

1 **Recent advances in isotopes as palaeolimnological proxies**

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10 *isotopes, Chironomids, Palaeolimnology*

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 <sup>13</sup>C-<sup>18</sup>O 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.

23

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  $^{13}\text{C}$ - $^{18}\text{O}$  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 H<sub>2</sub>O) 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  $^{18}\text{O}$  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 SiO<sub>4</sub> 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  $\text{ClF}_3$ ,  $\text{BrF}_5$  or  $\text{F}_2$ . The oxygen is then measured as  $\text{CO}_2$ ,  $\text{CO}$  or  $\text{O}_2$  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‰ ( $1\sigma$ ) 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  $-0.2\text{‰}/^\circ\text{C}$ . 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  $\text{SiO}_2$  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  $\delta\text{D}$  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  $\text{C}_{21}$  *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  $\text{C}_3$  (Calvin-Benson pathway) and  $\text{C}_4$  (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  $\text{C}_{29}$  *n*-alkane for  $\text{C}_3$  plants is  $-34.7\text{‰}$ , while the  $\text{C}_4$  plants are  $^{13}\text{C}$ -enriched  
275 with an average  $\delta^{13}\text{C}$  for  $\text{C}_{29}$  *n*-alkane of  $-21.4\text{‰}$  (*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  $\text{C}_4$  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  $\text{C}_3$  and  $\text{C}_4$  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,  $^{13}\text{C}$ -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  $\text{C}_4$ -dominated terrestrially derived organic  
295 matter, as well as the limitation of dissolved  $\text{CO}_2$  in lakes causing  $^{13}\text{C}$ -enrichment of  $\delta^{13}\text{C}_{\text{OM}}$   
296 as organisms begin to utilise bicarbonate ( $\text{HCO}_3^-$ ) 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  $^{13}\text{C}$ -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  $\text{CO}_2$  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  $\sim 7200$  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  $^{13}\text{C}$ -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 ( $\delta\text{D}$ ) 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  $\delta\text{D}$  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  $\delta D$  composition of leaf  
350 waxes, as precipitation is the source water for higher plants, while algal lipid  $\delta D$  values  
351 register a change in the hydrological balance of the lake as they record surface water  $\delta D$ . A  
352 number of studies have demonstrated on a continental to global scale that the  $\delta D$  of  
353 precipitation controls the  $\delta D$  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  $\delta D$  of lake water is an important control of algal lipid  $\delta D$  (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  $\delta D$  of various biomarkers can be a powerful palaeohydrological proxy in  
358 lake sediments. However, other factors have the potential to alter the  $\delta D$  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  $\delta D$  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  $\delta D$  proxies is  
368 essential for the interpretation of lake-sediment  $\delta D$  records. Down core variations in  $\delta D$  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  $\delta D$  can equally  
371 result from shifts in plant or algal species (ecological turnover) and the overall  $\delta D$  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  $\delta D$  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  $\delta D$  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  $\delta D$  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  $\delta D$  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  $\delta D$  reflect the  $\delta D$  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  $\delta D$   
393 of short-chain lipids in surface lake sediments, which are potentially from multiple sources,  
394 and lake water  $\delta D$  (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  $\delta D$  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  $\delta D$  as a proxy for evapotranspiration or water balance in some lakes. For example, a  
401  $30\%$  difference in  $\delta D$  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  $\delta D$ , but were enriched by  $30\%$  compared to the aquatic  
404  $n$ -alkane  $\delta D$ , the offset between the two (terrestrial  $\delta D$  mean =  $-128\%$ ; aquatic  $\delta D$  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  $\delta D$  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  $\Delta\delta\text{D C}_{29}\text{-C}_{23}$  is a good indicator of humid  
418 conditions, while a negative  $\Delta\delta\text{D C}_{29}\text{-C}_{23}$  reflects semi-arid to arid conditions and therefore  
419  $\Delta\delta\text{D C}_{29}\text{-C}_{23}$  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  $\delta\text{D}$   
423 can complicate the application of the  $\Delta\delta\text{D C}_{29}\text{-C}_{23}$  approach to lake sediments. Aichner et al.  
424 (2010b) tested the application of  $\Delta\delta\text{D C}_{29}\text{-C}_{23}$  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  $\delta\text{D}$  of terrestrial and aquatic *n*-alkanes and when applied to a  
427 lake sediment record (Lake Koucha), there was also no significant offset in  $\delta\text{D}$  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  $\Delta\delta\text{D C}_{29}\text{-C}_{23}$  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  $\delta\text{D}$ . 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  $\delta\text{D}$  of leaf wax, which they attribute to soil evaporation.  
435 However, McInerney et al. (2011) found no enrichment of  $\delta\text{D}$  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  $\delta\text{D}$  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  $\delta\text{D}$  of leaf waxes and meteoric  $\delta\text{D}$   
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,  $\delta\text{D}$  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  $\delta D$  composition of terrestrial and/or aquatic biomarkers is predominantly  
452 used in non-carbonate precipitating lake ecosystems, so it is rare to compare the  $\delta D$  of a leaf  
453 wax and the more traditional palaeohydrological indicator of  $\delta^{18}O$  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  $\delta D$  of fatty acid  $C_{28}$  ( $C_{28} \delta D$ ) as an indicator of  
457 precipitation  $\delta D$  and used the  $\delta^{18}O$  of lake carbonate ( $\delta^{18}O_{carb}$ ) as a proxy for regional water  
458 balance (see fig. 4). The  $\delta^{18}O_{carb}$  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  $C_{28} \delta D$  lags the  $\delta^{18}O_{carb}$   
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  $C_{28} \delta D$  and  $\delta^{18}O_{carb}$  between  $\sim 1500$  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  $C_{28} \delta D$  and  $\delta^{18}O_{carb}$  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  $\delta D$  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  $C_{28} \delta D$  and  $\delta^{18}O_{carb}$   
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 depleted 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  $\delta^{18}O$  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  $^{13}\text{C}$  and  $^{18}\text{O}$  into bonds with each other in the solid carbonate phase alone,  
502 independent of the  $^{18}\text{O}$  of the waters from which the mineral grew (e.g., Schauble et al. 2006;  
503 Eiler 2007). The  $^{13}\text{C}$ - $^{18}\text{O}$  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  $\text{CO}_2$ . The latter, termed the  $\Delta_{47}$  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 ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$ ,  $\delta\text{D}$ ) 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  $\delta\text{D}$  of the chironomids have been used as a proxy for the  $\delta^{18}\text{O}$  and  $\delta\text{D}$  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  $^{18}\text{O}$ -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  $\delta\text{D}$  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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585

586

587

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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  $\delta D_{\text{wax}}$  and  $\delta^{18}\text{O}_{\text{carb}}$ . Error bars are indicated on the  $\delta D_{\text{wax}}$  record. It  
911 was established the  $\delta D$  of fatty acid  $\text{C}_{28}$  ( $\text{C}_{28}$   $\delta D$ ) as an indicator of precipitation  $\delta D$  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

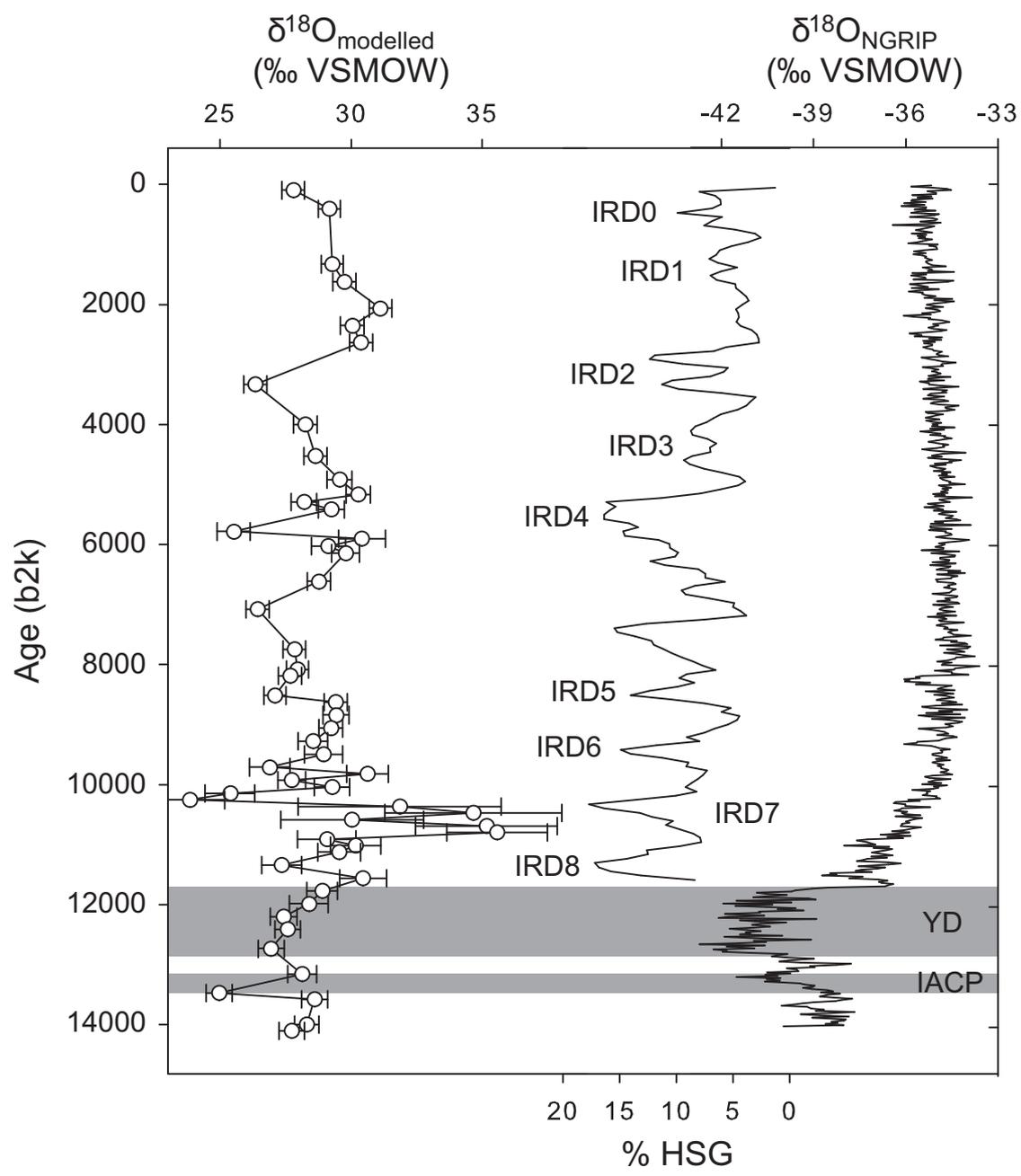


Figure 2

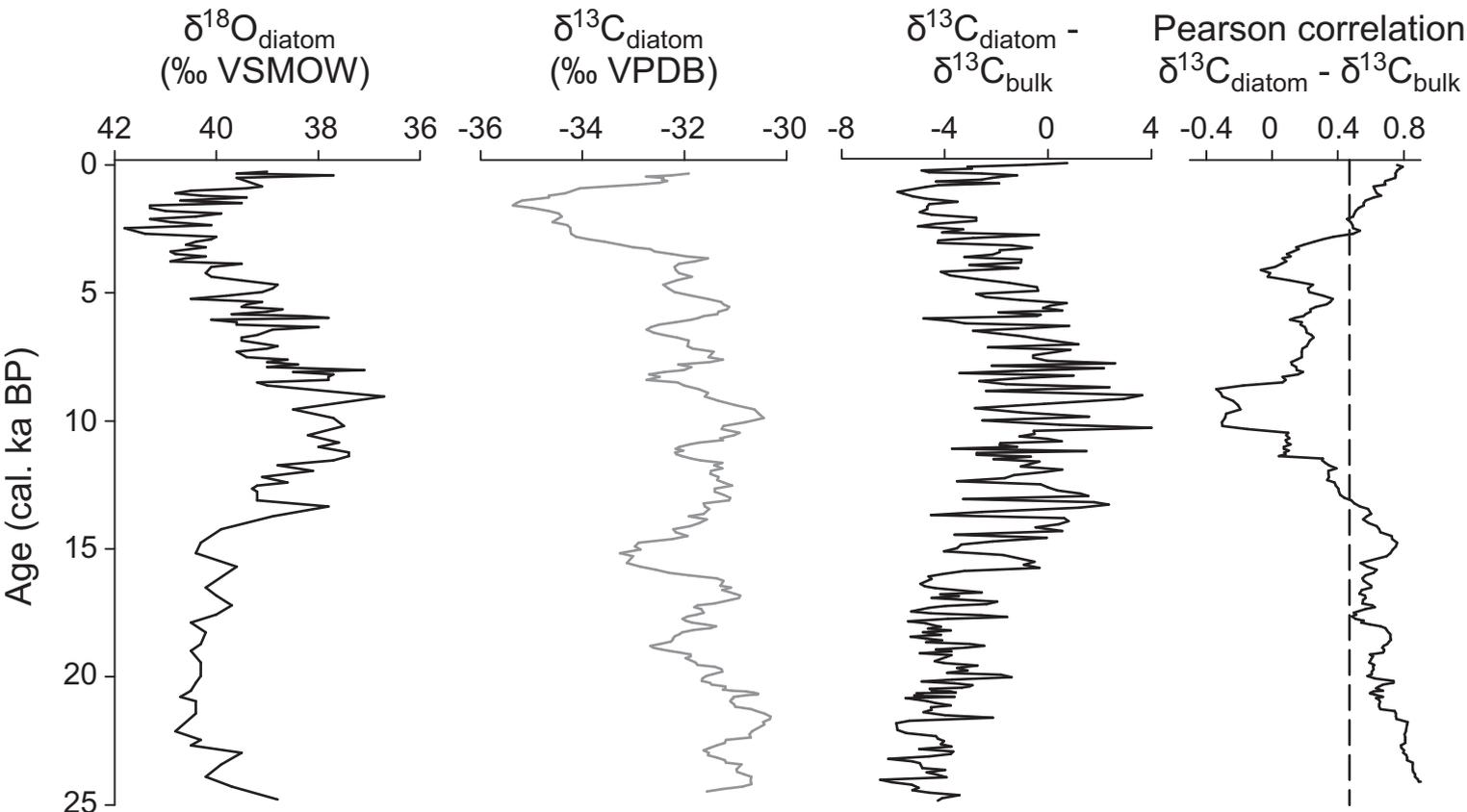


Figure 3

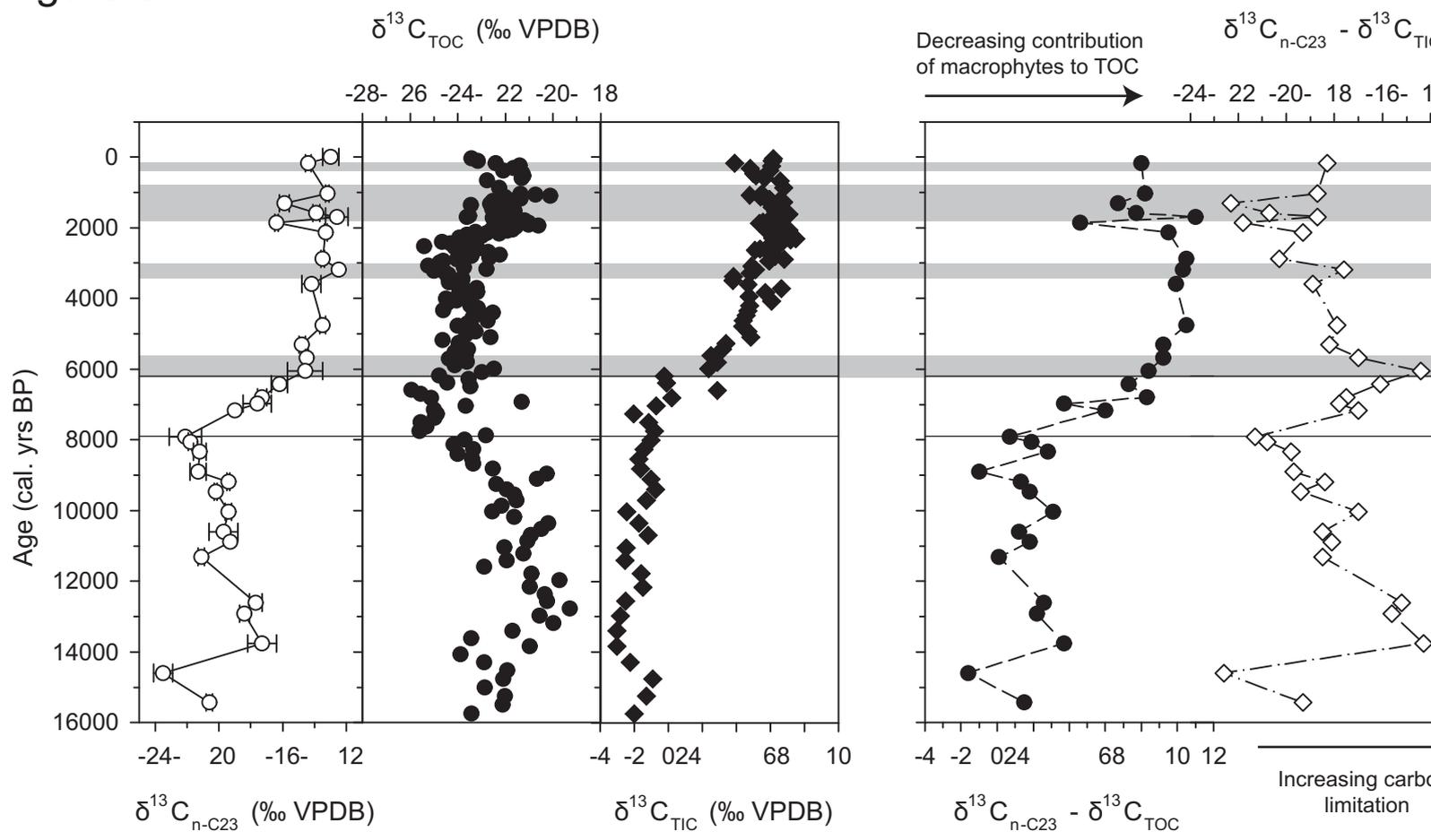


Figure 4

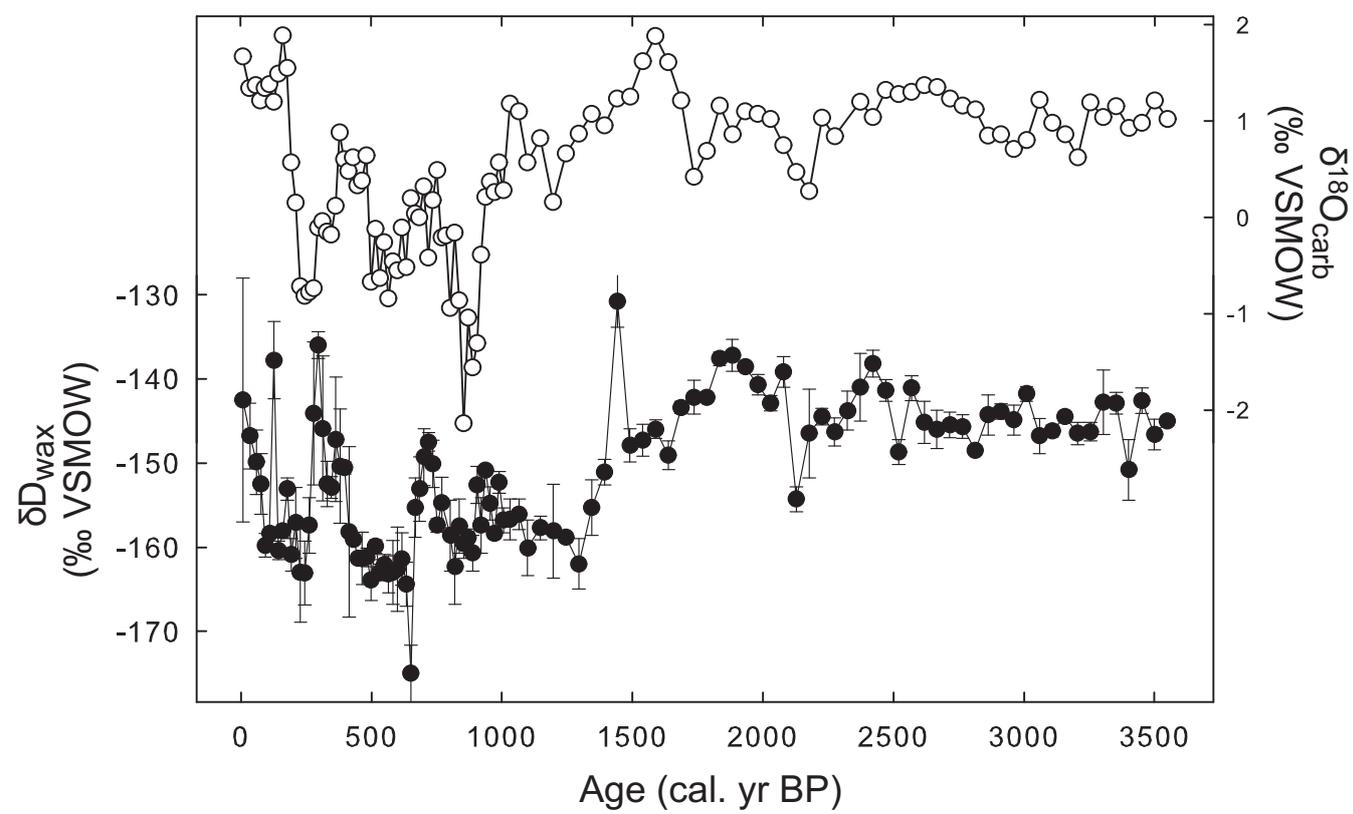


Figure 5

