

1 Recent advances in isotopes as palaeolimnological proxies

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11

12 **Abstract**

13 Isotope geochemistry is an essential part of environmental and climate change research and
14 over the last few decades has contributed significantly to our understanding of a huge array of
15 environmental problems, not least in palaeolimnology and limnogeology. Here we describe
16 some of the recent developments in the use of stable isotopes in palaeo-lake research. These
17 are: better preparation, analysis, and interpretation of biogenic silica oxygen and silicon
18 isotopes; extraction and characterisation of specific compounds such as leaf waxes and algal
19 lipids for isotope analysis; determining the excess of ¹³C-¹⁸O bonds in clumped isotopes; and
20 the measurement of multiple isotope ratios in chironomid chitin. These advances have
21 exciting prospects and it will be interesting to see how these techniques develop further and
22 consequently offer a real advancement in our science over the next decade.

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24

25 **Introduction**

26 Isotope geochemistry has become an essential part of environmental and climate change
27 research over the last few decades and has contributed significantly to our understanding of a
28 huge array of environmental problems, which span the whole of Earth system science and not
29 least in palaeolimnology and limnogeology. Continual improvements over time have been
30 made in preparatory methods and mass spectrometry (de Groot 2004), specifically the
31 development of simpler (less time consuming) preparation procedures, decreases in sample
32 size, improved accuracy of measurements and better international standardisation. Now, on-
33 line systems including continuous flow and laser technology are routine and require very
34 small sample sizes and can measure high numbers of samples often completely automatically.
35 In addition there is an increasing move towards combined measurements of several isotopes
36 in a sample. Here, we describe specific advancements that have, or will, in our opinion,
37 enable significant advancements in palaeolimnology. For example; (1) better preparation,
38 analysis, and interpretation of biogenic silica oxygen and silicon isotopes; (2) extraction and
39 characterisation of specific compounds such as leaf waxes and algal lipids for isotope
40 analysis; (3) determining the excess of ^{13}C - ^{18}O bonds in clumped isotopes; and (4)
41 measurement of multiple isotope ratios in chironomids. We summarise these significant
42 advancements, some of which are relatively new to palaeolimnology so there are few
43 published examples to draw upon. It will be interesting in the next decade to see which of
44 these techniques take off and offer a real advancement in our science.

45 **Biogenic silica oxygen and silicon isotopes**

46 The use of oxygen and silicon isotopes in biogenic silica was developed by oceanographers in
47 the 1970s and 80's (Labeyrie 1974; Labeyrie and Juillet 1982; Labeyrie et al. 1984)
48 following techniques developed forty to fifty years ago (see Clayton and Mayeda 1963). The
49 oxygen ($\delta^{18}\text{O}$), silicon ($\delta^{30}\text{Si}$), carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) compositions of biogenic
50 silica are increasingly being used as proxies for environmental change. $\delta^{18}\text{O}$ tends to be used
51 as a measure of temperature/water composition variation, $\delta^{30}\text{Si}$ for productivity, and $\delta^{13}\text{C}$ and
52 $\delta^{15}\text{N}$ for nutrient cycling/source investigation. Biogenic silica is a structurally complex
53 mineral especially for $\delta^{18}\text{O}$ measurement; carbon and nitrogen (for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) occur in
54 very small quantities in organic material hosted within the structure and can be difficult to
55 extract, while the measurement of $\delta^{30}\text{Si}$ is relatively simple (in comparison to $\delta^{18}\text{O}$, $\delta^{13}\text{C}$,

56 $\delta^{15}\text{N}$), but there are still uncertainties over the interpretation of the $\delta^{30}\text{Si}$ signal in
57 palaeolimnology, largely as a result of the paucity of studies. However, the recent renewed
58 effort in using biogenic silica in palaeoenvironmental research (especially diatom silica in
59 palaeolimnology; Leng and Barker 2006) has highlighted new ways of dealing with the many
60 issues that accompany its use. Specifically these issues are: contamination; the hydrous layer
61 and associated maturation of diatom silica; controls on the $\delta^{18}\text{O}$; the also the forward
62 potential of $\delta^{30}\text{Si}$ and occluded $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in diatom silica.

63

64 *Contamination of biogenic silica*

65

66 Much effort has been placed on diatom purification and methodological issues prior to
67 isotope analysis (Shemesh et al. 1995; Morley et al. 2004; Lamb et al. 2005; Schleser et al.
68 2001; Rings et al. 2004; Brewer et al. 2008; Tyler et al. 2007; van Bennekom and van der
69 Gaast 1976; Mackay et al. 2011) as almost pure biogenic silica is required since oxygen and
70 silicon are common elements in other components found in lake sediments (clay, silt, tephra,
71 carbonates) and these can affect the isotope signal or in the case of organic carbon interfere
72 with methodological procedures. While standard chemical leaching and physical separation
73 approaches (sieving, heavy liquids) work well for samples with a high proportion of diatom
74 silica (>10%) more sophisticated and time consuming approaches are required to clean
75 relatively diatom poor (<10%) material, where sample sizes are small or where the
76 contaminant is similar in size and density to the diatom silica. Relatively new approaches
77 include SPLITT (gravitational split-flow lateral-transport), micromanipulation, and chemical
78 mass balance modelling. SPLITT is an approach similar to heavy liquid separation (Giddings
79 1985) whereby individual particles within a sample are separated under laminar flow of water
80 on the basis of their density, size and shape. This approach has been successfully applied to
81 the separation of diatoms from other particles (Schleser et al. 2001; Rings et al. 2004; Leng
82 and Barker 2006). Perhaps most time consuming of all is the use of a micro-manipulation
83 device attached to an inverted microscope with a cellular micro-injector system used to
84 extract individual non diatom particles from a sample. Whilst time consuming, it potentially
85 allows for the final stage removal of particles that are chemically and physically identical to
86 diatoms as well as potentially allowing the separation of diatom species (Snelling et al. in
87 press). Finally, where all other methods fail there is mass balance chemical modelling,
88 whereby a combination of whole-rock geochemistry and electron-optical imaging provides a

89 method for the identification, estimation of the amounts and subsequent removal of the
90 effects of different types of contamination (Lamb et al. 2005; Brewer et al. 2008; Mackay et
91 al. 2011; Fig. 1). This approach only works well in samples where the contamination is low
92 relative to the amount of diatom (<50%) and the contaminant has a uniform and consistent
93 chemical composition.

94

95 *The hydrous layer and maturation of oxygen isotopes in biogenic silica*

96

97 Biogenic silica has an amorphous structure containing Si-O-Si bonds, Si-OH bonds and
98 crystallization water (Knauth and Epstein 1982). These oxygen-bearing compounds (-OH
99 and H₂O) can exchange freely with water in their environment, for example with porewater
100 during burial of diatoms (Mopper and Garlick 1971; Kawabe 1978; Mikkelsen et al. 1978;
101 Schmidt et al. 1997; Brandriss et al. 1998; Moschen et al. 2006) or even with water used in
102 the preparation of the material in the laboratory. Because of the ready exchangeability of the
103 hydrous layer, and potentially wide ranging alteration of its isotope composition, it must be
104 removed prior to $\delta^{18}\text{O}$ measurement (Leng and Sloane 2008) making it a complex mineral to
105 analyse. Also the presence of this hydrous layer means that $\delta^{18}\text{O}$ may be influenced by
106 secondary processes that lead to early diagenetic changes. Schmidt et al. (2001) described the
107 influence of silica condensation on the isotopic composition of sedimented opal due to
108 isotope exchange. Moschen et al. (2006) ascribed ^{18}O enrichment of the diatomaceous silica
109 as an effect of biogenic silica maturation (dehydroxylation i.e. reduction of Si-OH groups)
110 after removal of the organic coatings. Sedimentary diatomaceous silica is likely to be affected
111 by secondary processes (especially the hydrous parts), however, the predominant portion of
112 the oxygen (c. 90%) should be bound to silicon in SiO₄ tetrahedrons (forming the structurally
113 bound oxygen and this oxygen should be more resistant to alteration). In addition progressive
114 silica maturation does not appear to occur within sedimentary archives because we would
115 expect (but do not see) a trend in $\delta^{18}\text{O}$ leading to a successive isotopic change through time.
116 It might be there is a very slow progression of the maturation process after a fast initial phase
117 of signal alteration and so that some of the $\delta^{18}\text{O}$ signal is in fact acquired soon after the
118 formation of the biogenic silica, during early diagenesis in the water column and during early
119 sediment burial. Dodd and Sharp (2010) showed that maturation is a process that occurs in
120 the water column or at the latest at the sediment-water interface. They comment that in the
121 case of deep lacustrine environments, where the bottom water remains at a nearly constant

122 temperature of 4°C, the re-equilibration of diatom silica with bottom conditions could reduce
123 or remove the conflating effects of temperature on $\delta^{18}\text{O}$ recorded by palaeo-diatom silica and
124 provide direct information on the $\delta^{18}\text{O}$ of the lake water. Overall though lake sediment
125 records are most likely site specific and as our knowledge of the likely effects of maturation
126 increases so does the effort in ensuring analysis is only undertaken of the tetrahedrally-
127 bonded oxygen.

128

129 The removal of the effect of the exchangeable oxygen contained in hydrous groups is perhaps
130 still the greatest analytical issue and there are two main approaches - chemical removal using
131 a fluorination reagent, and controlled isotopic exchange with subsequent removal by a mass
132 balance calculation. These two analytical protocols are the basis of four methods that have
133 been established to measure $\delta^{18}\text{O}$ in biogenic silica (Chapligin et al. 2011). These methods
134 have been developed relatively independently because there is no commercially available
135 “off the shelf” equipment that allows for dehydration of biogenic silica prior to extraction of
136 the oxygen isotopes and mass spectrometry. A brief summary of the methods is given below:

137

138 (1) The Controlled Isotopic Exchange (CIE) method where loosely-bound oxygen is
139 exchanged with oxygen from water vapour (and later mass balanced) before reaction with a
140 fluorinating reagent; (2) StepWise Fluorination (SWF) method in which the biogenic
141 material is step heated and reacted with a fluorinating reagent; (3) inductive High-
142 Temperature carbon reduction (iHTR) in which the silica is thermally dehydrated under
143 vacuum; and (4) inert Gas Flow Dehydration (iGFD) during which exchangeable oxygen is
144 thermally removed under a continuous flow of helium.

145

146 Generally, after the removal or fixing of the exchangeable oxygen by the methods described
147 above, the tetrahedrally-bonded oxygen from the Si-O-Si structure is liberated either by
148 conventional (slow reaction in heated nickel cylinders) or laser (fast reaction by laser heating)
149 fluorination with ClF_3 , BrF_5 or F_2 . The oxygen is then measured as CO_2 , CO or O_2 by mass
150 spectrometry. Chapligin et al. (2011) undertook an isotope standard comparison study, which
151 shows for the first time that despite procedural and methodological differences across the
152 eight laboratories that participated in the standard comparison study (using the 4 different
153 methods on six working standards), they produced data on working standards that have
154 standard deviations for $\delta^{18}\text{O}$ between 0.3 and 0.9‰ (1σ) and shows that the exchangeable

155 oxygen issue, at least analytically, is dealt with relatively consistently.

156

157 *Oxygen isotopes in diatom silica*

158

159 To date the most successful studies have been conducted on diatom silica in areas where the
160 $\delta^{18}\text{O}_{\text{diatom}}$ registers changes in the $\delta^{18}\text{O}$ composition of the lake water (rather than
161 temperature) which is then related to other aspects of climate, for example the
162 precipitation/evaporation balance (Rioual et al. 2001), the amount of precipitation in the
163 tropics (Barker et al. 2001), and changes in the source of precipitation in Northern Europe
164 (Shemesh et al. 2001a,b; Rosqvist et al. 2004; Jones et al. 2004). However, diatom oxygen
165 signals do contain a component of temperature, although empirical studies of the $\delta^{18}\text{O}$ of
166 diatom frustules have indicated a discrepancy in the silica–water fractionation factor between
167 modern/cultured diatoms and sediment-derived diatom records (e.g. Schmidt et al. 2001;
168 Moschen et al. 2005; 2006; Tyler et al. 2008). $\delta^{18}\text{O}$ values of modern diatoms collected as
169 living specimens from natural, freshwater environments record a temperature dependent
170 silica–water fractionation nearly identical to that reported by Brandriss et al. (1998) and
171 Moschen et al. (2005) from cultured diatom samples, both indicating a mineral–water
172 temperature coefficient of $-0.2\text{‰}/\text{°C}$. However, caution is needed, as described previously,
173 some studies suggest that the signal is incorporated during sinking in the water column and/or
174 in the bottom waters or at the sediment surface under different temperatures. Overall, while
175 $\delta^{18}\text{O}_{\text{diatom}}$ is likely not a good proxy for temperature alone there are many other aspects of
176 climate this it is ideally suited.

177

178 *Silicon isotopes in diatom silica*

179

180 In oceans there have been studies that have confirmed the link between diatom silicon
181 utilization and $\delta^{30}\text{Si}$ compositions (De La Rocha et al. 2000; Varela et al. 2004; Cardinal et
182 al. 2005). Consequently, $\delta^{30}\text{Si}$ data from the sedimentary records have been used as a proxy
183 to reconstruct palaeoproductivity (or more strictly marine silicic acid use by diatoms relative
184 to initial dissolved silicic acid concentrations; i.e. De La Rocha et al. 1998; Brzezinski et al.
185 2002). In continental waters, there have been relatively fewer $\delta^{30}\text{Si}$ investigations, and those
186 reported tend to be on major rivers (De La Rocha et al. 2000; Ding et al. 2004; 2011). Silicon
187 in rivers comprises both dissolved and particulate matter, and measurement of both allows an

188 assessment of weathering as well as productivity-related fractionation. These studies, whilst
189 highlighting the complexity of lake systems (potentially having several weathering
190 component fluxes) show that $\delta^{30}\text{Si}$ values are consistent with Si concentrations (De La Rocha
191 et al. 2000). They also suggest that Si isotope fractionation is independent of species and
192 (direct) temperature, offering potential information on changes in nutrient supply and
193 limnology. In palaeolimnology, studies have focused on the relationship between climate,
194 diatom productivity, and lake mixing regimes (Alleman et al. 2005) though to date there are
195 rather few studies to draw upon. Given the current interest in $\delta^{30}\text{Si}$ in palaeolimnology, we
196 are likely to see a surge of studies in the years to come especially accompanying the use of
197 $\delta^{18}\text{O}$ in diatom silica (Leng and Barker 2006).

198

199 One of the first studies of lacustrine $\delta^{30}\text{Si}$ is from Lake Rutundu, Mt. Kenya, over the last ~38
200 ka, which described a small, well studied lake/catchment in order to highlight processes that
201 may have operated at biome to continental scales during the Late Quaternary (Street-Perrott
202 et al. 2008). A combination of lake sediment fluxes and stable isotope ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$,
203 $\delta^{30}\text{Si}$) data showed that under glacial conditions high diatom productivity was maintained by
204 substantial transport of dissolved SiO_2 and soil nutrients from a sparse, leaky, terrestrial
205 ecosystem. During a period of high monsoon rainfall and seasonality rapid Si cycling by fire-
206 prone grassland was associated with substantial aeolian transport of opal phytoliths by smoke
207 plumes, but greatly reduced nutrient flux in runoff. Invasion of tall, subalpine shrubs
208 subsequently enhanced landscape stability, leading to very low sediment fluxes of both
209 phytoliths and diatoms. In another study Chen et al. (2012) measured $\delta^{30}\text{Si}$ from diatoms in
210 sediments from Lake Huguangyan, a closed crater lake in China. The results show a
211 relationship between $\delta^{30}\text{Si}_{\text{diatom}}$, biogenic silica content and local temperature records over the
212 last 50 years and northern hemisphere palaeotemperatures over the last 2000 years. They
213 interpret higher $\delta^{30}\text{Si}_{\text{diatom}}$ as an indication of greater dissolved silicic acid utilization at higher
214 temperature while lower $\delta^{30}\text{Si}_{\text{diatom}}$ reflects decreased utilization at lower temperature.

215

216 *Carbon and nitrogen isotopes in occluded organic matter in diatom silica*

217

218 Increasingly researchers are using isotope methods based on single organisms that use
219 dissolved carbon and nitrogen for photosynthesis to reveal changes in the carbon and nitrogen
220 cycle rather than bulk methods. In this context diatom frustule contains proteins (pleuralins,

221 silaffins and long chain polyamines) incorporated during growth that are central to silica
222 sequestration and become entombed within the silica cell wall structure (Hecky et al. 1973;
223 Kroger and Poulson 2008; Bridoux et al. 2010). Analyses of $\delta^{13}\text{C}_{\text{diatom}}$ (Jacot Des Combes et
224 al. 2008; Schneider-Mor et al. 2005; Shemesh et al. 1995) and $\delta^{15}\text{N}_{\text{diatom}}$ (e.g., Crosta and
225 Shemesh 2002; Crosta et al. 2005) in Southern Ocean cores highlight the feasibility and
226 applicability of these techniques in palaeoceanography. Pre-analysis the diatom silica has to
227 be prepared by removing all external mucilage organic matter. Since the amount of occluded
228 organic matter is small within diatom silica, several milligrams of material is used for the
229 analysis. However, these methods are not generally used in palaeolimnology where more
230 complex nutrient cycling can pose significant challenges for the interpretation of the
231 palaeoenvironmental record. There are advantages in using the isotope composition of
232 occluded organic matter within the silica of diatoms. It has been suggested that the isotope
233 composition within the diatom cell walls is not affected by post depositional degradation and
234 therefore potentially preserves an unaltered signal of surface water conditions during diatom
235 growth (Brenner et al. 1999; Ficken et al. 2000) and in addition avoids the generally
236 heterogeneous nature of bulk sedimentary organic matter (Hurrell et al. 2010). There are
237 unresolved analytical issues outstanding especially with $\delta^{15}\text{N}_{\text{diatom}}$ measurements, in particular
238 the ongoing discussion of issues around the persulfate-denitrifier technique (Leng and Swann
239 2010). Few studies exist of isotopes in occluded organic matter in diatom silica. In a recent
240 study of a 25,000-year sediment record from Lake Challa, Mt. Kilimanjaro, $\delta^{13}\text{C}_{\text{diatom}}$ was
241 used in comparison with other proxy data to make inferences about the three major phases in
242 the history of the lake (Fig. 2; Barker et al., in press). From 25 ka to 15.8 ka years BP and
243 from 5.5 ka to present, $\delta^{13}\text{C}_{\text{diatom}}$, $\delta^{13}\text{C}_{\text{bulk}}$ and high diatom content suggests high aquatic and
244 terrestrial productivity. From 15.8 to 5.5 ka the correlation between $\delta^{13}\text{C}_{\text{diatom}}$ and $\delta^{13}\text{C}_{\text{bulk}}$
245 breaks down, suggesting carbon supply to the lake satisfied or exceeded demand from
246 productivity. The tripartite division of the data interpreted alongside $\delta^{18}\text{O}_{\text{diatom}}$ show that the
247 demand exerted by lake productivity regulated by nutrient availability and changes in carbon
248 supplied from the catchment is forced by climate as indicated hydrological interpretation of
249 $\delta^{18}\text{O}_{\text{diatom}}$ (Fig. 2; Barker et al. in press).

250

251 **Compound specific isotope analysis**

252

253 There has been enormous growth in the application of compound specific isotope analysis

254 (CSIA) to lacustrine sediments; in particular, the use of $\delta^{13}\text{C}$ to reconstruct changes in
255 vegetation and primary productivity, and δD to track changes in aridity and precipitation. The
256 CSIA approach relies on the extraction and quantification of organic molecules and on the
257 assumption they can be related back to a precursor organism, especially the straight-chain
258 hydrocarbons of *n*-alkanes, *n*-alkanols and *n*-alkanoic acids. The long-chain ($\text{C}_{27}\text{--}\text{C}_{35}$) *n*-
259 alkanes are a main component of the epicuticular waxes of higher plants (Eglinton and
260 Hamilton 1967). In contrast, aquatic algae are dominated by shorter-chain homologues ($\text{C}_{17}\text{--}$
261 C_{21} *n*-alkanes), while the mid-chain homologues ($\text{C}_{23}\text{--}\text{C}_{25}$ *n*-alkanes) are a dominant
262 component of submerged aquatic macrophytes (Giger et al. 1980; Cranwell et al. 1987;
263 Ficken et al. 2000). On the whole, these molecules are well preserved in lake sediments and
264 once they have been identified and quantified by GC and GC/MS their isotopic composition
265 can be determined using GC-IRMS. Both terrestrial plant leaf waxes and algal lipids
266 therefore, can provide important insights into past environments.

267 *Carbon isotopes in leaf waxes and algal lipids*

268 The carbon isotope composition ($\delta^{13}\text{C}$) of specific compounds has predominantly been used
269 in two ways. Firstly, the $\delta^{13}\text{C}$ of terrestrial plant leaf waxes (long-chain *n*-alkanes and *n*-
270 acids) have been used to distinguish between vegetation using different photosynthetic
271 pathways such as C_3 (Calvin-Benson pathway) and C_4 (Hatch-Slack pathway) plants. As $\delta^{13}\text{C}$
272 of the *n*-alkanes differs depending on the photosynthetic pathway utilised by the plant, their
273 isotopic composition can be used to examine changes in vegetation type. For example, the
274 average $\delta^{13}\text{C}$ for C_{29} *n*-alkane for C_3 plants is -34.7‰ , while the C_4 plants are ^{13}C -enriched
275 with an average $\delta^{13}\text{C}$ for C_{29} *n*-alkane of -21.4‰ (cf. Castañeda et al. 2009a). As changes in
276 vegetation type are largely related to the prevailing climatic conditions such as temperature,
277 aridity and/or atmospheric carbon dioxide ($p\text{CO}_2$) concentrations, the $\delta^{13}\text{C}$ of plant waxes can
278 be used to reconstruct qualitative changes in past climate. These $\delta^{13}\text{C}$ data can then be used
279 further by taking a semi-quantitative approach using end member-mixing models to
280 reconstruct the proportion of C_4 plants present in past vegetation (and therefore the degree of
281 aridity or $p\text{CO}_2$) by using the $\delta^{13}\text{C}$ values constrained for C_3 and C_4 plants (e.g. Huang et al.
282 2006; Castañeda et al. 2007; Sinnighe Damsté et al. 2011).

283

284 The second way lipid biomarker $\delta^{13}\text{C}$ can be used is by examining the $\delta^{13}\text{C}$ of aquatic
285 biomarkers, such as short- and mid-chain *n*-alkanes related to algae and submerged and

286 emergent macrophytes respectively, to reconstruct changes in primary productivity and
287 carbon source. Analysis of $\delta^{13}\text{C}$ *n*-alkanes has several advantages over the traditional proxy
288 for past aquatic productivity – $\delta^{13}\text{C}$ of bulk organic matter ($\delta^{13}\text{C}_{\text{OM}}$) – as the compound
289 specific approach does not incorporate several sources of carbon (terrestrial, aquatic and
290 bacterial) and other processes such as pH and dominant inorganic carbon species, which have
291 the potential to influence $\delta^{13}\text{C}_{\text{OM}}$. In fact, it is difficult to deconvolute all of the confounding
292 factors that determine $\delta^{13}\text{C}_{\text{OM}}$ in a lake. For example, ^{13}C -enriched values of $\delta^{13}\text{C}_{\text{OM}}$ tend to
293 indicate increased productivity (Hollander and McKenzie 1991; Leng and Marshall 2004),
294 but enriched $\delta^{13}\text{C}_{\text{OM}}$ values can also be caused by C_4 -dominated terrestrially derived organic
295 matter, as well as the limitation of dissolved CO_2 in lakes causing ^{13}C -enrichment of $\delta^{13}\text{C}_{\text{OM}}$
296 as organisms begin to utilise bicarbonate (HCO_3^-) as a function of pH (Meyers 2003; Aichner
297 et al. 2010a). Conversely, the breakdown of organic matter at the water-sediment interface
298 produces ^{13}C -deplete $\text{CO}_2(\text{aq})$ that can be incorporated into the carbon pool of the lake and the
299 subsequent $\delta^{13}\text{C}_{\text{OM}}$ would be isotopically lighter, even during a time of greater productivity
300 (Hollander and McKenzie 1991). $\delta^{13}\text{C}_{\text{OM}}$ can also be modified by the abundance of aquatic
301 macrophytes, which can be submerged or emergent and this leads to utilisation of CO_2 from
302 the atmosphere and inorganic carbon from the water, thus complicating the $\delta^{13}\text{C}_{\text{OM}}$ signal
303 with respect to palaeoproductivity (Aichner et al. 2010a). Furthermore, in lakes that receive a
304 high proportion of terrestrial organic matter, $\delta^{13}\text{C}_{\text{OM}}$ will not reflect aquatic productivity at
305 all, but rather changes within the lakes catchment (e.g., Lake Malawi; Castañeda et al.
306 2009b). Therefore, examining the $\delta^{13}\text{C}$ of different component *n*-alkanes can elucidate the
307 processes occurring within a lake's carbon cycle.

308

309 A recent study by Aichner et al. (2010a) analysed a sediment core from Lake Koucha, eastern
310 Tibetan Plateau, for $\delta^{13}\text{C}$ of total organic carbon ($\delta^{13}\text{C}_{\text{TOC}}$), total inorganic carbon ($\delta^{13}\text{C}_{\text{TIC}}$)
311 and the $\delta^{13}\text{C}$ of aquatic macrophyte-derived *n*-alkanes ($\delta^{13}\text{C}_{n\text{-C}23}$), to investigate the controls
312 on the lake's carbon cycle over the deglacial and Holocene. The authors found a correlation
313 between TOC, total amount of *n*-C23 and $\delta^{13}\text{C}_{\text{TOC}}$ and $\delta^{13}\text{C}_{n\text{-C}23}$, which indicates Lake
314 Koucha was macrophyte-dominated before 8000 cal. years BP (Fig. 3). Subsequent to this,
315 the lake switched from a saline to freshwater system ~ 7200 cal. years BP and the lake began
316 to be colonised with phytoplankton, before being dominated by algae, in particular, diatoms
317 from 6100 cal. years BP (Fig. 3; Aichner et al., 2010a). There is a large range of $\delta^{13}\text{C}_{n\text{-C}23}$

318 values through the record (-23.5% to -12.6%), with lowest values during the greatest
319 macrophyte abundance and the highest during the period of phytoplankton dominance.
320 Therefore, Aichner et al. (2010a) argue $\delta^{13}\text{C}_{n\text{-}C23}$ is an excellent indicator of carbon-limiting
321 conditions that lead to the assimilation of isotopically ^{13}C -enriched carbon species (Fig. 3).

322

323 The cause of this carbon limitation may be very localised (eg. within a single macrophyte
324 bed) or could be induced by higher primary productivity. A comparison of $\delta^{13}\text{C}_{n\text{-}C23}$ with
325 $\delta^{13}\text{C}_{\text{TOC}}$ from Lake Koucha (Fig. 3) and the offset between the two ($\delta^{13}\text{C}_{n\text{-}C23} - \delta^{13}\text{C}_{\text{TOC}}$)
326 provides information about the relative contributions of aquatic macrophytes to the overall
327 organic carbon pool. The isotopic signature of bicarbonates assimilated by macrophytes show
328 large variations, as indicated by $\delta^{13}\text{C}_{\text{TOC}}$ (Fig. 3), while the $\delta^{13}\text{C}_{\text{TIC}}$ increases by 8% during
329 the phytoplankton maximum (period III, Fig. 2), suggesting the more positive $\delta^{13}\text{C}_{n\text{-}C23}$ values
330 during this time are caused by enrichment of the inorganic carbon pool (Aichner et al.,
331 2010a). However, as there are multiple sources of inorganic carbon in a lake, $\delta^{13}\text{C}_{\text{TIC}}$ will
332 vary according to the source. Therefore the offset between $\delta^{13}\text{C}_{n\text{-}C23}$ and $\delta^{13}\text{C}_{\text{TIC}}$ provides a
333 more robust assessment for changes in carbon-limiting conditions within Lake Koucha (Fig.
334 3).

335

336 The $\delta^{13}\text{C}$ analysis of long-, mid- and short-chain lipid biomarkers deposited in lake sediments
337 has elucidated our understanding of past environmental changes, in particular, changes in
338 terrestrial and aquatic vegetation. As the studies discussed above highlight, the ability to
339 analyse specific compounds for $\delta^{13}\text{C}$ means for the first time we are beginning to understand
340 the different parts of the carbon cycle within lake systems.

341

342 *Hydrogen isotopes in leaf waxes and algal lipids*

343

344 The deuterium isotope composition (δD) of long-chain leaf waxes and short-chain algal lipids
345 e.g. *n*-alkanes and *n*-alkanoic acids, is increasingly being used as a proxy for past
346 hydrological fluctuations. The δD composition of meteoric water is influenced by a number
347 of environmental factors, such as temperature, source moisture, amount of precipitation, and
348 continental rainout (Bowen and Revenaugh 2003). Therefore, changes in climate that affect

349 the isotopic composition of precipitation can be tracked using the δD composition of leaf
350 waxes, as precipitation is the source water for higher plants, while algal lipid δD values
351 register a change in the hydrological balance of the lake as they record surface water δD . A
352 number of studies have demonstrated on a continental to global scale that the δD of
353 precipitation controls the δD of plant leaf waxes (e.g., Bi et al. 2005; Sachse et al. 2006;
354 Smith and Freeman 2006; Hou et al. 2008; Rao et al. 2009; Polissar and Freeman 2010),
355 while the δD of lake water is an important control of algal lipid δD (e.g., Sauer et al. 2001;
356 Huang et al. 2004; Sachse et al. 2004). These first-order relationships over a wide spatial
357 scale suggest the δD of various biomarkers can be a powerful palaeohydrological proxy in
358 lake sediments. However, other factors have the potential to alter the δD of leaf wax or algal
359 lipids, such as biosynthetic fractionations, (evapo)transpiration and ecological turnover, and
360 must be considered before full palaeoclimatic inferences can be made.

361

362 As in many isotope systems, there is a fractionation between the isotope composition of water
363 and the isotope composition of the biomarker. In the case of leaf waxes and algal lipids a
364 biosynthetic fractionation of δD from prevailing meteoric and lake water has been
365 documented in higher plants (Smith and Freeman 2006; Hou et al. 2008; Feakins and
366 Sessions 2010) and algae (Zhang and Sachs 2007; Zhang et al. 2009). Determining this
367 apparent fractionation between meteoric and/or lake water and lake sediment δD proxies is
368 essential for the interpretation of lake-sediment δD records. Down core variations in δD can
369 be interpreted as fluctuations in hydrology, if we assume biosynthetic fractionation of the
370 same lipid derived from a different species is identical. If not, changes in δD can equally
371 result from shifts in plant or algal species (ecological turnover) and the overall δD trends will
372 therefore be driven by differences in isotopic fractionation. For example, the fractionation
373 between meteoric water and leaf waxes originating from grasses and woody species range –
374 73‰ to –242‰ and from –57‰ to –220‰, respectively (Liu and Yang 2008), although
375 smaller fractionations have been documented in arid and semi-arid environments (up to –
376 90‰; Feakins and Sessions 2010). These fractionations are further complicated depending on
377 the photosynthetic pathway used by the plant (C_3 vs. C_4 vegetation), as studies have shown n -
378 alkanes from C_4 grasses to have more positive δD values than those from C_3 grasses (Smith
379 and Freeman 2006; Liu et al. 2006a; Liu and Yang 2008; McInerney et al. 2011). However,
380 some studies suggest C_3 plants have increased δD values compared to their C_4 counterparts
381 (Chikaraihsi and Narako 2003), while others haven't observed any difference between C_3 and
382 C_4 plants (Bi et al. 2005; Rao et al. 2009). Liu and Yang (2008) suggest the main influence

383 on δD is the vegetation-form (i.e. tree, shrub or grass as they utilise very different source
384 waters). A recent study by Rao et al. (2009) however found no apparent relationship between
385 δD and vegetation type (e.g. forest vs. grassland or C_3 vs. C_4 plants). In an aquatic setting,
386 culture studies have demonstrated that while algal lipid δD reflect the δD of the source water,
387 there were systematic variations in the fractionation between the isotope composition of
388 water and different lipid homologues within a single species (Zhang and Sachs 2007).
389 Perhaps more importantly, Zhang and Sachs (2007) document a -90% to -100% difference
390 in hydrogen fractionation within a single lipid class across five species of algae. The authors
391 therefore advised caution against the use of lipids that have multiple sources (Zhang and
392 Sachs 2007). Despite this, a number of studies have shown a strong relationship between δD
393 of short-chain lipids in surface lake sediments, which are potentially from multiple sources,
394 and lake water δD (Huang et al. 2004; Sachse et al. 2004; Shuman et al. 2006; Hou et al.
395 2008; Henderson et al. 2010a).

396

397 The CSIA approach means it is possible to measure the δD composition of multiple
398 compounds within the same stratigraphic layer and as a result some studies have begun to
399 explore the use of long-chain (terrestrially-derived) and short-chain (aquatic-derived) n -
400 alkane δD as a proxy for evapotranspiration or water balance in some lakes. For example, a
401 30% difference in δD between terrestrially- and aquatic-derived n -alkanes was observed
402 across a range of small, groundwater-fed lakes in Europe (Sachse et al. 2004). As the
403 terrestrial n -alkanes record meteoric δD , but were enriched by 30% compared to the aquatic
404 n -alkane δD , the offset between the two (terrestrial δD mean = -128% ; aquatic δD mean = $-$
405 157%) is thought to derive from evapotranspiration in the terrestrial plant leaf (Sachse et al.
406 2004). This means there is the potential to reconstruct changing evapotranspiration through
407 time, although this assumes the moisture source for a lake doesn't change. Mügler et al.
408 (2008) found terrestrial n -alkanes (C_{29}) enriched by $\sim 30\%$ compared to aquatic n -alkanes
409 (C_{23}) in Lake Holzmaar, a groundwater fed lake in Germany, supporting the analysis by
410 Sachse et al. (2004). However, in the semi-arid and arid setting of the Tibetan Plateau,
411 terrestrial n -alkanes were isotopically depleted by $\sim 60\%$ compared to aquatic n -alkanes in
412 two lakes (Nam Co, Jiana Co) (Mügler et al. 2008). The difference in offset between C_{29} and
413 C_{23} n -alkanes in Germany compared to Tibet results from the moisture balance of the lakes.
414 The δD of Lake Holzmaar surface water is in isotopic equilibrium with input water
415 (precipitation and groundwater), while Nam Co and Jiana Co are isotopically enriched by
416 30% to 50% , suggesting evaporative concentration of lake waters at these two sites. As a

417 result Mügler et al. (2008) suggest a positive $\Delta\delta D_{C_{29}-C_{23}}$ is a good indicator of humid
418 conditions, while a negative $\Delta\delta D_{C_{29}-C_{23}}$ reflects semi-arid to arid conditions and therefore
419 $\Delta\delta D_{C_{29}-C_{23}}$ can potentially be used as a palaeoaridity indicator and to estimate the
420 evaporation to inflow ratio to reconstruct past water balance.

421
422 The influence of relative humidity and evaporative enrichment of soil and leaf water on δD
423 can complicate the application of the $\Delta\delta D_{C_{29}-C_{23}}$ approach to lake sediments. Aichner et al.
424 (2010b) tested the application of $\Delta\delta D_{C_{29}-C_{23}}$ to Tibetan lakes by examining surface sediment
425 and aquatic macrophytes from a number of sites across the NE Tibetan Plateau. There was no
426 observable offset between the δD of terrestrial and aquatic *n*-alkanes and when applied to a
427 lake sediment record (Lake Koucha), there was also no significant offset in δD between the
428 *n*-alkanes down core (Aichner et al. 2010b). The authors suggest this results from the
429 evapotranspiration of soil and leaf water in tandem with evaporative enrichment of lake water
430 in particularly arid environments and therefore the palaeoaridity proxy of $\Delta\delta D_{C_{29}-C_{23}}$ is not
431 uniformly applicable. A number of studies have also assessed the influence of relative
432 humidity and evaporative enrichment of soil and leaf water on δD . Hou et al. (2008) found
433 that halving the relative humidity from 80% to 40% in a growth chamber study produced
434 only ~7‰ enrichment in the δD of leaf wax, which they attribute to soil evaporation.
435 However, McInerney et al. (2011) found no enrichment of δD of leaf waxes in a growth
436 chamber, but documented enrichment in a field study as a result of changes in relative
437 humidity. They suggest this is explained by D-enrichment of the grass source water by
438 evaporation from soils, rather than within leaf evapotranspiration. In juxtaposition to this is a
439 study of an arid ecosystem that suggests leaf transpiration is responsible for the D-enrichment
440 of δD in leaf waxes, rather than soil evaporation as many plant species take up groundwater
441 and precipitation without fractionation (Feakins and Sessions 2010). While a latitudinal study
442 of 28 catchments of varying climate, ecosystem and vegetation types by Polissar and
443 Freeman (2010) highlights net fractionation between the δD of leaf waxes and meteoric δD
444 varies according to the ecosystem. Open grasslands were more sensitive to changes in aridity,
445 whereas closed forested ecosystems have reduced soil water loss and therefore less sensitivity
446 to evaporative enrichment (Polissar and Freeman 2010).

447
448 Despite the complications highlighted above, δD is still a very useful palaeolimnological tool
449 and continues to be successfully applied in a number of regions. However, important

450 consideration of the myriad of confounding factors is essential to make climatic
451 interpretations. The δD composition of terrestrial and/or aquatic biomarkers is predominantly
452 used in non-carbonate precipitating lake ecosystems, so it is rare to compare the δD of a leaf
453 wax and the more traditional palaeohydrological indicator of $\delta^{18}\text{O}$ of carbonate. In a recent
454 study, Liu et al. (2008) used a multi-isotope proxy record generated from the same core
455 (QHN3/1) to disentangle the late Holocene climatic impact on Lake Qinghai, a large lake on
456 the NE Tibetan Plateau. They established the δD of fatty acid C_{28} (C_{28} δD) as an indicator of
457 precipitation δD and used the $\delta^{18}\text{O}$ of lake carbonate ($\delta^{18}\text{O}_{\text{carb}}$) as a proxy for regional water
458 balance (see fig. 4). The $\delta^{18}\text{O}_{\text{carb}}$ record is in good accordance with other high-resolution late
459 Holocene isotope records from Lake Qinghai (Henderson et al. 2010b). The comparison
460 highlights coherent trends between the two proxies, although the C_{28} δD lags the $\delta^{18}\text{O}_{\text{carb}}$
461 record, which might reflect the time taken for the integration of terrestrial leaf waxes from the
462 catchment into the sediment record compared to autochthonous deposition of carbonates.
463 However, the records show coupled reductions in C_{28} δD and $\delta^{18}\text{O}_{\text{carb}}$ between ~ 1500 to 1250
464 calendar years BP, which is coincident with a regional Little Ice Age (LIA) that has
465 previously been documented in the lake (*cf.* Henderson et al. 2010b). In addition, a
466 comparison of the C_{28} δD and $\delta^{18}\text{O}_{\text{carb}}$ records with independent temperature and salinity
467 proxy data based on alkenones from the same QHN3/1 core (Liu et al. 2006) suggest this LIA
468 reduction in δD values resulted from changes in moisture source to the region, rather than an
469 increase in monsoon precipitation. The alkenone data show the LIA to be cold and arid, while
470 the isotope records suggest Lake Qinghai was fresher. As variations in C_{28} δD and $\delta^{18}\text{O}_{\text{carb}}$
471 can also be caused by a change in the isotope composition of input water, Liu et al. (2008)
472 suggest there was more westerly-derived moisture, which is isotopically deplete as a result of
473 continental rainout. This interpretation of the isotope data during the LIA is further
474 supported by other high-resolution records from other basins within the Lake Qinghai region
475 (Henderson et al. 2010b), suggesting regionally coherent patterns in climate change during
476 the LIA.

477

478 **Clumped isotopes in lake carbonates**

479 Stable isotope geochemistry has been used as an indicator of palaeoclimate since the work of
480 McCrea (1950) and Urey et al. (1951) highlighted the potential for $\delta^{18}\text{O}$ to be used for
481 palaeotemperature reconstruction. The technique has been routinely applied in

482 palaeolimnology of all geological ages (Leng and Marshall 2004). In lacustrine
483 environments, stratigraphic changes in $\delta^{18}\text{O}$ values are commonly attributed to changes in
484 temperature, air mass or precipitation/evaporation ratio. However, a perennial problem
485 though in the quantitative interpretation of geochemical proxies for climate is that the
486 systems are essentially underdetermined: in other words the variables that can be measured in
487 the sediments (generally $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ in carbonates, biogenic silica, organic matter) are
488 influenced by a wide range of interlinked environmental processes rather than a single factor.
489 For example, a change in temperature will produce a shift in the equilibrium $\delta^{18}\text{O}$ of
490 carbonate forming in a lake. However, the same temperature change will affect the $\delta^{18}\text{O}$ of
491 the rainfall and may also affect rates of evaporation, both in the lake and in the catchment.
492 All these factors will influence the $\delta^{18}\text{O}$ of the lacustrine components so a single variable
493 cannot be ascribed. In general, therefore, it is impossible to measure the $\delta^{18}\text{O}$ of carbonates
494 and silicates and translate the values into absolute or even relative temperature variation
495 without making some very significant assumptions (Leng and Marshall 2004). Thus, this
496 conventional approach amounts to solving several unknowns (most specifically temperature
497 and $\delta^{18}\text{O}$ of water) with a single constraint (i.e., $\delta^{18}\text{O}$ of carbonate)

498 Carbonate clumped-isotope thermometry has the potential to directly constrain both
499 temperature and $\delta^{18}\text{O}$ of carbonate independently. Carbonate clumped-isotope thermometry
500 constrains carbonate precipitation temperatures based on the temperature-dependent
501 ‘clumping’ of ^{13}C and ^{18}O into bonds with each other in the solid carbonate phase alone,
502 independent of the ^{18}O of the waters from which the mineral grew (e.g., Schauble et al. 2006;
503 Eiler 2007). The ^{13}C - ^{18}O bond enrichment relative to the ‘stochastic’, or random, distribution
504 of all C and O isotopes among all possible isotopologues is determined by digesting pure
505 carbonates and measuring the $\delta^{18}\text{O}$, $\delta^{13}\text{C}$, and abundance of mass-47 isotopologues (mostly
506 $^{13}\text{C}^{18}\text{O}^{16}\text{O}$) in product CO_2 . The latter, termed the Δ_{47} value, varies with carbonate growth
507 temperature (Ghosh et al. 2006a). Few applications have been made (or at least published)
508 within palaeolimnology to date, but one study calculated the timing of the Colorado Plateau
509 uplift by estimating depositional temperatures of Tertiary lake sediments that blanket the
510 plateau interior and adjacent lowlands using the carbonate clumped-isotope
511 palaeothermometer (Huntington et al. 2010). Comparison of modern and ancient samples
512 deposited near sea level provided an opportunity to quantify the influence of climate, and
513 therefore assess the contribution of changes in elevation to the variations of surface

514 temperature on the plateau. Analysis of modern lake calcite from 350-3300 m elevation
515 revealed a lake water carbonate temperature (LCT) lapse rate of $4.2 \pm 0.6^\circ\text{C}/\text{km}$, while analysis
516 of Miocene deposits from lower elevation suggests that ancient LCT lapse rate was
517 $4.1 \pm 0.7^\circ\text{C}/\text{km}$, and temperatures were $7.7 \pm 2.0^\circ\text{C}$ warmer at any elevation in comparison to
518 temperatures predicted by the modern trend. The inferred modern cooling is consistent with
519 other Pliocene temperature estimates, and the consistency of lapse rates through time
520 supports the interpretation that there has been little or no elevation change (Huntington et al.
521 2010).

522 **Multiple isotopes in chironomid head capsules**

523 The stable isotope composition of chironomid head capsules is being increasingly used in
524 palaeoenvironmental studies. Recent advances in chironomid isotope research means that
525 multiple isotope ratios ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, δD) can be measured from the same aliquot (Fig. 5).
526 The advantage of the use of chironomids is that in Northern Europe in particular, carbonate
527 lakes tend to be rare, especially those that are not evaporative, and for this reason
528 chironomids (along with diatom silica and aquatic cellulose) are used. Chironomids have the
529 slight advantage over diatom silica in that the individuals tend to be big enough to hand pick
530 (so easier to clean), and unlike aquatic cellulose can be identified under microscopy. $\delta^{18}\text{O}$ and
531 δD of the chironomids have been used as a proxy for the $\delta^{18}\text{O}$ and δD of the water in which
532 they lived, from which there is the potential to reconstruct $\delta^{18}\text{O}$ precipitation (air
533 temperature/source region) in certain types of lakes (Wooller et al. 2004; Wang et al. 2009;
534 Verbruggen et al. 2010). In the latter study the authors explored whether $\delta^{18}\text{O}$ of chironomid
535 capsules can be compared to $\delta^{18}\text{O}$ of bulk carbonates from Lateglacial and early Holocene
536 sediments from Rotsee (Switzerland). Chironomid $\delta^{18}\text{O}$ indicated depleted lake water $\delta^{18}\text{O}$
537 during the Oldest Dryas period, the Aegelsee and Gerzensee Oscillations, and the Younger
538 Dryas, whereas ^{18}O -enriched $\delta^{18}\text{O}$ values were associated with sediments deposited during
539 the Lateglacial interstadial and the early Holocene. Differences in the amplitude of variations
540 in bulk carbonate $\delta^{18}\text{O}$ and chironomid $\delta^{18}\text{O}$ were attributed to differential temperature
541 effects on oxygen isotope fractionation during the formation of carbonates and chironomid
542 head capsules or seasonal changes of lake water $\delta^{18}\text{O}$, potentially affecting $\delta^{18}\text{O}$ to different
543 extents. $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and δD in chironomids are thought to reflect the isotopic composition of
544 the invertebrate food, modified by trophic level, and can thus be used as a proxy for feeding
545 ecology (Wooller et al. 2008; van Hardenbroek et al. 2010). In lakes in particular, chironomid

546 $\delta^{13}\text{C}$ is dependent on whether the larvae fed predominantly on organic matter generated in the
547 photic zone or whether the larvae incorporate methanotropic microorganisms (Jones et al.
548 2008) due to living and burrowing into the sediment (Heiri et al. 2009). One of the potential
549 down sides identified by Heiri et al. (2009) is the possibility for alteration to the chemical
550 composition of the chitin by degradational or diagenetic processes as well as a need to
551 investigate further the chironomid-water/food fractionation processes.

552 **Summary**

553
554 Isotope geochemistry has become more routine within palaeolimnology over the last few
555 decades and it has been employed to address a diverse array of environmental issues ranging
556 from reconstructing past climate to understanding the dynamics of food webs through time.
557 Despite being established as an excellent palaeolimnological tool, continual improvements
558 have occurred in the understanding of the controls on isotope ratios, sample preparation and
559 refining of mass spectrometry techniques. But, perhaps the most important development has
560 been the move away from workhorse ‘bulk’ isotope methods based on carbonates and organic
561 matter, to the establishment of new proxies from lake sediments, such as, the multiple
562 isotopes that can be extracted from biogenic silica and chironomid chitin, the carbon and
563 hydrogen isotope analysis of specific compounds and the first steps in developing the
564 application of carbonate isotologues in lakes. These have opened up numerous possibilities
565 for the application of stable isotopes to new lakes. It is clear that over the last decade we have
566 taken huge steps in understanding isotope systems, but there still remain a number of
567 questions, especially for their application within palaeolimnology, as each lake is unique and
568 comes with it’s own set of specific circumstances. It will be exciting to see how the field
569 develops over the next decade as these techniques are more widely applied to lake sediment
570 records.

571

572

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882

883 **Figures**

884

885 Figure 1. Stratigraphic profiles of proxies highlighted in the text, plotted on a radiocarbon-
886 calibrated age scale: $\delta^{18}\text{O}_{\text{modelled}}$ profile with associated errors linked to mass-balancing
887 isotope measurements from Lake Baikal (see text for details); four stacked records of relative
888 abundance of haematite- stained grains (%HSG) in North Atlantic sediments indicative of
889 ice-rafted debris events; and $\delta^{18}\text{O}$ from NGRIP ice core. IRD numbers are according to those
890 given in Bond et al. (2001). YD (Younger Dryas) and IACP (intra-Allerød cold period) are
891 also given. Redrawn using data from Mackay et al. (2011).

892

893 Figure 2. 25,000-year multi-isotope records from the sediments of crater Lake Challa on the
894 eastern flank of Mt. Kilimanjaro. The combined data suggest three major phases in the
895 history of the lake's carbon cycle in particular the demand exerted by lake productivity
896 regulated by nutrient availability and changes in carbon supplied from the catchment forced
897 by climate as indicated hydrological interpretation of $\delta^{18}\text{O}_{\text{diatom}}$. Oxygen-isotope ratios ($\delta^{18}\text{O}$)
898 in diatom silica are plotted against a reversed axis, the $\delta^{13}\text{C}_{\text{diatom}}$ are a 10-sample running
899 mean. Also plotted is the difference between $\delta^{13}\text{C}_{\text{diatom}}$ and $\delta^{13}\text{C}_{\text{bulk}}$, the Pearson correlation
900 coefficient (R) between $\delta^{13}\text{C}_{\text{diatom}}$ and $\delta^{13}\text{C}_{\text{bulk}}$ are based on 3000-yr moving windows of the
901 respective data series. Redrawn using data from Barker et al. (in press).

902

903 Figure 3. Comparison of $\delta^{13}\text{C}_{n\text{-}C23}$, $\delta^{13}\text{C}_{\text{TOC}}$ and $\delta^{13}\text{C}_{\text{TIC}}$ from Lake Koucha, Tibetan Plateau.
904 The offset between $\delta^{13}\text{C}_{n\text{-}C23}$ and $\delta^{13}\text{C}_{\text{TOC}}$: higher values indicate a lower contribution to the
905 organic carbon pool from aquatic macrophytes. The offset between $\delta^{13}\text{C}_{n\text{-}C23}$ and $\delta^{13}\text{C}_{\text{TIC}}$:
906 high values are representative of carbon-limited conditions in the lake. Grey horizontal bars
907 signify cooling episodes documented on the Tibetan Plateau. Redrawn using data from
908 Aichner et al. (2010a) and Mischke et al. (2008).

909

910 Figure 4. Lake Qinghai δD_{wax} and $\delta^{18}\text{O}_{\text{carb}}$. Error bars are indicated on the δD_{wax} record. It
911 was established the δD of fatty acid C_{28} ($\text{C}_{28} \delta D$) as an indicator of precipitation δD and used
912 the $\delta^{18}\text{O}$ of lake carbonate ($\delta^{18}\text{O}_{\text{carb}}$) as a proxy for regional water balance. The comparison
913 highlights coherent trends between the two proxies, although the $\text{C}_{28} \delta D$ lags the $\delta^{18}\text{O}_{\text{carb}}$
914 record, which might reflect the time taken for the integration of terrestrial leaf waxes from the
915 catchment into the sediment record compared to autochthonous deposition of carbonates.
916 Redrawn using data from Liu et al. (2008).

917

918 Figure 5. Multiple isotope data from chironomid head capsules from a lake in northeastern
919 Iceland. (a) $\delta^{13}\text{C}$ and (b) $\delta^{15}\text{N}$ of chironomid head capsules plotted alongside the $\delta^{15}\text{N}$ and
920 $\delta^{13}\text{C}$ of the sediments; and (c) $\delta^{18}\text{O}$ of mixed insect remains, chironomid head capsules and
921 chironomid adult remains. Large magnitude changes in $\delta^{18}\text{O}$ occurred during the Holocene at
922 the site. Downcore shifts in $\delta^{18}\text{O}$ of chironomids do not correlate with measurements of the
923 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of chironomid head capsules, implying that the $\delta^{18}\text{O}$ changes were not
924 primarily driven by changes in chironomid diet during the Holocene but more likely changes
925 in the seasonality of precipitation, in the patterns of air masses supplying precipitation to
926 Iceland and in the dominant mode of the North Atlantic Oscillation. Redrawn using data from
927 Wooller et al. (2007).

928

Figure 1

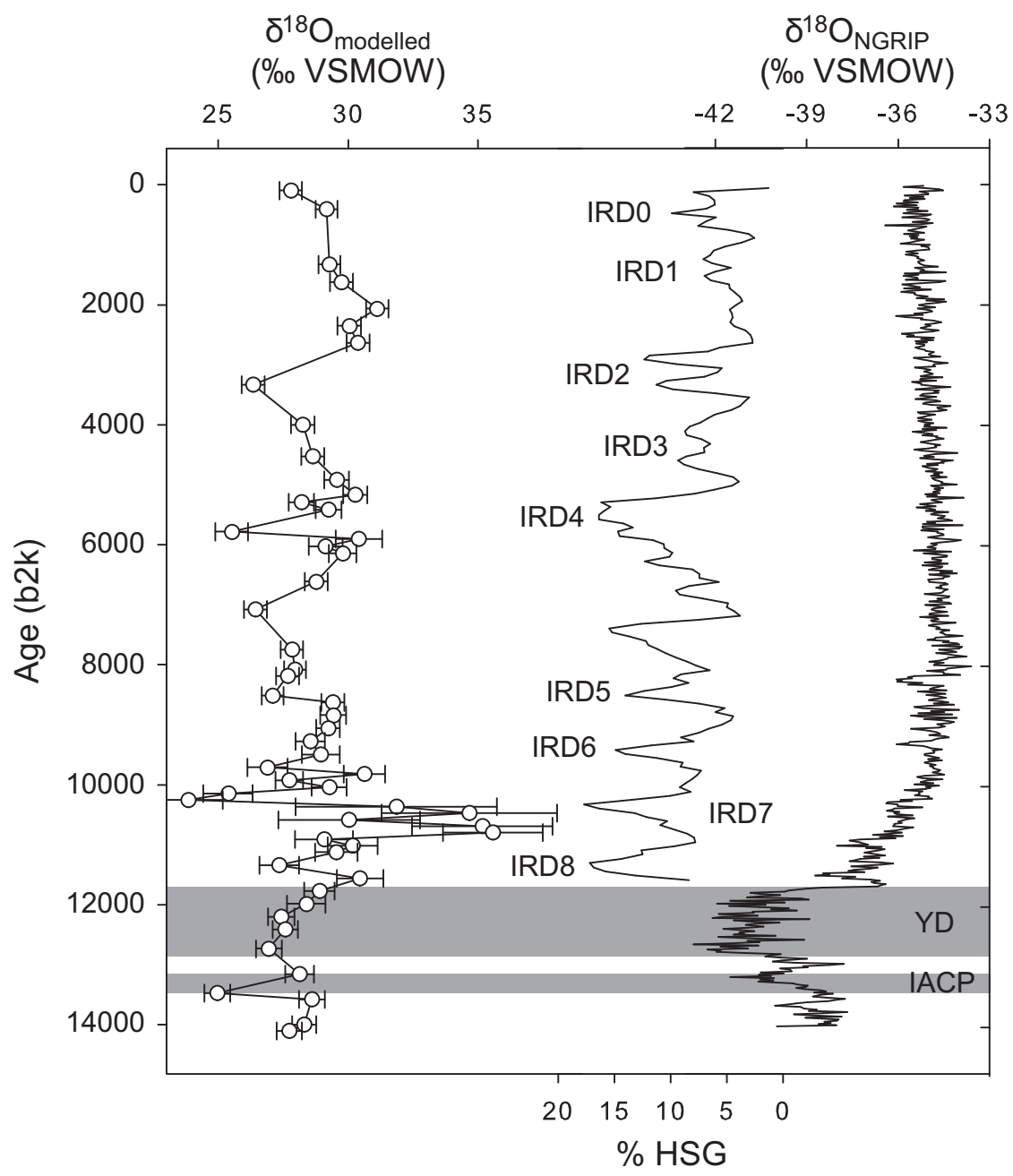


Figure 2

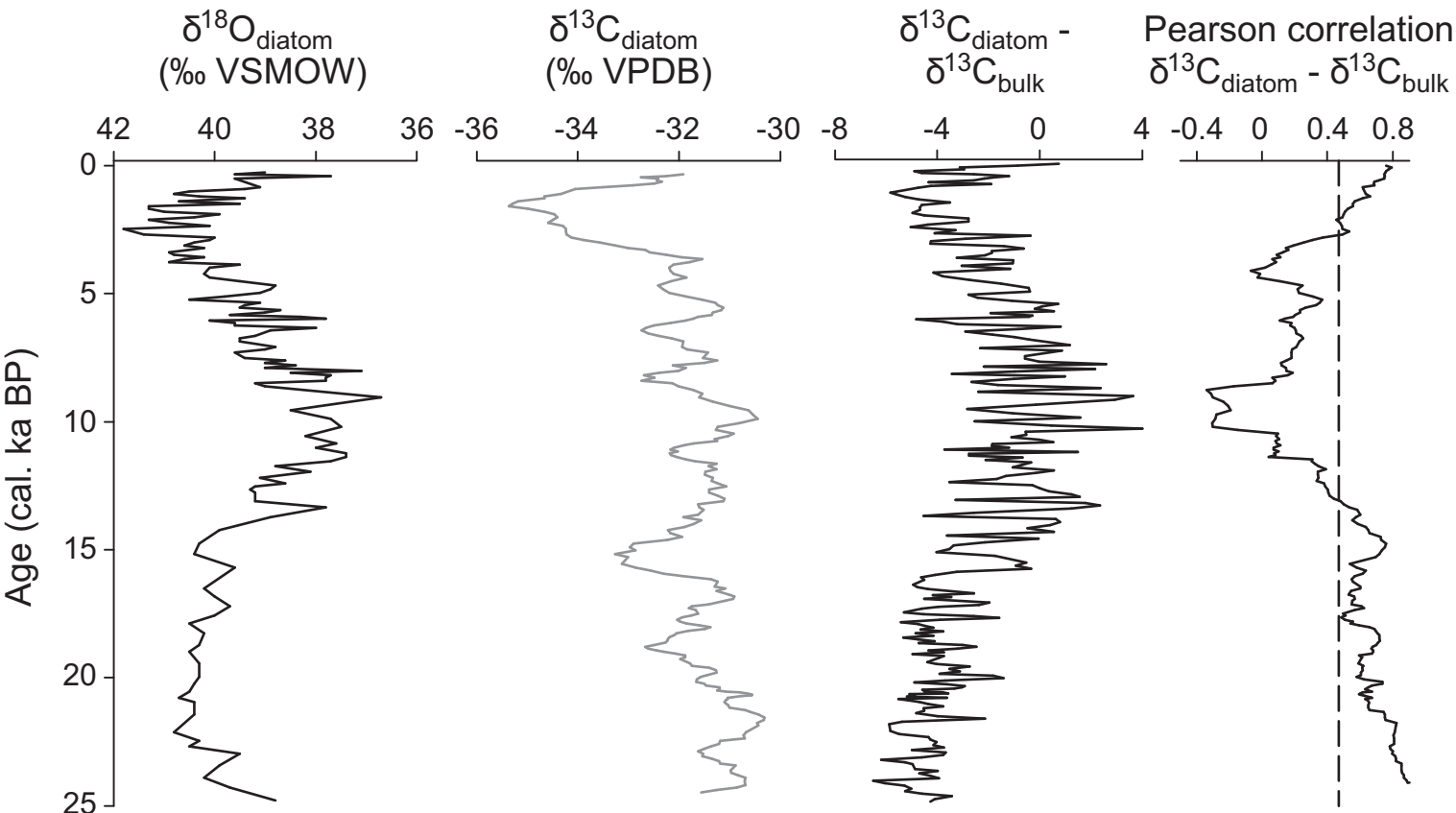


Figure 3

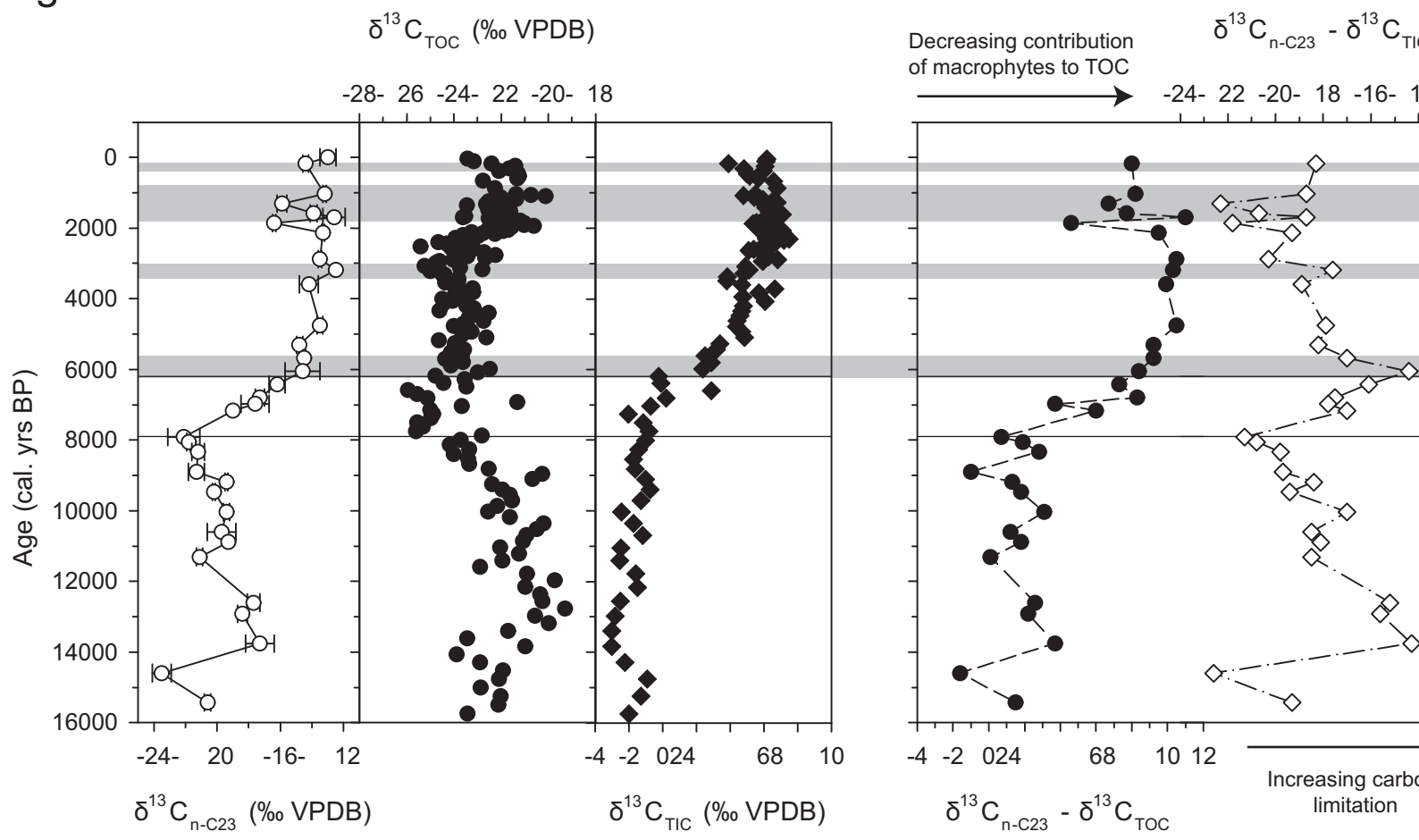


Figure 4

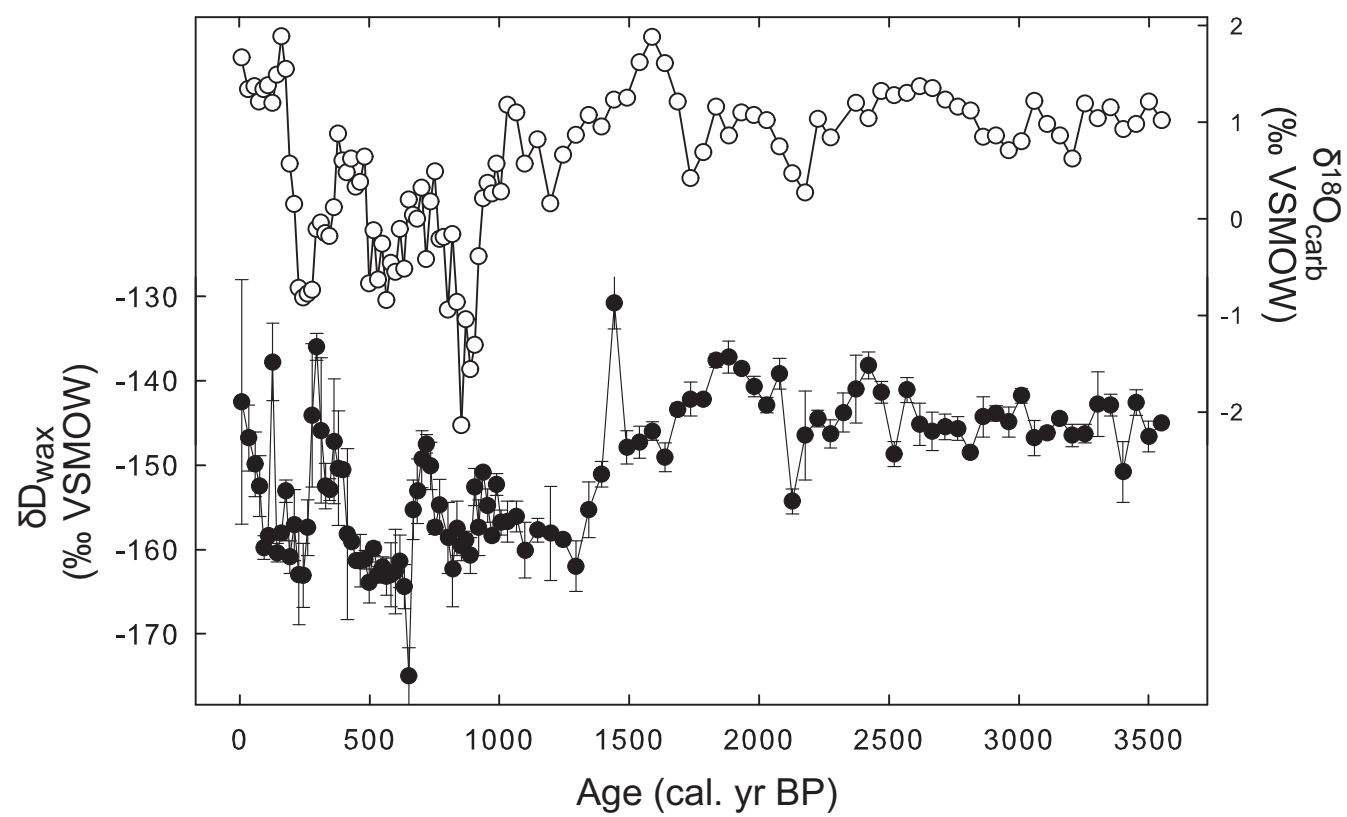


Figure 5

