent with previous studies on leaves
233 in Italy (Sachse et al., 2006) and on leaves and soils below *Quercus* in Central Spain
234 (Almendros et al., 1996). *Olea e.* also has a high c_{tot} , but shows a dominance of the *n*-
235 C_{33} alkane, which is normally regarded as a marker for grasses and herbs. Since
236 *Olea e.* is a species adapted to pronounced variations in temperature and
237 precipitation (Pantaléon-Cano et al., 2003), the preferential synthesis of longer
238 chain leaf waxes might be a distinct protective mechanism compared to other
239 deciduous trees. Other studies have found a dominance of *n*- C_{31} in *Olea* (Bianchi et
240 al., 1992; Mihailova et al., 2015). Also, Bianchi et al. (1992) showed that *Oleas* leaf
241 wax production was quite unique in the sense that it produces pentacyclic
242 triterpenes as dominant leaf wax group. The samples of *Thymus v.* and *Stipa t.* have
243 high *n*-alkane concentrations. Both species show a dominance of long chains (*n*- C_{33} :
244 *Thymus v.* and *n*- C_{31} : *Stipa t.*), which is typical for grasses and herbs.

245
246 In summary, these results suggest that *n*-alkanes may be useful biomarkers to infer
247 past environmental conditions. Shorter chains (C₂₇ and C₂₉) may indicate the
248 presence of deciduous trees, as well as *Quercus i.* and *c.*, whereas longer chains (C₃₁
249 and C₃₃) may indicate the presence of grasses and herbs, as well as trees, such as
250 *Olea* and *Juniperus*, which are particularly well adapted to arid conditions. Conifers
251 other than *Juniperus* are probably not represented in the sedimentary archives.

252
253 As *n*-alkane homologue patterns have been shown to be affected by degradation,
254 Zech et al. (2009) proposed an endmember model for corrections. Endmember plots
255 use a normalized *n*-alkane ratio on the y-axis, and the OEP as a proxy for
256 degradation (e.g. Tipple and Pagani, 2010; Vogts et al., 2012; Wang et al., 2014) on
257 the x-axis. In general, *n*-alkane ratios are wider for grasses and soils from
258 grasslands, and narrower for samples from deciduous trees and respective soils.
259 However, with increasing degradation (lower OEPs), differences become less,
260 illustrated and quantitatively described by “degradation lines” (Fig. 2a). In
261 principal, the endmember model can be used to quantify the leaf wax contributions
262 from deciduous trees and grasses, but one should keep in mind the wide variability
263 of the homologue patterns.

264 Due to the relatively high amounts of *n*-alkanes C₃₁ and C₃₃, *Olea e.* plots far above
265 the degradation line for deciduous trees from Central Europe, and it even plots
266 above the degradation line for grasslands (Fig. 2a). Both *Quercus* species likewise
267 plot above the degradation line for deciduous trees. As mentioned above, this likely

268 reflects an adaptation to arid and warm conditions in Central Spain. *Stipa t.* and
269 *Thymus v.* plot close to the degradation line for grasslands. The *Juniperus* samples
270 also plot close to the grassland degradation line. We chose not to plot the *Pinus*
271 samples, due to their low *n*-alkane concentration, which makes them irrelevant
272 when interpreting sediment *n*-alkane patterns.

273
274 All in all, Fig. 2a illustrates that the endmember model for Central Europe can be
275 used in Iberia to evaluate and correct degradation effects on *n*-alkane patterns in
276 sedimentary archives. But it also shows that quantitative interpretations (in terms
277 of deciduous trees vs. grass contributions) are likely biased by the adaptation of
278 plants to arid and warm conditions. High reconstructed and modelled “grass
279 percentages” can therefore be interpreted as signs of more arid conditions, but
280 should not be regarded as exclusive concerning drought-adapted species, such as
281 *Juniperus* and *Olea*. More plant samples from the Mediterranean region need to be
282 studied to refine this picture, but we will nevertheless try to use *n*-alkanes and the
283 endmember model in the LPS El Paraíso for paleoenvironmental reconstruction.

284 **4.2 *n*-Alkane patterns in the LPS El Paraíso**

285 C_{tot} in the samples from the LPS El Paraíso range from 4.7 $\mu\text{g/g}$ to 0.04 $\mu\text{g/g}$ dry
286 sediment (Fig. 3). Concentrations are higher in the upper part of the sequence yet
287 decrease with depth, which is probably due to enhanced degradation in the deeper
288 layers of the LPS. Interestingly, the pattern of the OEP does not follow this trend.
289 Overall, OEP values >4 throughout the whole profile show good preservation (Zech

290 et al., 2009), but values are lowest in the MIS 2 loess (~1.4 to 1.9 m depth) and then
291 increase again with depth. The *n*-alkanes that survived degradation in the lower
292 part of the profile are possibly stabilized by a mechanism that prevents preferential
293 degradation of odd vs. even chains, which is typically found during degradation of
294 fresh plant material. ACL ranges from 30.6 to 29.3, and the lowest values are at
295 ~1.4 to 1.9 m depth (Fig. 3). The *n*-alkane ratio $(n\text{-C}_{31}+n\text{-C}_{33})/(n\text{-C}_{27}+n\text{-C}_{31}+n\text{-C}_{33})$
296 strongly correlates with the ACL, and also has its lowest values in the MIS 2 loess
297 sediments.

298

299 Before interpreting these chain-length patterns paleoecologically, we evaluated the
300 degree to which they may have been biased by degradation effects. In the
301 endmember plot (Fig. 2b), most samples fall close to the degradation line of grasses
302 and only a few data points are closer to the degradation line of deciduous trees.
303 Although we calculated “grass contribution %” for all samples from the endmember
304 model, and plotted the respective values (Fig. 3), we explicitly emphasize that they
305 are *not* to be interpreted in terms of leaf wax inputs from grasses. We say this,
306 because *Juniperus* and *Olea* are characterized by longer chain lengths. Instead, the
307 endmember calculations simply show that the homologue patterns in the LPS El
308 Paraíso are not only an artefact of degradation. “Grass contribution %” closely
309 correlates with the ACL and the *n*-alkane ratio. All three plots suggest that grasses
310 and drought-adapted species, like *Juniperus* and *Olea*, were likely dominant at our
311 research site during most of the Late Quaternary. Deciduous trees *less* adapted to
312 drought seem to have been more prevalent only during MIS 2. Our results and

313 interpretation are in line with fluvial and lacustrine records from Iberia, which
314 suggest there were more humid conditions during the global LGM, probably due to a
315 southward shift of the westerlies and storm tracks (Moreno et al., 2012; Moreno et
316 al., 2014; Beghin et al., 2015). Our results and interpretation are also in line with
317 genetic features of recent European tree populations, which show that tree species
318 survived in glacial refugia in Southern Europe during the last glacial (Carrión et al.,
319 2003; González-Sampériz et al., 2010). At first glance, all these findings seem to
320 contradict pollen records, which indicate steppe vegetation during the last glacial
321 (Pantaléon-Cano et al., 2003; González-Sampériz et al., 2006; González-Sampériz et
322 al., 2010). Therefore future studies should investigate the complementary nature of
323 the various archives and proxies. Local, microclimatic and ecological differences and
324 variability have to be considered. Wolf and Faust (2015), for example, have shown
325 strong spatial heterogeneity of physiographic and climatic conditions on the Iberian
326 Peninsula for the Holocene epoch. Specific strengths, weaknesses and potential
327 pitfalls, e.g. pollen and leaf wax production during the last glacial, when CO₂
328 concentrations were lower and winds probably stronger, also have to be considered.
329 One obvious limitation of the *n*-alkane proxy is that it is nearly blind for conifers
330 (*Juniperus* is an exception). Since pines are believed to be an important component
331 of glacial forests in the Iberian Peninsula (Rodríguez-Sánchez et al., 2010), this
332 motivated us to also explore the potential of *n*-alkanoic acids as leaf wax biomarkers
333 for paleoenvironmental reconstructions.

334

335 4.3 *n*-Alkanoic acid patterns in plants

336 C_{tot} of the long chain *n*-alkanoic acids ranges from 12.6 to 62.5 $\mu\text{g/g}$ dry weight (Tab.
337 2). The maximum long chain *n*-alkanoic acid in the two *Juniperus* species is *n*- C_{28}
338 (Tab. 2), which is longer than in the study of Almendros et al. (1996), who found a
339 dominance of *n*- C_{22} and *n*- C_{24} in soils below *Juniperus*. On the other hand, both
340 *Pinus* species have a clear dominance of C_{24} , which is consistent with the previous
341 study. The two *Quercus* species show maxima for *n*- C_{28} and *n*- C_{24} , and Almendros et
342 al. (1996) found a *n*- C_{24} dominance in *Quercus*. In *Olea e.* we found a dominance of *n*-
343 C_{32} , whereas Bianchi et al. (1992) reported *n*- C_{28} to be predominant in leaves of *Olea*
344 in Italy. *Thymus v.* has a dominance of *n*- C_{24} and *Stipa t.* shows a high amount of *n*-
345 C_{28} .

346
347 In the following section, we compare the *n*-alkanoic acid patterns with those from
348 the transect study in Central Europe (Schäfer et al., submitted). There, we found C_{24}
349 acid to be particularly abundant in conifers, while *n*- C_{28} appeared to be
350 preferentially synthesized by deciduous trees, and grasslands showed relatively high
351 amounts of *n*- C_{32} and *n*- C_{34} . In a CDG ternary plot (Fig. 4a), samples accordingly
352 plot in distinct clusters. Here, we marked only the position of the respective means
353 for conifers, deciduous trees and grasses.

354
355 The two *Pinus* species from Central Spain have very high C indices and plot in the
356 “correct” corner for coniferous trees. Our *Juniperus* samples, however, plot closer to
357 the endmember of deciduous trees. As noted above, Almendros et al. (1996) found a

358 dominance of C₂₂ and C₂₄ in soils under *Juniperus*, so their samples would plot
359 closer to the expected location based on comparison with the endmembers from
360 Central Europe. Our *Olea* sample has a very high G index. Again, previously
361 reported *n*-alkanoic acid patterns are different, as Bianchi et al. (1992) reported C₂₈
362 dominance for *Olea* from Italy. As for our *n*-alkanes, we suggest that *Juniperus* and
363 *Olea* adapt to the arid and warm conditions in Central Spain by synthesizing longer
364 *n*-alkanoic acids. *Quercus c.* has a very high D index, but to our surprise *Quercus i.*
365 has a dominance of shorter homologues and a high C index. *Stipa t.* has a high D
366 index and plots unexpectedly far from the grass endmember, whereas *Thymus v.* has
367 a low D index and plots relatively close to the coniferous endmember. Overall, these
368 findings illustrate the variability of the *n*-alkanoic acid homologue patterns. They
369 also demonstrate the need to substantially enlarge the number of plant samples
370 from Iberia and Southern Europe before the CDG indices can be robustly used in
371 paleoenvironmental reconstructions.

372 **4.4 *n*-Alkanoic acid patterns in the LPS El Paraíso**

373 We evaluated the *n*-alkanoic acid variability in the LPS El Paraíso. C_{tot} of *n*-alkanoic
374 acids range from 9.5 µg/g in the uppermost sample to 0.01 µg/g dry sediment in the
375 deepest one (Fig. 5). Concentrations drop dramatically below 3 m depth. Low leaf
376 wax biomarker concentrations could reflect very arid climate conditions and low
377 vegetation density, but we suspect that degradation is also very relevant. EOPs are
378 extremely low (close to 1, apart from 2 samples) in the upper part of the sequence,
379 which indicates poor preservation of *n*-alkanoic acids, and EOPs could not even be

380 calculated below 3 m depth, because not all necessary compounds were above the
381 detection limit.

382

383 In general, preservation of lipids in soils depends on various chemical and
384 environmental factors, particularly soil pH (e.g. Nierop and Verstraten, 2003). While
385 *n*-alkane preservation is favoured by higher soil pH, *n*-alkanoic acids are better
386 preserved in more acidic soils (Bull et al., 2000). The arid conditions in Central
387 Spain and high soil pH might thus explain the poor preservation status of the *n*-
388 alkanoic acids in the LPS El Paraíso, particularly below ~3 m depth, i.e. before ~30
389 ka. More arid conditions before ~30 ka are in accordance with the interpretation of
390 sedimentary *n*-alkanes and the presence of carbonate concretions below ~4 m depth.

391

392 Despite the wide variability of the *n*-alkanoic acid indices C, D and G in our modern
393 vegetation samples, we calculated and plotted these indices for the LPS El Paraíso.
394 We did so in order to check for systematic patterns and changes in paleovegetation.
395 The *n*-alkanoic acid concentrations are high enough to calculate the indices only in
396 the upper part of the sequence. The sample PA55 (Fig. 5) has a high C indice and
397 plot close to the coniferous endmember in the CDG ternary plot. This suggests that
398 *Pinus* and *Quercus i.* may have contributed most to the sedimentary leaf waxes. For
399 the MIS 2 samples between ~1.4 and 1.9 m depth, relatively high D indices coincide
400 with lower G indices, whereas generally lower D indices and higher G indices
401 characterize the sediments and soils before ~30 ka. In the CDG ternary plot, the
402 “MIS 2” samples therefore plot lower than the “MIS 3” samples (Fig. 4b). Although

403 assigning specific sources and robustly reconstructing past changes in vegetation
404 are not possible at this stage, we speculate that the MIS 2 samples contain more
405 input from *Quercus i.* and *c.*, and possibly also from deciduous trees, whereas the
406 longer chain lengths in the MIS 3 samples reflect adaptations to more arid
407 conditions. Such an interpretation would be consistent with the *n*-alkanes and
408 would lend support to the assumption of an arid MIS 3 in Spain as documented by
409 Álvarez-Lao et al., 2015.

410 **5. Conclusions**

- 411 •The *n*-alkane homologue patterns in plants from Central Spain are generally
412 consistent with earlier observations and confirm the hypothesis that broadleaf
413 trees, particularly deciduous trees, produce shorter chains than grasses and
414 herbs. However, plants in Central Spain seem to adapt to the arid and warm
415 conditions by synthesizing relatively long chains, as evidenced for example by
416 the C₃₃ dominance in *Olea* and *Juniperus*.
- 417 •*n*-Alkane patterns in the LPS El Paraíso are characterized by shorter chain
418 lengths during MIS 2, which lends support to the hypothesis that climate
419 conditions in the Mediterranean region were *not* particularly arid. In fact, our
420 findings, climate modelling studies, fluvial and lacustrine records, as well as
421 glacial refugia for temperate trees in Southern Europe, all suggest the
422 conditions were more humid than previously suggested mainly based on
423 palynology.

424 •The *n*-alkanoic acid patterns of the plants vary substantially and, like the *n*-
425 alkane patterns, indicate that adaptation to arid and warm climate involves
426 preferential synthesis of longer chains. Longer chains in the LPS El Paraíso
427 can tentatively be interpreted as confirming more arid conditions before ~30
428 ka, whereas more humid conditions can again be tentatively inferred for the
429 MIS 2.

430 Many more plant samples from Central Spain and from Southern Europe are
431 needed to corroborate the proposed relationships between leaf wax patterns and
432 climate. This is particularly the case for the *n*-alkanoic acids. In the future, we plan
433 to carry out compound-specific δD and $\delta^{13}C$ analyses on the leaf waxes from the LPS
434 El Paraíso in order to obtain supplementary paleoclimatic and -hydrologic
435 information (Sachse et al., 2012; Zech et al., 2013). Compound-specific ^{14}C analyses
436 shall also be carried out to establish a more precise and robust chronology (Häggi et
437 al., 2014).

438

439 **Acknowledgements**

440 We thank M. Suhr, S. Lutz, L. I. Schweri and L. Wüthrich for help in the laboratory.

441 This project was funded by SNSF (PP00P2 150590).

442

443 **References**

- 444 Almendros, G., Sanz, J., Velasco, F., 1996. Signatures of lipid assemblages in soils
445 under continental Mediterranean forests. *European Journal of Soil Science* 47,
446 183-196.
- 447 Álvarez-Lao, D.J., Ruiz-Zapata, M.B., Gil-García, M.J., Ballesteros, D., Jiménez-
448 Sánchez, M., 2015. Palaeoenvironmental research at Raxidora Cave: New
449 evidence of cold and dry conditions in NW Iberia during MIS 3. *Quaternary*
450 *International* 379, 35-46.
- 451 Beghin, P., Charbit, S., Kageyama, M., Combourieu-Nebout, N., Hatté, C., Dumas,
452 C., Peterschmitt, J.Y., 2015. What drives LGM precipitation over the western
453 Mediterranean? A study focused on the Iberian Peninsula and northern Morocco.
454 *Clim Dyn*, 1-21.
- 455 Benito, G., Sopena, A., Sánchez-Moya, Y., Machado, M.a.J., Pérez-González, A.,
456 2003. Palaeoflood record of the Tagus River (Central Spain) during the Late
457 Pleistocene and Holocene. *Quaternary Science Reviews* 22, 1737-1756.
- 458 Bianchi, G., Vlahov, G., Anglani, C., Murelli, C., 1992. Epicuticular wax of olive
459 leaves. *Phytochemistry* 32, 49-52.
- 460 Bout-Roumazielles, V., Combourieu Nebout, N., Peyron, O., Cortijo, E., Landais, A.,
461 Masson-Delmotte, V., 2007. Connection between South Mediterranean climate
462 and North African atmospheric circulation during the last 50,000 yr BP North
463 Atlantic cold events. *Quaternary Science Reviews* 26, 3197-3215.

464 Brincat, D., Yamada, K., Ishiwatari, R., Uemura, H., Naraoka, H., 2000. Molecular-
465 isotopic stratigraphy of long-chain *n*-alkanes in Lake Baikal Holocene and glacial
466 age sediments. *Organic Geochemistry* 31, 287-294.

467 Bull, I.D., Bergen, P.F.v., Nott, C.J., Poulton, P.R., Evershed, R.P., 2000. Organic
468 geochemical studies of soils from the Rothamsted classical experiments—V. The
469 fate of lipids in different long-term experiments. *Organic Geochemistry* 31, 389-
470 408.

471 Bush, R.T., McInerney, F.A., 2013. Leaf wax *n*-alkane distributions in and across
472 modern plants: Implications for paleoecology and chemotaxonomy. *Geochimica et*
473 *Cosmochimica Acta* 117, 161-179.

474 Bush, R.T., McInerney, F.A., 2015. Influence of temperature and C₄ abundance on *n*-
475 alkane chain length distributions across the central USA. *Organic Geochemistry*
476 79, 65-73.

477 Calvo, J.P., Alonso-Zarza, A.M., Garcia del Cura, M.A., Ordoñez, S., Rodriguez-
478 Aranda, J.P., Sanz Montero, M.E., 1996. Sedimentary evolution of lake systems
479 through the Miocene of the Madrid Basin: paleoclimatic and paleohydrological
480 constraints, In: Friend, P. F. and Dabrio, C. J. (ed.): *Tertiary basins of Spain: the*
481 *stratigraphic record of crustal kinematics*, pp. 272–277.

482 Carrión, J.S., Yll, E.I., Walker, M.J., Legaz, A.J., Chaín, C., López, A., 2003. Glacial
483 refugia of temperate, Mediterranean and Ibero-North African flora in south-
484 eastern Spain: new evidence from cave pollen at two Neanderthal man sites.
485 *Global Ecology and Biogeography* 12, 119-129.

486 Clark , P. U., Dyke, A. S., Shakun, J. D., Carlson, A. E., Clark, J., Wohlfarth, B.,
487 Mitrovic, J. X., Hostetler, S. W., Marshall McCabe, A. 2009. The Last Glacial
488 Maximum. *Science* 325, 710-714.

489 Cranwell, P.A., 1973. Chain-length distribution of *n*-alkanes from lake sediments in
490 relation to post-glacial environmental change. *Freshwater Biology* 3, 259-265.

491 Diefendorf, A.F., Freeman, K.H., Wing, S.L., Graham, H.V., 2011. Production of *n*-
492 alkyl lipids in living plants and implications for the geologic past. *Geochimica et*
493 *Cosmochimica Acta* 75, 7472-7485.

494 Eglinton, G., Hamilton, R.J., 1967. Leaf epicuticular waxes. *Science* 156, 1322-1335.

495 Eglinton, T.I., Eglinton, G., 2008. Molecular proxies for paleoclimatology. *Earth and*
496 *Planetary Science Letters* 275, 1-16.

497 Ficken, K.J., Barber, K.E., Eglinton, G., 1998. Lipid biomarker, $\delta^{13}\text{C}$ and plant
498 macrofossil stratigraphy of a Scottish montane peat bog over the last two
499 millennia. *Organic Geochemistry* 28, 217-237.

500 Garcia Giménez, R., Vigil de la Villa, R., González Martín, J.A., 2012.
501 Characterization of loess in central Spain: a microstructural study.
502 *Environmental Earth Science* 65, 2125-2137.

503 García, R., Petit-Domínguez, M.D., Rucandio, M.I., González, J.A., 2011. Provenance
504 of loess from the Spanish central region: chemometric interpretation. *Geological*
505 *Magazine* 148, 481-491.

506 Giorgi, F., Lionello, P., 2008. Climate change projections for the Mediterranean
507 region. *Global and Planetary Change* 63, 90-104.

508 González-Sampériz, P., Leroy, S.A.G., Carrión, J.S., Fernández, S., García-Antón,
509 M., Gil-García, M.J., Uzquiano, P., Valero-Garcés, B., Figueiral, I., 2010. Steppes,
510 savannahs, forests and phytodiversity reservoirs during the Pleistocene in the
511 Iberian Peninsula. *Review of Palaeobotany and Palynology* 162, 427-457.

512 González-Sampériz, P., Valero-Garcés, B.L., Moreno, A., Jalut, G., García-Ruiz,
513 J.M., Martí-Bono, C., Delgado-Huertas, A., Navas, A., Otto, T., Dedoubat, J.J.,
514 2006. Climate variability in the Spanish Pyrenees during the last 30,000 yr
515 revealed by the El Portalet sequence. *Quaternary Research* 66, 38-52.

516 Gülz, P.-G., 1994. Epicuticular Leaf Waxes in the Evolution of the Plant Kingdom.
517 *Journal of Plant Physiology* 143, 453-464.

518 Häggi, C., Zech, R., McIntyre, C., Zech, M., Eglinton, T.I., 2014. On the stratigraphic
519 integrity of leaf-wax biomarkers in loess paleosols. *Biogeosciences* 11, 2455-2463.

520 Hoefs, M.J.L., Rijpstra, W.I.C., Sinninghe Damsté, J.S., 2002. The influence of oxic
521 degradation on the sedimentary biomarker record I: evidence from Madeira
522 Abyssal Plain turbidites. *Geochimica et Cosmochimica Acta* 66, 2719-2735.

523 Lewis, C.J., McDonald, E.V., Sancho, C., Peña, J.L., Rhodes, E.J., 2009. Climatic
524 implications of correlated Upper Pleistocene glacial and fluvial deposits on the
525 Cinca and Gállego Rivers (NE Spain) based on OSL dating and soil stratigraphy.
526 *Global and Planetary Change* 67, 141-152.

527 Maffei, M., Badino, S., Bossi, S., 2004. Chemotaxonomic significance of leaf wax n-
528 alkanes in the Pinales (Coniferales). *Journal of Biological Research* 1, 3-19.

529 Mihailova, A., Abbado, D., Pedentchouk, N., 2015. Differences in n-alkane profiles
530 between olives and olive leaves as potential indicators for the assessment of olive

531 leaf presence in virgin olive oils. *European Journal of Lipid Science and*
532 *Technology* 117, 1480-1485.

533 Moreno, A., González-Sampériz, P., Morellón, M., Valero-Garcés, B.L., Fletcher,
534 W.J., 2012. Northern Iberian abrupt climate change dynamics during the last
535 glacial cycle: A view from lacustrine sediments. *Quaternary Science Reviews* 36,
536 139-153.

537 Moreno, A., Svensson, A., Brooks, S.J., Connor, S., Engels, S., Fletcher, W., Genty,
538 D., Heiri, O., Labuhn, I., Perşoiu, A., Peyron, O., Sadori, L., Valero-Garcés, B.,
539 Wulf, S., Zanchetta, G., 2014. A compilation of Western European terrestrial
540 records 60–8 ka BP: towards an understanding of latitudinal climatic gradients.
541 *Quaternary Science Reviews* 106, 167-185.

542 Nierop, K.G.J., Verstraten, J.M., 2003. Organic matter formation in sandy
543 subsurface horizons of Dutch coastal dunes in relation to soil acidification.
544 *Organic Geochemistry* 34, 499-513.

545 Pallàs, R., Rodés, Á., Braucher, R., Carcaillet, J., Ortuño, M., Bordonau, J., Bourlès,
546 D., Vilaplana, J.M., Masana, E., Santanach, P., 2006. Late Pleistocene and
547 Holocene glaciation in the Pyrenees: a critical review and new evidence from ¹⁰Be
548 exposure ages, south-central Pyrenees. *Quaternary Science Reviews* 25, 2937-
549 2963.

550 Pantaléon-Cano, J., Yll, E.-I., Pérez-Obiol, R., Roure, J.M., 2003. Palynological
551 evidence for vegetational history in semi-arid areas of the western
552 Mediterranean (Almería, Spain). *The Holocene* 13, 109-119.

553 Poynter, J.G., 1989. Molecular stratigraphy: the recognition of palaeoclimatic
554 signals in organic geochemical data. University of Bristol, Bristol.

555 Poynter, J.G., Farrimond, P., Robinson, N., Eglinton, G., 1989. Aeolian-derived
556 higher plant lipids in the marine sedimentary record: links with paleoclimate,
557 Paleoclimatology and Paleometeorology: Modern and Past Patterns of Global
558 Atmospheric Transport. Kluwer Academic, Hingham, Mass, Dordrecht, pp. 435–
559 462.

560 Prentice, I.C., Guiot, J., Harrison, S.P., 1992. Mediterranean vegetation, lake levels
561 and palaeoclimate at the Last Glacial Maximum. *Nature* 360, 658-660.

562 Rodríguez-Sánchez, F., Hampe, A., Jordano, P., Arroyo, J., 2010. Past tree range
563 dynamics in the Iberian Peninsula inferred through phylogeography and
564 palaeodistribution modelling: A review. *Review of Palaeobotany and Palynology*
565 162, 507-521.

566 Rommerskirchen, F., Plader, A., Eglinton, G., Chikaraishi, Y., Rullkötter, J., 2006.
567 Chemotaxonomic significance of distribution and stable carbon isotopic
568 composition of long-chain alkanes and alkan-1-ols in C₄ grass waxes. *Organic*
569 *Geochemistry* 37, 1303-1332.

570 Roquero, E., Silva, P.G., Goy, J.L., Zazo, C., Massana, J., 2015. Soil evolution indices
571 in fluvial terrace chronosequences from central Spain (Tagus and Duero fluvial
572 basins). *Quaternary International* 376, 101-113.

573 Sachse, D., Billault, I., Bowen, G.J., Chikaraishi, Y., Dawson, T.E., Feakins, S.J.,
574 Freeman, K.H., Magill, C.R., McInerney, F.A., van der Meer, M.T.J., Polissar, P.,
575 Robins, R.J., Sachs, J.P., Schmidt, H.-L., Sessions, A.L., White, J.W.C., West,

576 J.B., Kahmen, A., 2012. Molecular Paleohydrology: Interpreting the Hydrogen-
577 Isotopic Composition of Lipid Biomarkers from Photosynthesizing Organisms.
578 Annual Review of Earth and Planetary Sciences 40, 221-249.

579 Sachse, D., Radke, J., Gleixner, G., 2006. δD values of individual n-alkanes from
580 terrestrial plants along a climatic gradient – Implications for the sedimentary
581 biomarker record. Organic Geochemistry 37, 469-483.

582 Schäfer, I.K., Lanny V., Eglinton, T.I., Zech, M., Zech, R., submitted. The role of
583 vegetation, degradation and climate for leaf waxes in litter and topsoils in
584 Europe.

585 Schwark, L., Zink, K., Lechterbeck, J., 2002. Reconstruction of postglacial to early
586 Holocene vegetation history in terrestrial Central Europe via cuticular lipid
587 biomarkers and pollen records from lake sediments. Geology 30, 463-466.

588 Seager, R., Liu, H., Henderson, N., Simpson, I., Kelley, C., Shaw, T., Kushnir, Y.,
589 Ting, M., 2014. Causes of Increasing Aridification of the Mediterranean Region
590 in Response to Rising Greenhouse Gases. Journal of Climate 27, 4655-4676.

591 Tarasov, P.E., Müller, S., Zech, M., Andreeva, D., Diekmann, B., Leipe, C., 2013.
592 Last glacial vegetation reconstructions in the extreme-continental eastern Asia:
593 Potentials of pollen and n-alkane biomarker analyses. Quaternary International
594 290–291, 253-263.

595 Tipple, B.J., Pagani, M., 2010. A 35 Myr North American leaf-wax compound-
596 specific carbon and hydrogen isotope record: Implications for C₄ grasslands and
597 hydrologic cycle dynamics. Earth and Planetary Science Letters 299, 250-262.

598 Tipple, B.J., Pagani, M., 2013. Environmental control on eastern broadleaf forest
599 species' leaf wax distributions and D/H ratios. *Geochimica et Cosmochimica Acta*
600 111, 64-77.

601 Vogts, A., Schefuß, E., Badewien, T., Rullkötter, J., 2012. *n*-Alkane parameters from
602 a deep sea sediment transect off southwest Africa reflect continental vegetation
603 and climate conditions. *Organic Geochemistry* 47, 109-119.

604 Wang, N., Zong, Y., Brodie, C.R., Zheng, Z., 2014. An examination of the fidelity of
605 *n*-alkanes as a palaeoclimate proxy from sediments of Palaeolake Tianyang,
606 South China. *Quaternary International* 333, 100-109.

607 Wolf, D., Faust, D. (2015): Western Mediterranean environmental changes:
608 Evidences from fluvial archives. *Quaternary Science Reviews*
609 (QSR) 122, 30 – 50

610 Zech, M., Andreev, A., Zech, R., Müller, S., Hambach, U., Frechen, M., Zech, W.,
611 2010. Quaternary vegetation changes derived from a loess-like permafrost
612 palaeosol sequence in northeast Siberia using alkane biomarker and pollen
613 analyses. *Boreas* 39, 540-550.

614 Zech, M., Buggle, B., Leiber, K., Marković, S., Glaser, B., Hambach, U., Huwe, B.,
615 Stevens, T., Sümege, P., Wiesenberg, G., Zöller, L., 2009. Reconstructing
616 Quaternary vegetation history in the Carpathian Basin, SE-Europe, using *n*-
617 alkane biomarkers as molecular fossils: Problems and possible solutions,
618 potential and limitations. *Eiszeitalter und Gegenwart. Quaternary Science*
619 *Journal* 58, 148-155.

620 Zech, M., Rass, S., Buggle, B., Löscher, M., Zöller, L., 2012. Reconstruction of the
621 late Quaternary paleoenvironments of the Nussloch loess paleosol sequence,
622 Germany, using *n*-alkane biomarkers. *Quaternary Research* 78, 226-235.

623 Zech, M., Zech, R., Buggle, B., Zöller, L., 2011. Novel methodological approaches in
624 loess research—interrogating biomarkers and compound-specific stable isotopes.
625 *Eiszeitalter & Gegenwart-Quatern Sci J* 60, 170-187.

626 Zech, R., Zech, M., Marković, S., Hambach, U., Huang, Y., 2013. Humid glacials,
627 arid interglacials? Critical thoughts on pedogenesis and paleoclimate based on
628 multi-proxy analyses of the loess–paleosol sequence Crvenka, Northern Serbia.
629 *Palaeogeography, Palaeoclimatology, Palaeoecology* 387, 165-175.

630 Zhang, Z., Zhao, M., Eglinton, G., Lu, H., Huang, C.-Y., 2006. Leaf wax lipids as
631 paleovegetational and paleoenvironmental proxies for the Chinese Loess Plateau
632 over the last 170 kyr. *Quaternary Science Reviews* 25, 575-594.

633 Zocatelli, R., Lavrieux, M., Disnar, J.-R., Le Milbeau, C., Jacob, J., Bréheret, J.,
634 2012. Free fatty acids in Lake Aydat catchment soils (French Massif Central):
635 sources, distributions and potential use as sediment biomarkers. *Journal Soils*
636 *Sediments* 12, 734-748.

637

638 **Captions**

639 Fig 1: (a) Map of the sample location, (b) photograph of the research area and (c) the
640 LPS El Paraíso

641 Fig. 2: Endmember plot modified after Zech et al. (2013) and Schäfer et al.
642 (submitted) with a) plant samples from Central Spain, and b) samples from the LPS
643 El Paraíso. Degradation line grass: $0.129 \cdot \log(\text{OEP}) + 0.5745$, degradation line
644 deciduous forests: $-0.171 \cdot \log(\text{OEP}) + 0.7459$.

645 Fig. 3: *n*-Alkane proxies in the LPS El Paraíso. From left to right: concentrations of
646 long-chain *n*-alkanes from *n*-C₂₅ to *n*-C₃₅, OEP, *n*-alkane ratio $(n\text{-C}_{31}+n\text{-C}_{33})/(n\text{-C}_{27}+n\text{-C}_{31}+n\text{-C}_{33})$ and grass contribution (%). Loess units are shaded in yellow.

648 Fig. 4: Ternary plot for *n*-alkanoic acid indices C, D and G with a) plant samples
649 from Central Spain, and b) samples from the LPS El Paraíso. Endmembers for
650 conifers (con), deciduous trees (dec) and grasses (grass) from Central Europe after
651 Schäfer et al. (submitted)

652 Fig. 5: *n*-Alkanoic acid proxies in the LPS El Paraíso. From left to right:
653 Concentrations of long-chain *n*-alkanoic acids (C₂₀-C₃₄), EOP, indices C, D and G.
654 Loess units are shaded in yellow.

Figure 1
[Click here to download high resolution image](#)



Figure 2

[Click here to download high resolution image](#)

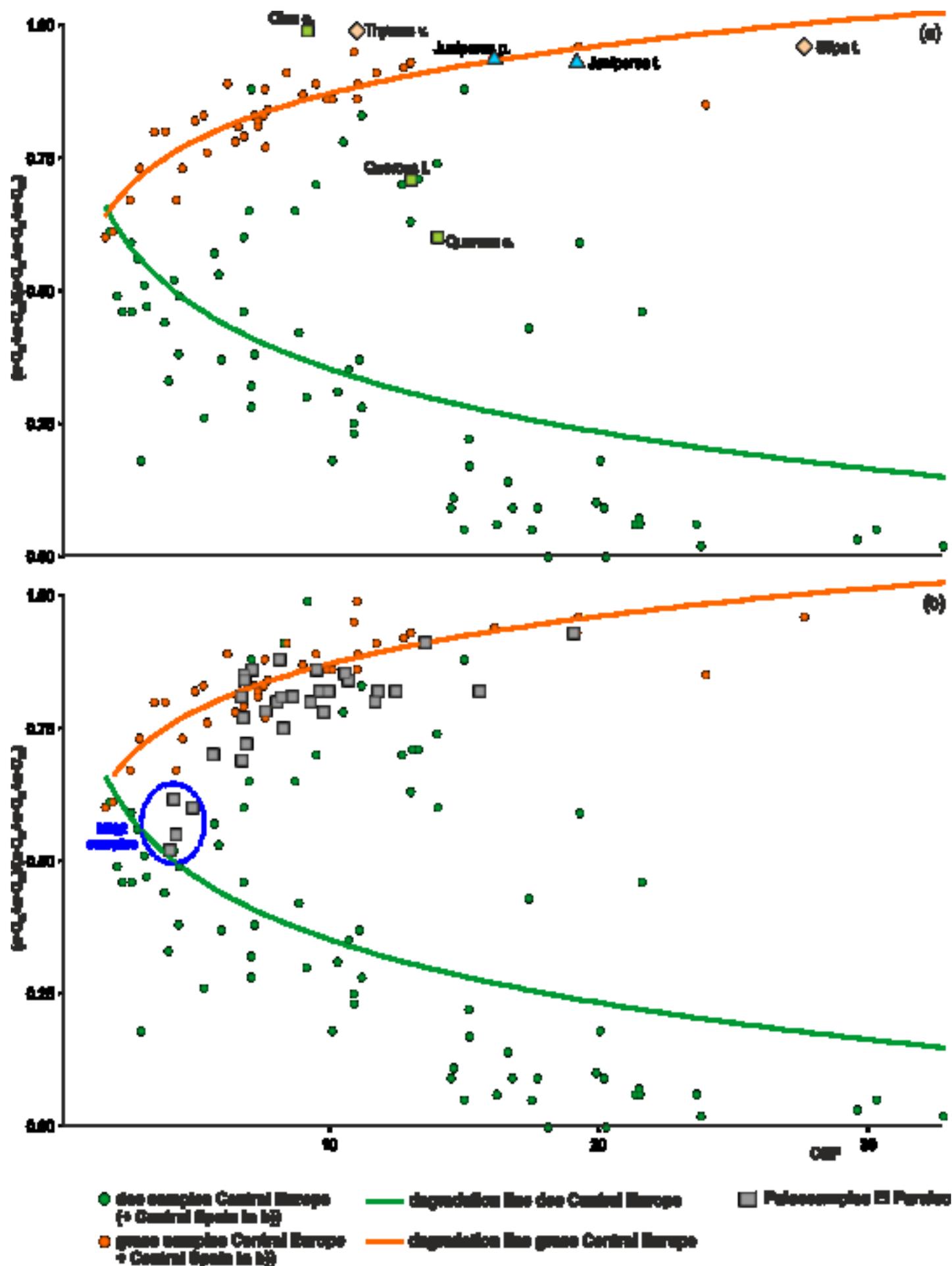
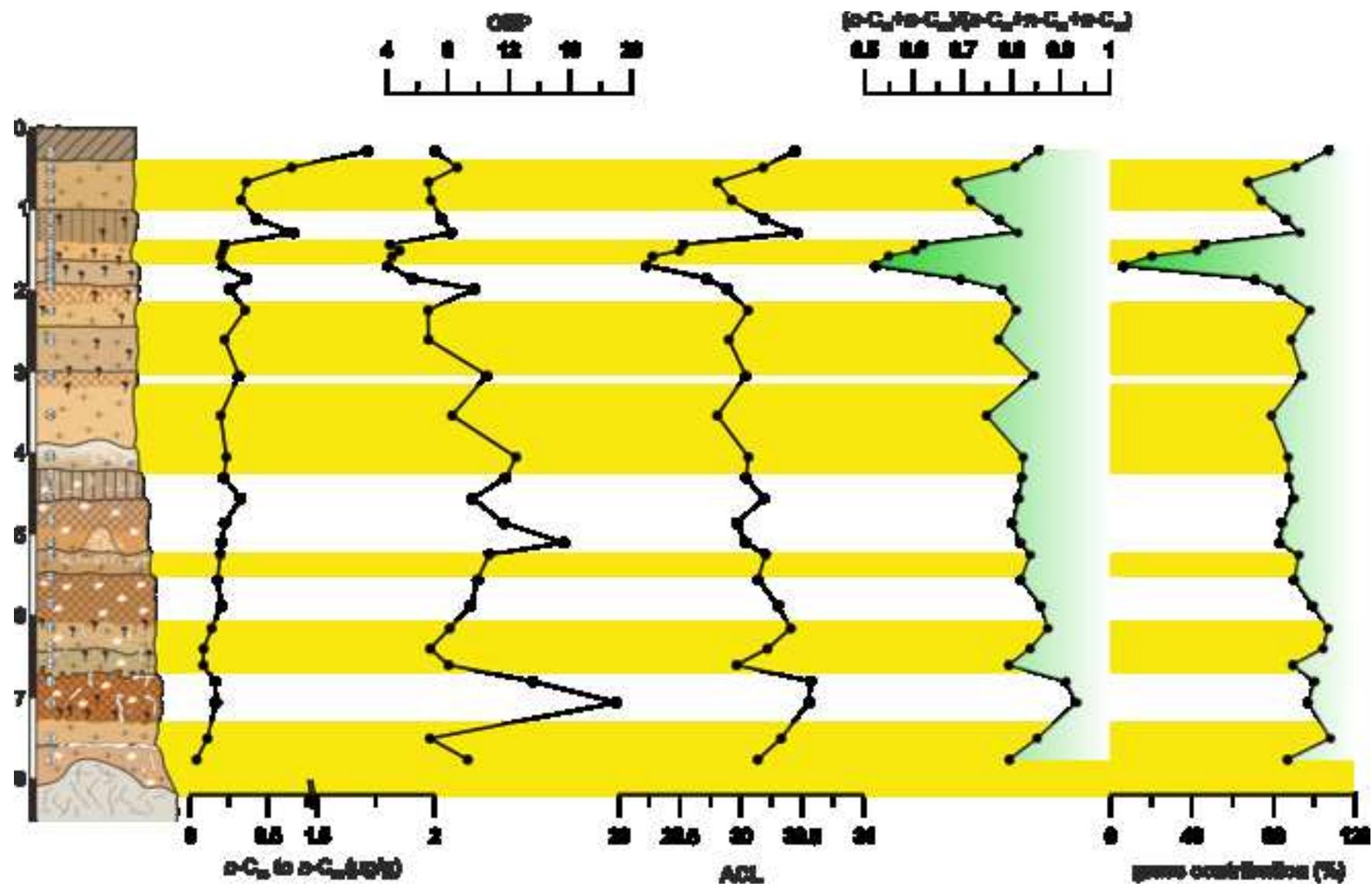


Figure 3
[Click here to download high resolution image](#)



- | | | |
|---|---|---|
|  colluvium |  tertiary marls |  bioturbation |
|  A-horizon |  calcareous/gypsey pseudo-myoella |  sample number |
|  rubefied B-horizon |  CaCO ₃ crust/ fragments | |
|  loess rich in fine sand |  small secondary concretions (CaCO ₃ /gypsum) | |

Figure 4
[Click here to download high resolution image](#)

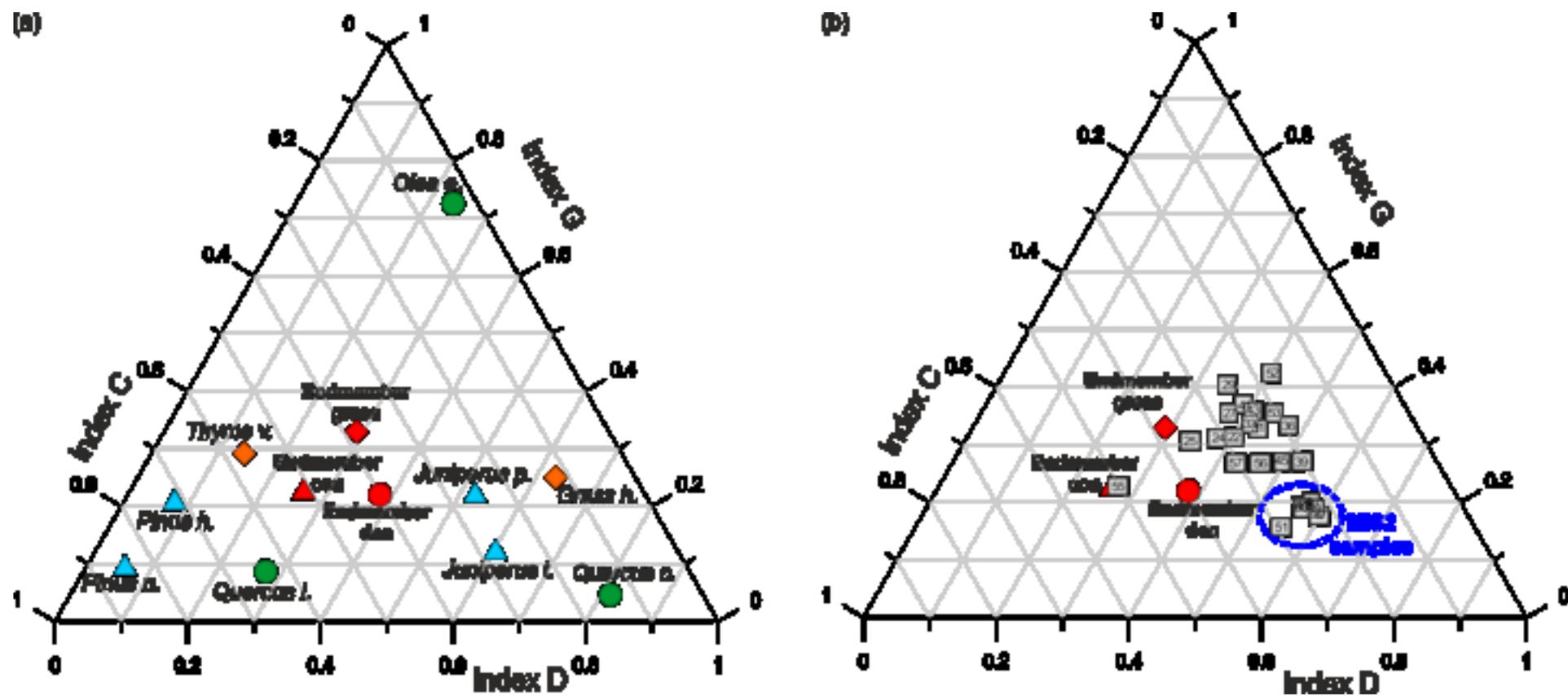


Figure 5
[Click here to download high resolution image](#)

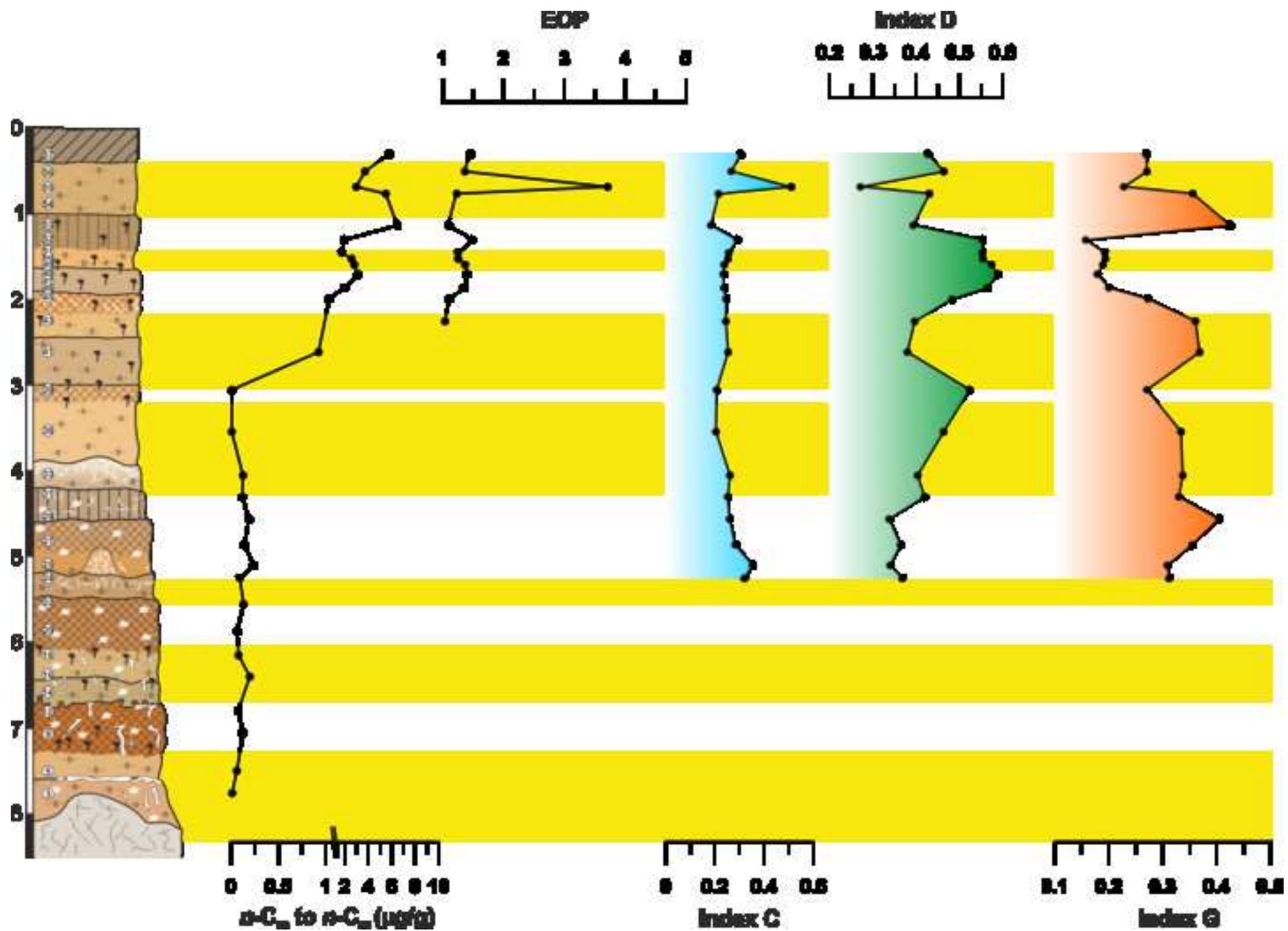


Table 1: *n*-alkane distribution in fresh plant material typical for the local vegetation at our sample location

sample	<i>n</i> -C ₂₅	<i>n</i> -C ₂₇	<i>n</i> -C ₂₉	<i>n</i> -C ₃₁	<i>n</i> -C ₃₃	<i>n</i> -C ₃₅	<i>c</i> _{tot}	OEP	ACL	$(n-C_{31}+n-C_{33})/(n-C_{27}+n-C_{31}+n-C_{33})$
<i>Juniperus phonicea</i>	0.42	3.55	1.88	5.60	50.13	17.29	86.11	16.13	32.35	0.94
<i>Juniperus thurifera</i>	0.66	3.95	3.50	12.09	80.13	13.37	121.89	19.17	32.38	0.96
<i>Pinus nigra</i>	0.35	0.38	0.44	0.45	3.32	0.41	6.81	4.29	31.93	0.91
<i>Pinus halepensis</i>	0.35	0.42	0.43	0.42	0.37	0.40	3.87	1.52	29.90	0.65
<i>Quercus coccifera</i>	3.14	26.67	52.41	38.84	1.51	0.45	131.97	14.02	29.25	0.60
<i>Quercus ilex</i>	0.71	7.44	63.76	17.17	0.82	0.42	97.55	13.07	29.25	0.71
<i>Olea europaea</i>	0.28	0.86	11.05	37.89	44.85	17.10	127.73	9.15	31.68	0.99
<i>Thymus vulgaris</i>	1.20	2.09	39.79	125.70	175.01	8.50	390.76	11.03	31.77	0.99
<i>Stipa tenacissima</i>	2.76	8.54	43.13	168.07	60.16	1.84	295.20	27.63	31.00	0.96

Table 2: *n*-alkanoic acid distribution in the fresh plant material from vegetation typical at our sampling location

sample	Concentration in $\mu\text{g/g}$ sample							EOP	Index C	Index D	Index G
	<i>n</i> -C ₂₄	<i>n</i> -C ₂₆	<i>n</i> -C ₂₈	<i>n</i> -C ₃₀	<i>n</i> -C ₃₂	<i>n</i> -C ₃₄	C _{tot}				
<i>Juniperus phonicea</i>	1.75	1.05	3.53	1.17	0.46	1.06	12.58	5.78	0.25	0.52	0.22
<i>Juniperus thurifera</i>	2.10	1.12	4.14	1.29	0.42	0.45	15.14	7.76	0.26	0.58	0.12
<i>Pinus nigra</i>	4.03	0.49	0.28	0.19	0.14	0.35	16.28	3.56	0.87	0.06	0.10
<i>Pinus halepensis</i>	5.06	0.72	0.56	0.67	0.56	1.02	32.38	5.00	0.74	0.08	0.22
<i>Quercus coccifera</i>	3.78	13.40	16.32	7.50	0.86	0.07	62.49	11.06	0.13	0.78	0.04
<i>Quercus ilex</i>	5.81	1.23	1.53	5.00	0.45	0.03	25.39	11.75	0.45	0.20	0.06
<i>Olea europaea</i>	0.85	1.56	4.80	8.10	9.64	5.03	35.80	8.25	0.04	0.24	0.72
<i>Thymus vulgaris</i>	8.95	6.61	1.92	4.10	2.06	1.97	37.91	10.47	0.53	0.13	0.27
<i>Stipa tenacissima</i>	1.21	2.30	4.74	3.02	1.59	0.30	17.76	11.29	0.11	0.60	0.24



^b
UNIVERSITÄT
BERN

OESCHGER CENTRE
CLIMATE CHANGE RESEARCH

Imke Kathrin Schäfer
Institute of Geography
University of Bern
Hallerstrasse 12
CH- 3012 Bern
+41(0) 31 631 8890
imke.schaefer@giub.unibe.ch

Dear editors of Quaternary International,

Please find attached the revised version of our manuscript entitled

Evidence for humid conditions during the last glacial from leaf wax patterns in the loess-paleosol sequence El Paraíso, Central Spain

by Imke K. Schäfer, Marcel Bliedtner, Daniel Wolf, Dominik Faust, Roland Zech

We thank the anonymous reviewer for his comments and suggestions. We revised our manuscript accordingly:

- 1: The English for our manuscript was corrected by a native speaker (service from the Oeschger Centre for Climate Change Research)
- 2: We deleted Lines 231-233 as suggested. We prefer, however, to keep lines 399-405. Those give an outlook for our ongoing work.
- 3: Lines 290-293: To our knowledge there are no published pollen record that support a humid LGM, which we clarified in the revised manuscript.
- 4: Lines 356-361: We agree with the reviewer that an arid climate and hence a low vegetation density can be an additional explanation for the low *n*-alkanoic acid concentrations. We now included this in the discussion.
- 5: As suggested by the reviewer we modified the discussion concerning the agreement of our results with previous findings. This now reads as: Our results and interpretation are in line with fluvial and lacustrine records from Iberia, which suggest there were more humid conditions during the global LGM, probably due to a southward shift of the westerlies and storm tracks (Moreno et al., 2012; Moreno et al., 2014; Beghin et al., 2015). Our results and interpretation are also in line with genetic features of recent European tree populations, which show that tree species survived in glacial refugia in Southern Europe during the last glacial (Carrión et al., 2003; González-Sampériz et al., 2010).
- 6: We replaced “Conclusion” with “Conclusions” as the reviewer requested

With many thanks for your efforts

Yours sincerely,

A handwritten signature in blue ink that reads "Imke Schäfer". The signature is written in a cursive style with a large, stylized 'S' at the end.

Imke Schäfer