1   **Biomarker approaches for reconstructing terrestrial environmental change**

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14   **Shortened running title***: Biomarkers in terrestrial environments

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Abstract

The response of the terrestrial biosphere to warming remains one of the most poorly understood and quantified aspects of the climate system. One way to test the behaviour of the Earth system in warm climate states is to examine the geological record. The abundance, distribution, and/or isotopic composition of source-specific organic molecules ("biomarkers") have been used to reconstruct terrestrial paleoenvironmental change over a range of geological timescales. Here, we review new or recently improved biomarker approaches for reconstructing: (i) physical climate variables (land temperature, rainfall), (ii) ecosystem state variables (vegetation, fire regime), and (iii) biogeochemical variables (soil residence time, methane cycling). This review encompasses a range of key compound classes (e.g., lipids, lignin, and carbohydrates). In each section, we explore the concept behind key biomarker approaches and discuss their successes as paleoenvironmental indicators. We emphasize that analyzing several biomarkers in tandem can provide unique insights into the Earth System.

1. Introduction

Human activity is fundamentally altering the chemical composition of the atmosphere and warming the planet. Higher temperatures coupled with changing rainfall patterns will alter vegetation distributions, soil residence time, and a variety of terrestrial biogeochemical processes, resulting in feedbacks that can amplify or reduce greenhouse gas forcing (Rohling et al 2012). Quantifying these feedbacks remains challenging because of large uncertainties associated with land surface processes and their response to carbon cycle perturbations (Luo 2007).

The geological record encompasses a wide range of climate states that can help explore the response of regional climate and terrestrial ecosystems to external forcing (Tierney et al 2020). However, direct instrumental records span only the last few centuries. In deeper time, researchers must rely upon indirect biological, chemical, or physical indicators ("proxies") preserved within the sedimentary record. Plant fossils (e.g., leaves, pollen, spores) reveal information about past ecosystems, but also inform climate through analysis of leaf physiognomy (e.g., leaf margin analysis; Wilf 1997) and leaf and/or pollen taxonomy. The stable and clumped isotopic composition of terrestrial carbonates (e.g., herbivore teeth, soil carbonates) also provide important constraints into terrestrial ecosystems and climate (e.g., Cerling 1984). However, these archives are often poorly preserved and/or discontinuous, such that additional techniques are often required.

The abundance, distribution, and/or isotope composition of source-specific organic molecules—termed "biomarkers" (Sidebar 1)—preserved in a variety of sedimentary

70 deposits provides an additional window into ancient terrestrial environments (Figure 1).

71 Here, we provide a holistic overview and synthesis of key terrestrial biomarker proxies.

72 We review biomarker approaches for reconstructing: (i) physical climate variables (land

73 temperature, rainfall), (ii) ecosystem state variables (vegetation, fire regime), and (iii)

74 biogeochemical variables (soil residence time, methane cycling). In each section, we

75 explore the concept behind key biomarker approaches—highlighting their limitations and

76 challenges—and discuss their successes as paleoenvironmental indicators. We also

77 highlight emerging terrestrial biomarker proxies and discuss potential for future studies.

78

79 **2. Reconstructing terrestrial environmental change using organic biomarkers**

80

81 **2.1. Temperature proxies**

82 Terrestrial temperature exerts a major control on vegetation distributions (Section 2.2),

83 the hydrological cycle (Section 2.3) and a variety of biogeochemical cycles (Section 2.4

84 to 2.6). Yet even for relatively recent, well-studied time intervals (e.g., the Holocene; 12

85 thousand years (ka) to present), large continental regions lack rigorous temperature

86 constraints (Kaufman et al 2020). Branched glycerol dialkyl glycerol tetraethers

87 (brGDGTs) are increasingly used to reconstruct land temperature. Branched GDGTs are

88 comprised of two *n*-alkyl chains, each containing 4-6 methyl groups and 0-2 cyclopentane

89 moieties (Sinninghe Damsté et al 2000). The stereochemistry of the glycerol units

90 confirms a bacterial—rather than archaeal—source organism, but the exact biological

91 source organism(s) remain(s) debated (Sinninghe Damsté et al 2018). Acidobacteria are

92 a potential source organism, due to their widespread abundance in soil and peat (Weijers

93   et al., 2009). Various Acidobacterial strains synthesize the presumed "building block" of

94   brGDGTs (*iso*-diabolic acid). However, only a few Acidobacterial strains have been

95   directly shown to produce brGDGTs (Sinninghé Damste et al., 2011; 2018; Halamka et

96   al., 2021). Recent work demonstrates that oxygen limitation can trigger brGDGT

97   production in at least one Acidobacterial strain, perhaps explaining why few brGDGTs

98   had previously been identified in the laboratory (Halamka et al., 2021). Using a global soil

99   database, Weijers et al. (2007b) first demonstrated that the number of methyl groups

100   (captured via the methylation of branched tetraethers; MBT) varied as a function of

101   temperature and pH (Weijers et al 2007b), whereas the number of cyclopentane moieties

102   (captured via the cyclization of branched tetraethers; CBT) varied as a function of pH

103   alone (Weijers et al 2007b). This led to the formulation of the MBT/CBT index, which was

104   correlated to mean annual air temperature (MAAT; i.e., measured 2 m above ground as

105   determined from climatological reanalysis data) (Weijers et al 2007b).

106

107   The MBT/CBT index was originally applied in a marginal marine setting (Weijers et al

108   2007a) and revealed that tropical African temperatures increased by ~4°C during the last

109   deglaciation (Weijers et al 2007a). However, later analytical refinements (Becker et al

110   2013, De Jonge et al 2013, Hopmans et al 2016) found that penta- and hexa-methylated

111   brGDGTs contained methyl groups at the C5 (5-methyl brGDGTs) or C6 position (6-

112   methyl brGDGTs) and that these co-eluted in the original method of Weijers et al (2007b).

113   This led to the formulation of the methylation index of 5-methyl brGDGTs (MBT'$_{5ME}$). This

114   metric removes the soil pH dependency that was present in the original MBT index

115   (Weijers et al 2007b) and yields more accurate MAAT estimates in soils (De Jonge et al

116   2014). However, there remains a poor correlation between MBT'$_{5ME}$ and MAAT in soils

117   with a high abundance of 6-methyl brGDGTs (i.e., arid and/or alkaline soils; Dang et al

118   2016). Some studies have suggested that samples with a high 6- over 5-methyl brGDGT

119   ratio (e.g., IR$_{6ME}$ > 0.5) are not reliable for temperature inference (Dang et al 2016),

120   although in a global analysis of the soil and peat dataset, Crampton-Flood et al., (2020)

121   did not find support for this particular cut-off. Only core tops with a very high IR$_{6Me}$ value

122   (> 0.8) showed a high residual error.

123

124   The MBT'$_{5ME}$ index correlates strongly with MAAT in a global peat dataset (Naafs et al

125   2017) and is comparable to the relationship observed in soils (Crampton-Flood et al

126   2020). The MBT'$_{5ME}$ index has been increasingly applied in peat archives to reconstruct

127   land temperatures and can reproduce millennial-scale Holocene climate events (i.e.,

128   Younger Dryas, Bølling-Allerød)   (Zheng et al 2017). However, peat and/or lignite

129   deposits can be subject to dramatic changes in local hydrology which may lead to an

130   apparent change in brGDGT-derived temperature estimates. For instance, brGDGT

131   distributions within a UK lignite deposit imply ~10°C cooling during the onset of the

132   Paleocene-Eocene Thermal Maximum (PETM) (Inglis et al 2019a). This coincided with

133   enhanced waterlogging and is consistent with in situ production of aquatic brGDGTs (see

134   below; Weber et al 2018). The application of MBT'$_{5ME}$ in dynamic terrestrial environments

135   (e.g., lake-mire transitions) should therefore be interpreted cautiously and within a multi-

136   proxy and/or data-modelling framework.

137

138   BrGDGTs respond differently in lakes than they do in soils. In particular, the application

139   of mineral soil or peat calibrations in lacustrine settings can yield up to 10°C colder-than-

140   predicted temperatures (Tierney et al 2010). In lake environments, brGDGTs can be

141   produced in situ—possibly by a distinct microbial community (Weber et al 2018)—and

142   regional or global lake-specific calibration models are therefore required (Martínez-Sosa

143   et al 2021). The relationship between MBT'$_{5ME}$ and temperature is stronger in lakes than

144   soils and peats; conversely, the latter have a stronger relationship to pH (Martínez-Sosa

145   et al 2021). This supports the hypothesis that different microbial communities are involved

146   in the production of these compounds. Notably, much like arid and/or alkaline soils,

147   lacustrine brGDGT distributions are distinct in hypersaline and/or alkaline lakes, with

148   higher relative abundance of brGDGT-*Ia* and more 6′ methyl isomers (Martinez-Sosa et

149   al., 2021). Hypersaline and/or alkaline lakes have higher average MBT$_{5ME′}$ values than

150   other lakes for a given temperature, implying that global lake calibrations may

151   overestimate temperature values in such environments. This potentially limits the

152   application of MBT'$_{5me}$ in these environments (Martínez-Sosa et al 2021, Tierney et al

153   2010).

154

155   One of the strengths of the MBT'$_{5ME}$ is that is can be applied in older geological time

156   intervals when other terrestrial temperature proxies (e.g., pollen transfer functions) may

157   be less certain. For example, Tibbett et al (2021) used MBT'$_{5ME}$ to constrain temperatures

158   across the Eocene-Oligocene transition (EOT) and found an abrupt ~4-6°C cooling in

159   East Antarctica (Prydz Bay) prior to the establishment of continental-scale ice sheets

160   (Figure 2). The branched GDGT-inferred cooling is larger, more abrupt and later than that

161 reconstructed from the rock-derived chemical weathering proxies. This is related to

162 catchment sourcing: rock erosion is dominated by the mountainous sectors of

163 catchments, whereas soil-derived biomarkers are dominated by the greater lowland

164 extent of catchments. The two signals together reveal the earlier cooling of the high

165 mountain regions in the rock-derived proxy, whereas the brGDGTs detect the later and

166 more abrupt lowland cooling.

167

168 Despite these successes, MBT'$_{5ME}$ values can be overprinted by brGDGT production in

169 rivers and/or marine sediments (e.g., upland soils, rivers, marine sediments; Crampton-

170 Flood et al 2018, Zell et al 2014). If these sources were substantial, for example in the

171 lowland Amazon River (Zell et al., 2014), they can bias brGDGT temperature estimates.

172 This can be partially resolved by screening for and excluding and/or correcting for

173 possible marine/riverine overprints (Crampton-Flood et al 2018). Marine settings are

174 typically characterised by a high degree of cyclisation of tetramethylated brGDGTs (i.e.,

175 high #rings$_{tetra}$ values; Sinninghé Damste, 2018). This is because marine environments

176 are relatively alkaline compared to soils and peat. As #rings$_{tetra}$ values do not exceed 0.7

177 in modern soils, higher values (i.e., >0.7) are evidence for marine *in situ* production

178 (Sinninghe Damsté, 2016). Rivers often have a higher contribution of 6-methyl brGDGT

179 isomers (de Jonge et al., 2015), yielding high Isomerisation Ratio values (IR; de Jonge et

180 al., 2015). However, diagnosing riverine input using the IR is challenging as both arid and

181 alkaline soils contain a high abundance of 6-methyl brGDGT isomers (Dang et al, 2016).

182

183   The MBT'$_{5ME}$ index also has a theoretical maximum temperature of ~25-30°C in soils and

184   peat (Crampton-Flood et al 2020, De Jonge et al 2014, Naafs et al 2017, Weijers et al

185   2007b), which limits use of this proxy in greenhouse climates (e.g., the Eocene).

186   Branched GDGT distributions in settings that are warmer than modern remain largely

187   unknown, although both soil (Chen et al 2018) and lake water (Martinez-Sosa et al 2020)

188   incubations indicate that organisms can synthesise abundant brGDGTs when grown at

189   temperatures above 35°C.

190

191   Isoprenoidal glycerol dibiphytanyl glycerol tetraethers (isoGDGTs) might help reconstruct

192   temperatures not represented in modern climates. IsoGDGTs are synthesised by archaea

193   and comprised of two isoprenoid side chains containing up to eight cyclopentane moieties

194   (although rarely more than 4 cyclopentane moieties; Schouten et al, 2013 and ref.

195   therein). The number of cyclopentane moieties increases at higher temperatures (De

196   Rosa et al 1980), resulting in a more densely packed and stable membrane. This is the

197   premise of the TEX$_{86}$ surface water temperature proxy (Schouten et al 2002). In some

198   lacustrine settings—generally large and deep lakes—TEX$_{86}$ is correlated to annual mean

199   lake temperature (Powers et al 2004) and can be used to infer continental temperatures

200   (Tierney et al 2008). However, various factors can influence TEX$_{86}$ values in lakes (e.g.

201   input of GDGTs from sources other than Thaumarchaeota) (Blaga et al 2009).

202

203   The degree of isoGDGT cyclisation is poorly correlated with temperature in soils (Coffinet

204   et al 2014) and peats (Naafs et al 2018), likely due to the wide range of potential source

205   organisms in these settings. However, recent work identified isoGDGTs with more than

206　　four cyclopentane moieties (e.g., GDGT-5 to -7) in tropical (>19.5°C) and acidic (pH <

207　　5.1) peats (Naafs et al 2018). These compounds had previously only been observed in

208　　extremophile cultures and/or hyperthermophilic settings (e.g., hot springs) (Schouten et

209　　al 2013), suggesting that their occurrence in tropical, acidic peats represents an

210　　adaptation to higher temperatures and/or lower pH. Highly-cyclised isoGDGTs (e.g.,

211　　isoGDGT-5, but also isoGDGT-6) have been detected in early Eocene (~56 to 48 Ma)

212　　lignite deposits (Naafs et al 2018). This confirms that mid-latitude temperatures were

213　　higher than 19.5°C during the early Eocene, consistent with elevated brGDGT

214　　temperature estimates (>22-29°C) from the same samples (Naafs et al 2018). The

215　　abundance of isoGDGT-5 in low latitude Eocene lignite deposits is higher than that found

216　　in any modern peat, implying MAATs were higher than presently found in the low latitudes.

217　　These results suggest that highly-cyclised isoGDGTs (e.g., isoGDGT-5 to -8) could

218　　potentially be used to reconstruct temperatures >30°C (c.f., the brGDGT temperature

219　　proxy). However, experimental approaches (e.g., mesocosm or microcosm experiments)

220　　are required to decipher the relationship between highly-cyclised isoGDGTs and MAAT

221　　beyond the modern range. Genomic approaches may provide additional insights into the

222　　physiological function of highly-cyclised GDGTs (e.g., Zeng et al., 2019).

223

2.2. Vegetation proxies

224

225　　The geological record can provide insights into the response of plant communities to CO_2-

226　　induced warming and associated hydroclimate changes. Leaf fossils document local

227　　vegetation change across major climate events (e.g. the PETM; Wing et al., 2005).

228　　Biomarkers are highly complementary sources of information and provide a more

229   spatially-integrated perspective. The most common biomarkers used to reconstruct

230   changes in the plant community are: i) long-chain *n*-alkyl compounds (e.g., *n*-alkanes, *n*-

231   alcohols, *n*-alkanoic acids) (Eglinton & Hamilton 1967), ii) lignin (e.g., lignin phenols;

232   (Hedges & Mann 1979), and iii) terpenoids (e.g. di- and triterpenoids; Otto & Simoneit

233   2001)

234

235   Long-chain *n*-alkane compounds typically have carbon chain lengths ranging from C_{25} to

236   C_{35} and exhibit high odd-over-even ratios, whereas long-chain *n*-alcohols or *n*-alkanoic

237   acids range from C_{24} to C_{34} and exhibit high even-over-odd ratios. Various factors

238   determine the abundance of *n*-alkanoic acids vs n-alkanes in the sedimentary record.

239   Over short timescales, there can be preferential loss of long-chain *n*-alkanes during

240   soil litter decomposition (Wu et al., 2018). Over longer timescales (i.e., millions of years)

241   or at elevated temperatures, *n*-alkanoic acids are expected to degrade faster due to their

242   functionalized nature. However, laboratory pyrolysis experiments show that they are

243   stable as long as thermal maturity is low (Diefendorf et al., 2015) and remain abundant in

244   some early Cenozoic (Hollis et al., 2021) and late Mesozoic sediments (van Dongen et

245   al., 2006). While both compound classes (*n*-alkanoic acids and *n*-alkanes) are commonly

246   reported separately in paleoclimatic studies, the decision is often guided by absolute

247   abundances, the extent of reworking or petroleum exposure, and/or co-elution with other

248   compounds. To assess this the extent of reworking, we strongly encourage future studies

249   to report thermal maturity ratios (e.g., the carbon preference index (CPI), hopane

250    $\beta\beta/(\alpha\beta+\beta\beta)$ ratios., etc). Where possible dual compound classes can be used to reveal

251 productivity or catchment sourcing differences (e.g. Hemingway et al., 2016, Feakins et

252 al., 2018; 2020).

253

254 Studies have looked for patterns in long-chain *n*-alkyl distributions between different plant

255 types (Bush & McInerney 2013, Diefendorf et al 2011) and across climate gradients

256 (Feakins et al 2016b), but recent meta-analyses have found limited value of summary

257 statistics (e.g., average chain length, odd-over-even ratios) as taxonomic or climatic

258 indicators (Bush & McInerney 2013, Diefendorf et al 2011). However, in some low-

259 diversity settings, interpretations of chain length ratios are able to detect plant type

260 variations (Bush & McInerney 2013). For example, in boreal *Sphagnum* moss-dominated

261 peats, the C_{23}/C_{29} or C_{23}/C_{31} *n*-alkane ratio has been used to differentiate between

262 *Sphagnum* moss (dominated by mid-chain homologues; e.g., C_{23}) and woody plants or

263 grasses (which are dominated by long-chain homologues; e.g., C_{29} or C_{31}) and reveals

264 changes in environmental conditions (Nott et al 2000). Certain studies also report a

265 greater predominance of C_{33} and/or C_{35} *n*-alkanes in graminoids (e.g., Bush and

266 McInerney, 2013). As tropical trees produce a substantially higher abundance of C_{29} and

267 C_{31} *n*-alkanes compared to grasses (Garcin et al., 2014), they dominate the sedimentary

268 C_{29} and C_{31} *n*-alkane pool. Thus, longer chain length alkanes (C_{33} and/or C_{35}) may more

269 strongly detect C_4 grass inputs into sediments (e.g., Schefuß et al., 2003). However, as

270 these chain lengths are not taxon-specific, additional evidence from pollen and spores

271 aids interpretations.

272

273 Other biomarkers can provide additional information on past vegetation. Lignin—a key

274 component of tree wood and other plant tissues—is a heterogeneous polymer containing

275 a variety of monomeric building blocks (see also Section 2.3 below). The ratio of different

276 lignin monomers (i.e., syringyl, vanillyl, and cinnamyl phenols) can differentiate between

277 non-woody angiosperms, woody angiosperms, and woody gymnosperms (Hedges &

278 Mann 1979). Terpenoids can also distinguish between angiosperms and gymnosperms.

279 Triterpenoids (e.g., ursane, lupane, oleanane) are mostly synthesised by angiosperms,

280 whereas diterpenoids (e.g., abietic acid, abietane, pimarane) are more abundant in

281 gymnosperms, especially evergreens (Diefendorf et al 2012). The relative abundance of

282 di- vs triterpenoids has been applied to reconstruct gymnosperms vs angiosperms

283 abundance in the past, although selective loss of triterpenoids can lead to an

284 overestimation of gymnosperms in the sedimentary record (Diefendorf et al 2014).

285

286 Machine learning methods have considerable potential to extract information from

287 vegetation biomarkers, especially the 'forest' of peaks present in homologous *n*-alkyl

288 series. Machine learning has detected chemotaxonomic classification power from plant

289 wax distributions, requiring the diagnostic information in molecular distributions across

290 two compound classes (*n*-alkanoic acids and *n*-alkanes) to sufficiently differentiate desert

291 shrub, conifer forest and macrophytes in modern ecosystems and in lake sediments

292 (Peaple et al 2021). These two compound classes are generalist biomarkers, but their

293 proportions and chain length distributions vary across taxa (Diefendorf et al 2011).

294

295   Presumably, additional compound classes and specialist biomarkers would add further

296   skill at chemotaxonomic separations. The machine learning approach has advantages

297   over summary metrics such as the average chain length or carbon preference index on a

298   single compound class (usually only the *n*-alkanes) and appears to perform better than

299   linear mixing models on dual compound classes (Gao et al 2011). Machine learning (e.g.,

300   non-negative matrix factorization, random forests, neutral networks) can also help

301   untangle source mixing and/or identify end-member distributions (Peaple et al., 2021;

302   Karp et al., 2021; Polissar et al. 2021). However, machine learning is subject to various

303   uncertainties (e.g., input of *n*-alkyl lipids from plants not represented in the training

304   dataset) or from taphonomic alterations between plant and sediment. Machine learning

305   should always be guided by physical and/or chemical knowledge of the relevant

306   biomarkers – otherwise, the outputs may not have clear physical meaning or may be

307   somewhat uncertain. Machine learning may also require training across a wider variety

308   of plant, soil and sedimentary settings and across ecosystems. It remains to be seen

309   whether it will perform well in ecosystems with greater diversity of plant taxa (e.g.,

310   rainforest) or lower leaf trait variability. It is also unclear how such machine learning

311   approaches will perform in non-analogue settings beyond the training set, although such

312   non-analogue questions also apply to other proxies. In order to enable further testing of

313   these approaches, we recommend collecting multi-compound plant wax abundances

314   (e.g., *n*-alkanoic acids, *n*-alkanes), including the broadest possible range of chain lengths

315   and the non-dominant odd/even chain lengths to develop information about plant wax in

316   a broader range of species and sedimentary settings.

317

318   Another widespread approach for vegetation reconstruction using biomarker methods

319   involves analysing the stable carbon isotopic composition (δ^{13}C) of long-chain *n*-alkyl

320   compounds. The δ^{13}C value of long-chain *n*-alkyl compounds can readily discern the

321   prevalence of C_3 and C_4 plant types (Huang et al 2001). This results from a large

322   fractionation in plant biosynthesis that differs according to photosynthesis pathway (n.b.,

323   the isotopic difference is larger in epicuticular waxes than in bulk tissues). Plant wax from

324   C_4 plants (most tropical grasses and some shrubs) are ^{13}C-enriched (~15 ‰) relative to

325   those produced by C_3 plants. This has been exploited to study the Neogene expansion

326   of C_4 grasslands (Freeman & Colarusso 2001, Polissar et al 2019). Plant wax δ^{13}C

327   records document major expansion of C_4 grasslands on the African continent ~ 10 Ma

328   (Polissar et al 2019). However, C_4 expansion is not globally uniform and plant wax δ^{13}C

329   records show that C_4 grassland expansion occurs in the Ganges-Brahmaputra catchment

330   ~3 Ma later (Figure 3) (Karp et al 2021).

331

332   Despite the substantial discriminatory difference between photosynthesis pathways, the

333   carbon isotopic composition (δ^{13}C) C_3 plants has a large spread which introduces

334   uncertainties into linear mixing model interpretations of past vegetation change

335   (Diefendorf et al., 2010). As a partial solution, (Cerling et al 2011) proposed a sinusoidal

336   regression between woody cover and δ^{13}C of soil organic matter, which was adapted for

337   plant waxes (Magill et al 2013). However, this only applies to post-Miocene, low-latitude

338   settings dominated by C_4 grasses. δ^{13}C variability *within* C_3 plants is also a valuable tool

339   for paleoenvironmental reconstruction. Within C_3 ecosystems there are discernable

340   signals including a primary sensitivity to mean annual precipitation and altitude, but only

341   in wet climates (Wu et al 2017). For further review of carbon isotopes in terrestrial

342   ecosystem reconstructions, other paleoenvironmental interpretations, and areas for

343   future research, readers are referred to a recent review by Diefendorf and Freimuth

344   (2017).

345

346   **2.3. Hydroclimate proxies**

347   Hydroclimate variability exerts an important influence on vegetation patterns (Section

348   2.2), soil residence time (Section 2.4) and a variety of other biogeochemical processes

349   (Section 2.5-2.6). However, there are few continuous and direct records of hydrological

350   change in deep time. The hydrogen isotopic composition (δ^2H) of long-chain *n*-alkyl lipids

351   (e.g., *n*-alkanes, *n*-alcohols, *n*-alkanoic acids) can provide important insights into the

352   hydrologic cycle. These compounds have a well-constrained source organism (i.e., higher

353   plants) and offer excellent preservation potential in a variety of sedimentary

354   environments. In addition, the hydrogen is bound to the alkyl carbon making it non-

355   exchangeable in most (<150°C) archives. Sachse et al (2004) first demonstrated an

356   empirical correlation between sedimentary long-chain *n*-alkyl lipid δ^2H (δ^2H$_{wax}$) and

357   precipitation δ^2H in European lakes. This was later corroborated across a variety of

358   environmental gradients (Balascio et al 2018, Daniels et al 2017, Feakins & Sessions

359   2010, Garcin et al 2012) and exploited to reconstruct hydroclimate in the geological record

360   (Bhattacharya et al 2018, Fornace et al 2014, Schefuß et al 2005, Tierney et al 2008,

361   Weijers et al 2007a).

362

363 δ^2H_{wax} is usually employed as a proxy for the isotopic composition of precipitation

364 (δ^2H_{precip}). This typically integrates a combination of climatic changes including

365 temperature, rainfall amount and character (e.g., stratiform vs. convective rainfall), as well

366 as atmospheric vapor transport. δ^2H_{wax} records thus benefit from interpretations based

367 on isotope-enabled climate model simulations, which can be used to identify the effects

368 of climatic changes on water isotope compositions (Lee et al 2007, Schmidt et al 2007).

369 However, despite the complexity of water isotopes, paleoclimate δ^2H_{wax} records capture

370 larger-scale hydroclimate shifts than local hydrological proxies (c.f. lake levels). The

371 variety of information in δ^2H_{precip} is also an asset; in the mid-latitudes δ^2H_{wax} can be used

372 to distinguish between different seasonal moisture sources (Bhattacharya et al 2018)

373 while in the tropics δ^2H_{wax} reveals general trends in aridity (Tierney & deMenocal 2013)

374 or atmospheric convection (Windler et al 2021). Comparisons between δ^2H_{wax} and ice

375 core or speleothem $\delta^{18}O$ demonstrates that δ^2H_{wax} captures similar information and also

376 records rapid climate changes such as the Younger Dryas cooling (Fornace et al 2014)

377 (Figure 4). Furthermore, δ^2H_{wax} can capture climatic changes in the historical period, as

378 demonstrated by the δ^2H_{wax} record from the Gulf of Aden, which documents increasingly

379 arid conditions over the last 200 years (Tierney et al 2015) (Figure 4). Tandem

380 measurements of $\delta^{13}C_{wax}$ show the presence of the Suess effect, indicating rapid

381 transport of plant waxes from the terrestrial source (the Horn of Africa) to the coastal

382 ocean (Tierney et al 2015). As discussed further below (Section 2.4), such records

383 demonstrate that a young, rapidly overturning plant wax pool allows for rapid climate

384 changes to be recovered despite the presence of a pre-aged and/or reworked plant wax

385 pool.

386

387 Many studies use δ^2H_{wax} as a qualitative indicator of climatic changes (e.g., Figure 4).

388 However, quantitative inference of δ^2H_{precip} is possible if reasonable constraints can be

389 made on the apparent fractionation between δ^2H_{wax} and δ^2H_{precip} (= $^2\varepsilon_{wax/w}$; see Sachse et

390 al 2012, Sessions 2016 for a detailed review). Three key factors have the potential to

391 impact $^2\varepsilon_{wax/precip}$: i) soil evaporation, ii) leaf-water transpiration iii) wax biosynthesis (see

392 below).

393

394 Although theoretical models have incorporated evaporative ^2H-enrichment of soil water

395 into predictions of $^2\varepsilon_{wax/w}$ (Konecky et al 2019, Smith & Freeman 2006), ecohydrological

396 data demonstrates that this effect is minimal. For instance, in drylands, where soil water

397 is readily evaporated and non-bioavailable, woody plants take up rainwater

398 opportunistically via shallow roots and/or seek more consistent water sources by deep

399 rooting strategies (Fan et al 2017), resulting in minimal xylem water ^2H-enrichment. In

400 contrast, leaf water ^2H-enrichment that occurs during transpiration is more variable,

401 depending on the species and the climatological setting (Daniels et al 2017, Feakins et al

402 2016a, Feakins & Sessions 2010, Kahmen et al 2013a). In mesic environments where

403 lake water evaporation is minimal, pairing of plant wax with aquatic biomarkers has

404 proved useful for disentangling the effect of transpiration on δ^2H_{wax} (Mügler et al 2008,

405 Rach et al 2014).

406

407 An empirical collection of $^2\varepsilon_{wax/w}$ values across species and climatic settings implies that

408 it varies widely (up to ~70 ‰) between plant life forms (i.e., trees, shrubs, forbs,

409 graminoids) and physiological groups (i.e., C_3, C_4, CAM) (Sachse et al 2012). Taken at

410 face value, this suggests that biosynthetic differences have a large impact on apparent

411 fractionation. However, these empirical data combine physiological/biochemical and

412 climatic differences, and separating these influences on fractionation can be challenging

413 (Feakins et al 2016a, Kahmen et al 2013b, Sachse et al 2012). Experimental approaches

414 enable biophysical vs. climatic differences on fractionation to be isolated and have found

415 that $^2\varepsilon_{wax/w}$ is relatively consistent between species under controlled environmental

416 settings, with the only major physiological/biochemical difference between grasses (which

417 have a more negative $^2\varepsilon_{wax/w}$) and eudicots (Gamarra et al 2016, Gao et al 2014). Since

418 paleoclimate records of $\delta^2 H_{wax}$ integrate across a landscape, this enables the use of plant

419 community-scale $^2\varepsilon_{wax/w}$ values in paleoclimate studies to infer $\delta^2 H_{precip}$.

420

421 Estimating $^2\varepsilon_{wax/precip}$ in the paleoenvironmental record must also account for any seasonal

422 bias relative to annual rainfall. In most environments, plant growth is stimulated by

423 seasonal availability of light and plants often grow using water from the previous season's

424 rainfall. Seasonal studies of modern plant ecohydrology reveal the seasonality of rainfall

425 and plant uptake (Griepentrog et al 2019) and this has been invoked to explain small

426 $^2\varepsilon_{wax/precip}$ values observed in alpine gymnosperm flora (Polissar and Freeman, 2010).

427 However, expanded observational and experimental data are required to resolve these

428 uncertainties.

429

430 Several strategies are available for improving $^2\varepsilon_{wax/w}$ estimates in the paleoenvironmental

431 record. One strategy employs pollen data from the same samples to calculate plant-

432　specific fractionation factors (Feakins 2013), thus opening the possibility of developing

433　region- and time-specific vegetation corrections based upon appropriate modern

434　analogues. When applied to the Miocene (Feakins 2013) and Eocene (Inglis et al 2020),

435　this method can shift inferred δ^2H_{precip} values by >30 ‰. However, pollen-corrected

436　δ^2H_{precip} reconstructions have large uncertainties due to the differential production of

437　pollen between wind and insect-pollinated plants (e.g., pines and grasses are prolific

438　pollen dispersers, whereas tropical forests disperse little pollen). This concern can be

439　qualitatively assessed (e.g., absence of rainforest pollen does not guarantee an absence

440　of rainforest) or quantitatively deconvolved with additional mixing model weighting factors.

441

442　A more common strategy for Neogene (~23 Ma to present) climate reconstructions

443　involves measuring *n*-alkyl lipid $\delta^{13}C$ ($\delta^{13}C_{wax}$) values alongside δ^2H_{wax} in order to infer C_3

444　vs. C_4 plant prevalence (Tipple & Pagani 2010). This is particularly effective in tropical

445　and subtropical regions, where C_4 grasslands are a major component of the ecosystems

446　(Tierney et al 2017, Windler et al 2020) and have a significantly different $^2\varepsilon_{wax/w}$ than C_3

447　dicots (e.g., Gao et al, 2014). Figure 3 demonstrates how $^2\varepsilon_{wax/w}$ can be inferred via pollen

448　data and lipid $\delta^{13}C$ values (Polissar et al 2021) and used to calculate δ^2H_w. Vegetation-

449　corrected δ^2H_w shows a 10 ‰ greater enrichment after 6 Ma than raw δ^2H_{wax} and follows

450　the large C_4 grass expansion that is documented in $\delta^{13}C_{wax}$ (Figure 3). While this is small

451　relative to the large amplitude of change in this particular record, in other settings where

452　δ^2H_{wax} variance is lower, changes in $^2\varepsilon_{wax/w}$ strongly influence overall trends (Tierney et al

453　2017).

454

In addition to δ^2H_{wax}, the hydrogen isotopic composition of lignin methoxy groups ($\delta^2H_{methoxy}$) holds promise as a novel paleohydrologic indicator (Keppler et al 2007). Lignin is a key component (30–50%) of wood and other plant tissues and is a heterogeneous polymer containing a variety of monomeric building blocks. Each monomeric building block contains 0, 1 or 2 methoxy groups (-OCH_3), which are attractive targets for hydrogen isotope analysis because the hydrogens are in non-exchangeable positions. Methoxy groups can be released from wood (Keppler et al 2007), peat (Lee et al 2019a), and lignite (Lee et al 2019a), and spans a wide isotopic range (-325 to -150 ‰) in tree trunk samples (Keppler et al 2007). However, variability between species and trees may complicate detection of hydrologic signals, as shown in a coastal saltwater to freshwater gradient (Feakins et al 2013). Calibration and process-based understanding of drivers of biological variability in $^2\varepsilon_{methoxy/w}$ remains in the early stages, but recent analytical method development (Greule et al 2008, Lee et al 2019a) has enabled successful applications in Eocene-aged wood (Anhäuser et al 2018) and Neogene-aged sediments (Lee et al 2019b).

2.4. Terrestrial carbon cycling proxies

Carbon export from the terrestrial biosphere is a significant component of the global carbon cycle (Hilton & West 2020). One of the main unquantified processes is the lateral transport of terrestrial organic carbon (OC) along the aquatic continuum from upland terrestrial ecosystems to the ocean. Biomarker abundance (Bianchi et al 2004, Goñi et al 1997), $\delta^{13}C$ values (Feakins et al 2018, Hemingway et al 2016) and radiocarbon (^{14}C) content (Feng et al 2013, Kusch et al 2010) can be used to study changes in the storage,

478   aging, and mobilization of different components of the terrestrial OC cycle. This may

479   encompass plants (e.g., plant wax, lignin) or soils (e.g., brGDGTs), and can reveal the

480   spatial and temporal scale of their integration before delivery to depositional basins (Feng

481   et al 2013, Hemingway et al 2016, Kusch et al 2010).

482

483   Plant or soil derived biomarkers transported by rivers are often assumed to reflect a

484   catchment-integrated signal, but the spatial and temporal integration may vary by

485   compound type and/or change through time. Soils and rivers are typically dominated by

486   long-chain *n*-alkanoic acids (Feakins et al., 2018) due to preferential loss of long-chain *n*-

487   alkanes during soil litter decomposition (Wu et al., 2018). Studies also suggest that long-

488   chain *n*-alkanoic acids respond more rapidly to proximal changes in climate, whereas

489   long-chain *n*-alkanes have a greater spatial and temporal range and also have more risk

490   of petrogenic input (Feakins et al 2018, Hemingway et al 2016) (see Section 2.2). In a

491   global river dataset, Eglinton et al (2021) found that the age of exported plant wax (*n*-

492   alkanoic acids) ranged from 1-2ka in the tropics to up to 8ka in the high latitudes. They

493   found the strongest correlation between climatic variables (MAAT and MAP) and fluvial

494   *n*-alkanoic acid ^{14}C ages occurred when using an *e*-folding distance of ~500 km, implying

495   that this is roughly the spatial extent of plant wax *n*-alkanoic acid integration in large river

496   systems. The *e*-folding distance will likely be different for other compound classes (e.g.,

497   *n*-alkanes, lignin) and requires additional investigation.

498

499   Bomb-spike ^{14}C (produced during nuclear weapons detonation and testing that began in

500   1945 and increased until 1963) can also be detected in OC, including plant wax

501 biomarkers in soils and sediments. Biomarker $^{14}C/^{12}C$ ratios in soils or river sediments

502 that are higher than modern (natural) concentrations indicate the presence of bomb-

503 produced ^{14}C and can be used to track the mean age of biomarkers in storage or fluvial

504 transit. Using a two-pool mixing model approach, French et al. (2018) estimates that

505 ~80% of the *n*-alkanoic acids in the Bengal Fan have a reservoir age of ~1000 years, with

506 the remainder stored on the landscape for only ~15 yr (French et al 2018). This approach

507 has been extended to other river catchments (including the Mackenzie River, Saanich

508 Inlet, and Cariaco Basin) and shows a similar distribution of "fast-cycling" vs. "slow-

509 cycling" *n*-alkanoic acids (Vonk et al 2019), with high-latitude (permafrost-dominated)

510 catchments exhibiting the longest carbon storage. However, this approach requires

511 assumptions about the age distributions of each compound class and requires further

512 validation.

513

514 Carbon storage on land has profound implications for modern carbon cycle feedbacks

515 and may be illuminated by the study of past climate perturbations. Pairing of $\delta^2 H_{wax}$ and

516 $^{14}C_{wax}$ (e.g., across the last deglaciation; Figure 5) provides a powerful tool to probe the

517 relationships between climate and the carbon cycle, from the deglaciation into the

518 Anthropocene. In the low-latitudes, there is a negative relationship between $\delta^2 H_{wax}$ and

519 mean transit times across the deglaciation (reported as $F^{14}R$, or the plant-wax ^{14}C content

520 relative to that of the contemporaneous atmosphere at the time of deposition (Fornace

521 2016, Hein et al 2020, Schefuß et al 2016) (Figure 5). This implies wetter conditions are

522 associated with shorter OC residence times in both large river systems (Hein et al 2020,

523 Schefuß et al 2016) and in a more restricted lake catchment (Fornace 2016). The

524   observations that large portions of the plant wax pool being "old" (i.e., 1000 yr residence

525   times) and the inference that plant waxes are mobilized more quickly in wetter climates

526   raises the possibility that δ^2H_{wax} records are affected by changing spatiotemporal

527   integration in terrestrial catchments. The effect of "pre-aging" of plant wax would be to

528   smooth, dampen, and delay the original δ^2H_{precip} signature (French et al 2018). Some

529   δ^2H_{wax} records (e.g., the time series from the Congo Basin) have a smooth character that

530   might imply substantial spatiotemporal averaging (Schefuß et al 2005) compared to the

531   smaller and more abrupt changes recorded elsewhere (e.g., Lake Tanganyika; Tierney

532   et al., 2008). However, in many cases δ^2H_{wax} captures rapid climate changes with little

533   apparent delay, including decadal-scale historical climate shifts (Figure 4). Fornace

534   (2016) found plant wax reservoir ages of ~8,000 years in Lake Titicaca, yet δ^2H_{wax} shows

535   rapid changes and millennial-scale events similar to the Huascarán ice core (Figure 4),

536   indicating that the "decadal" plant-wax pool must be very responsive to climate change.

537   In the open ocean, but also in arid (coastal) regions, plant waxes may be exported

538   primarily via aeolian processes (Pagani et al., 2000; Eglinton et al., 2002) (e.g., Gulf of

539   Aden, Figure 4a). Wind transport of plant wax is able to capture seasonal variations in C_3

540   vs C_4 vegetation (Conte & Weber 2002) and provides another explanation for why δ^2H_{wax}

541   captures rapid climate change.

542

543   Beyond the late Pleistocene (ca. 40 to 50 ka), ^{14}C-based dating techniques are not

544   applicable and additional approaches are required to assess terrestrial OC cycling. The

545   ratio of brGDGTs to crenarchaeol (i.e., the Branched-versus-Isoprenoid Tetraether (BIT)

546   index) tracks the amount of terrestrial input in marine environments and ranges between

547   0 (marine-dominated) and 1 (terrestrial-dominated) (Hopmans et al 2004). The BIT index

548   typically decreases from the river, through the estuary, and into the open shelf (Hopmans

549   et al 2004). However, it can be controlled strongly by crenarchaeol—rather than

550   brGDGT—concentrations, leading to a decoupling between BIT values and other

551   terrestrial OC tracers (e.g., lignin concentrations) (Smith et al 2012). Due to these

552   challenges, brGDGT concentrations (rather than the BIT index) may be a more robust

553   tracer for soil OC (Smith et al 2012) and has shown promise in mountainous catchments

554   (Kirkels et al 2020). However, branched GDGTs degrade more quickly than other soil OC

555   tracers (e.g., long-chain *n*-alkanes, lignin phenols) (Zhu et al 2013) and caution is required

556   when applying this approach (and/or the BIT index) to represent bulk soil OC, especially

557   when the system is characterized by extensive OM degradation (e.g., large floodplains).

558   We therefore argue that future studies should employ multiple proxies when attempting

559   to trace soil OC export (e.g., lignin, *n*-alkyl lipids, brGDGTs).

560

561   **2.5. Fire proxies**

562   Reconstructing fire regimes usually relies upon the preservation of charcoal. However,

563   charcoal abundance can be over- or under exaggerated by preservation biases (Vachula

564   & Cheung 2021). Biomarkers offer a complementary view on the relationships between

565   fire regime, climate, and vegetation in past terrestrial ecosystems. The most common

566   approach involves analyzing the distribution of polycyclic aromatic hydrocarbons (PAHs),

567   which can form rapidly at high (>300°C) temperatures (i.e., during the combustion of fossil

568   fuels or biomass) or slowly at low (<150°C) temperatures (i.e., during petroleum

569   formation). Previous studies have argued that PAH distributions are linked to changes in

570   fire temperature and/or intensity (e.g., Denis et al. 2012). However, a recent meta-

571   analysis of PAHs in natural burn experiments (Karp et al., 2020) reveals that PAH

572   distributions are linked to burn phase, not temperature. PAHs formed in smoke are

573   typically smaller than those formed in residues. PAH size distributions also reflect

574   transport processes. As smaller PAHs are preferentially emitted into the aerosol phase,

575   they can be aerially transported far from the original combustion source. In contrast, larger

576   PAHs are less volatile and more likely to be stored in soils. This approach was employed

577   by Lyons et al. (2020) to show that PAHs were transported long distances following the

578   Cretaceous/Paleogene asteroid impact.

579

580   PAHs also provide insights into the fuel source: combusted vegetation yields PAHs with

581   no alkyl substitutions, whereas fossil carbon (e.g., oil or coal) is associated with one or

582   more alkyl substitutions. This is assessed via the methylphenanthrene to phenanthrene

583   ratio (Yunker et al 2002), non-negative matrix factorization (Karp et al 2021), and/or the

584   alkylated PAH derivative index (APDI; Karp et al, 2018). Positive APDI values (>10; i.e.,

585   no alkyl substitutions) imply biomass burning, whereas negative APDI values (< -10; one

586   or more alkyl substitutions) imply a fossil fuel carbon source (Karp et al 2020). However,

587   burned conifers can exhibit negative APDI values and may therefore resemble a fossil

588   fuel carbon source. PAH studies reveal that Neogene fire dynamics differed between

589   continents (Karp et al 2018, Karp et al 2021) and that fire occurrence often coincided with

590   hydrologic shifts and C_4 expansion (Figure 3). Carbon isotopic measurements of specific

591   PAH molecules provide additional information about the fuel source (Karp et al 2021). For

592   example, $\delta^{13}C$ analysis of pyrene indicates an increasing proportion of C_4 grasses burning

593 between 6 and 8 Ma, coeval with a shift in plant wax $\delta^{13}C$ to more C_4-like values (Figure

594 3).

595

596 Levoglucosan and its isomers (mannosan, galactosan) provide further detail about the

597 fire regime. Levoglucosan is a thermal by-product of cellulose or hemicellulose generated

598 during biomass burning and forms at relatively low temperatures (~100 to 400°C)

599 (Simoneit et al 1999). This compound is relatively labile and thus geological applications

600 may be limited to late Quaternary sediments. However, recent methodological advances

601 have lowered the limits of detection (~5 pg) (Schreuder et al 2018) and may enable

602 detection in older sediments and/or settings where oxygen exposure is low (e.g., anoxic

603 peats, water-saturated soils). Benzene polycarboxylic acids (BPCAs; benzene rings with

604 a differing number of carboxylic-acid groups) offer an additional approach and are more

605 likely to represent the bulk pyrogenic carbon pool (Glaser et al 1998). BPCAs form at

606 relatively high temperatures (>300-600°C, but up to 1000°C) and are produced by

607 oxidative chemical degradation of the condensed aromatic phase. BPCA distributions are

608 unable to differentiate between fuel sources (fossil fuel vs. biomass), but improved source

609 apportionment in modern or Holocene-aged samples is possible using ^{14}C dating since

610 different fuel sources (e.g., vegetation, pre-aged soils, fossil carbon) carry unique [14]C

611 signatures (Coppola et al 2018). The combined analysis of PAHs, levoglucosan, and

612 BPCAs can differentiate between low (<300°C) and high temperature fires (>300-

613 1000°C) and thus can provide a nuanced perspective on fire dynamics (Hanke et al 2016).

614

615 **2.6. Methane cycling proxies**

616 Atmospheric methane (CH_4) is a potent greenhouse gas second only to carbon dioxide

617 in its importance to climate change (Dean et al 2018). Higher temperatures and

618 associated changes in rainfall patterns are expected to enhance CH_4 emissions, resulting

619 in further warming. However, there have been few tools to test these predictions. Below

620 we discuss biomarker-based approaches used to study two key aspects of the methane

621 cycle: 1) methanogenesis (Section 2.6.1) and ii) methanotrophy (Section 2.6.2).

622

623 **2.6.1. Methanogenesis**

624 Methanogens thrive within water-saturated and anoxic environments (e.g., peatlands,

625 permafrost, anoxic lakes) and synthesise a suite of diether- and/or tetraether membrane

626 lipids (Schouten et al 2013). Archaeol (2,3-diphytanyl-O-sn-glycerol) is the most common

627 archaeal lipid in cultured methanogens (Bauersachs et al 2015, Koga et al 1993) and

628 shows promise as an indicator of methanogen biomass (Pancost et al 2011, Zheng et al

629 2014). Archaeol has been applied to reconstruct methanogen abundance in Holocene-

630 aged peat archives and reveals a minimum in methanogenesis in China between ~6 and

631 4 ka (Zheng et al, 2014). This suggests that archaeol may be a useful methanogen

632 biomarker in older (> 1 Ma) sediments. However, direct estimates of methanogen

633 biomass from archaeol should be approached with caution as there can be differing

634 concentrations of archaeol per methanogen cell (McCartney et al 2013).The acyclic

635 isoGDGT (i.e., isoGDGT-0) is also abundant in methanogens (Bauersachs et al 2015,

636 Koga et al 1993, Schouten et al 2013) and may provide complementary insights into

637 methanogenesis (e.g., peatlands, permafrost, lakes).

638 Both isoGDGT-0 and archaeol have diverse source organisms, perhaps limiting their

639 utility as methanogen biomarkers. In contrast, a sub-set of methanogens (e.g.,

640 Methanococcus, Methanosarcina) are known to synthesise *sn*-2-hydroxyarchaeol (Koga

641 et al 1993); this is structurally similar to archaeol but contains a hydroxyl group at the C-

642 3 position of the *sn*-2 phytanyl chain (Hinrichs et al 2000). Due to the labile nature of sn-

643 2-hydroxyarchaeol, this biomarker holds promise as a marker for living methanogen

644 biomass (Pancost et al 2011). There is also growing evidence that methanogens may

645 synthesise unusual butanetriol and pentanetriol dibiphytanyl glycerol tetraethers (BDGTs

646 and PDGTs, respectively). BDGTs and PDGTs have been identified in different

647 environments—including wetlands (Blewett et al 2020) —and have been assigned to the

648 methanogen order Methanomassiliicoccales (Becker et al 2016). Analogous to other

649 archaeal lipids (e.g., archaeol, GDGT-0), BDGTs and PDGTs in wetlands increase in

650 concentration below the anoxic layer and are nearly absent from oxygenated layers. This

651 is consistent with observations that only methanogens synthesize BDGTs and PDGTs

652 (Becker et al 2016). These compounds—alongside other 'minor' GDGTs (Bauersachs et

653 al 2015)—are promising methanogen-specific biomarkers that warrant further study.

654

655 **2.6.2. Methanotrophy**

656 Microbes capable of consuming methane are known as methanotrophs. Aerobic

657 methanotrophs can synthesise diagnostic hopanoids, including 35-

658 aminobacteriohopanepentol (i.e., aminopentol) and 35-aminobacteriohopanetetrol (i.e.,

659 aminotetrol) (Rush et al 2016, Talbot & Farrimond 2007). Both compounds are present in

660 terrestrial environments, especially peats (Van Winden et al 2012) and lakes (Talbot &

661 Farrimond 2007), and can persist in the sedimentary record for >50 Ma under favorable

662 conditions (e.g., anoxia). Both compounds have been used to reconstruct aerobic

663 methanotrophy during the Quaternary (Talbot et al 2014) and early Eocene (Talbot et al

664 2016), but typically lose their functionalized amino side group during diagenesis.

665

666 Analysis of hopanoid $\delta^{13}C$ provides an alternative means to reconstruct methanotrophy.

667 Hopanoid $\delta^{13}C$ is primarily determined by the $\delta^{13}C$ of the substrate, carbon assimilation

668 pathways and an organism's source ecology (Hayes 1993, Pancost & Sinninghe Damsté

669 2003). Heterotrophic organisms consuming organic substrates (e.g., organic acids,

670 sugars) will typically yield hopanoid $\delta^{13}C$ values which are similar to the food source (ca.

671 -20 to -30 ‰), whereas methanotrophic organisms (consuming methane) will yield

672 hopanoid $\delta^{13}C$ values which are lower (ca. -40 to -60 ‰, but up to -100 ‰) and more

673 variable (Pancost & Damsté 2003). A recent survey of hopanoid $\delta^{13}C$ values in peatlands

674 from different geographic regions shows that the $\delta^{13}C$ composition of the C_{31} hopane—

675 one of the most abundant hopanoids in peats—spans a relatively narrow range (−22 to

676 −32 ‰ VPDB) and is ^{13}C-enriched relative to bulk organic matter and co-occurring plant

677 wax biomarkers (Inglis et al 2019b). This suggests the C_{31} hopane is derived from a

678 heterotrophic source organism(s) and has limited utility as a methanotroph biomarker. In

679 contrast, other hopanoids (e.g., hop-22(29)-ene) yields lower $\delta^{13}C$ values (e.g., up to −45

680 ‰) (Inglis et al 2019b), suggesting that in some settings this compound is derived from a

681 mixed suite of bacterial sources consuming both ^{13}C-enriched carbohydrates and [13]C-

682 depleted, methane-derived CO_2. Low hopanoid $\delta^{13}C$ values (ca. -60 to -80 ‰) have also

683 been found in modern (e.g., Naeher et al 2014), Holocene (e.g., Elvert et al 2016, Naeher

684   et al 2014), and Eocene lakes (e.g., Collister et al 1992, Freeman et al 1990). These

685   values are considerably lower than in wetlands and indicate more vigorous methane

686   consumption in lakes.

687

688   Hopanoid δ^{13}C values have successfully been applied as a proxy for methanotrophy

689   across the Paleocene-Eocene Thermal Maximum (PETM). Pancost et al (2007) first

690   observed a sudden decrease in hopanoid δ^{13}C values (to -75 ‰) in the Cobham lignite,

691   UK, an ancient wetland deposited during the onset of the PETM (Figure 6). These low

692   values lie outside the modern range (Inglis et al 2019b) and coincided with an increase in

693   methanotroph (e.g., aminoBHPs) (Talbot et al 2016) and methanogen biomarkers

694   (GDGT-0) (Inglis et al 2019a). This implies enhanced methane cycling at this site during

695   the PETM. Recent work provides additional evidence that enhanced methanotrophy

696   occurred the onset of the PETM, with low hopanoid δ^{13}C values observed in PETM-aged

697   terrestrial/marine deposits from New Zealand (up to -60 ‰) (Inglis et al 2021). Hopanoid

698   δ^{13}C values rapidly returned to pre-event values even though brGDGT-derived

699   temperatures remain high for the duration of the PETM (Inglis et al, 2021) (Figure 6). This

700   suggests it is the onset of rapid global warming that is particularly disruptive to methane

701   cycling in wetlands, a finding that is particularly concerning given the rapid global warming

702   we are currently experiencing.

703

704   Methane can also be oxidised anaerobically (anaerobic oxidation of methane; AOM) by

705   a consortium of anaerobic methanotrophic archaea and sulfate reducing bacteria (SRB)

706   (Hinrichs et al., 1999). Anaerobic methanotrophs produce diagnostic isoGDGT

707   distributions, with a high abundance of isoGDGT-0 to -3 relative to crenarchaeol (Pancost

708   et al 2000). This is captured in the Methane Index (Zhang et al 2011), whereby high values

709   (> 0.5) suggest extensive anaerobic methanotrophy. This ratio is mainly used in marine

710   settings but has utility in terrestrial settings where AOM is elevated (e.g., freshwater

711   wetlands). To confirm the presence of AOM, other biomarkers can be analysed (e.g., [13]C-

712   depleted pentamethylicosane and/or crocetane)

713

714

715   **Summary Points:**

716

717   1. **Temperature** – Bacterial-derived branched GDGTs provide insights into terrestrial

718   temperatures up to the maximum theoretical limit of present calibrations (~30°C).

719   Mesocosm experiments and new GDGT proxies may offer insights into higher

720   terrestrial temperatures observed in the geological past.

721

722   2. **Plant ecosystems** – Lignin and plant wax molecular compositions and their stable

723   carbon isotopic composition ($\delta^{13}C$) carry signals of plant community composition

724   and paleoenvironmental change. Machine learning of higher plant biomarkers

725   offers promise in terms of reconstructing ecosystem turnover.

726

727   3. **Hydroclimate –** Recent advances, including tandem reconstruction of ecosystem

728   change with pollen or plant wax $\delta^{13}C$ and paired analysis of isotope-enabled model

729   simulations, have improved constraints on the interpretation of plant wax $\delta^{2}H$

730   values in the geological record. Lignin methoxy δ^2H values similarly records the

731   hydrological cycle, but the proxy is in comparatively early stages of development.

732

733   **4. Carbon cycling** – Plant - and soil-derived biomarkers provide insights into the

734   storage, aging, and mobilization of different terrestrial OC pools along the

735   terrestrial-aquatic continuum. Pairing of plant wax δ^2H and ^{14}C provides a powerful

736   tool to probe the relationships between climate and the carbon cycle during the

737   late Quaternary.

738

739   **5. Fire regime** - The analysis of multiple fire biomarkers provides a holistic

740   perspective on fire dynamics and can reveal the temperature history, fuel source

741   and burn conditions.

742

743   **6. Methane cycling** – The δ^{13}C of bacterial hopanoids provides unique (qualitative)

744   insights into methanotrophy throughout the geological record. Coupling this

745   approach alongside archaeal biomarkers for methanogenesis may corroborate

746   detection of changes in the terrestrial methane cycle.

747

752   feedback improved this review. We are grateful to the organic geochemical community

753   whose innovative work inspired this review.

754

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1216 **[Sidebar 1]**

1217 **Title**: **Organic biomarkers**

1218 Organisms produce a wide range of organic compounds, including proteins,

1219 carbohydrates and lipids. Organic compounds undergo various structural transformations

1220 during diagenesis, but can retain structural or isotopic information that enables insights

1221 into their biological precursor organism ("biomarkers"). Treibs (1934) first demonstrated

1222 that organic pigments (porphyrins) preserved within ancient soil, shale and coal deposits

1223 were derived from chlorophyll-a, a light-harvesting pigment found in all photosynthetic

1224 organisms. In general, a robust biomarker must be: (i) diagnostic of some species, taxa,

1225 or process; (ii) well preserved over long timescales (10^3 to 10^8 yr); and/or (iii) responsive

1226 to environmental perturbations (e.g., temperature).

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1239 **Summary phrases:**

1240   -   Biomarkers can be used to reconstruct terrestrial environmental change over a

1241   range of geological timescales

1242   -   Analyzing several biomarkers in tandem can provide unique insights into the Earth

1243   System

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Captions

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1263 Figure 1 Key terrestrial biomarkers in the environment. Biomarkers provide insights into

1264 physical climate variables (land temperature, rainfall), ecosystem state variables

1265 (vegetation, fire regime), and biogeochemical variables (soil residence time, methane

1266 cycling). Abbreviation: brGDGT, branched glycerol dialkyl glycerol tetraether.

1267 Figure 2 brGDGTs indicate a rapid drop in terrestrial temperatures in Prydz Bay, East

1268 Antarctica across the Eocene–Oligocene transition (~34 million years ago). (*a*) Benthic

1269 foraminiferal $\delta^{18}O$ values (Westerhold et al. 2020). (*b*) brGDGT-inferred MAAT

1270 estimates (Tibbett et al. 2021). The shaded region in panel *b* represents 1σ uncertainty.

1271 Abbreviations: brGDGT, branched glycerol dialkyl glycerol tetraether; MAAT, mean

1272 annual air temperature.

1273 Figure 3 Coupling of hydrology, vegetation, and fire regime on the Indian subcontinent

1274 during the late Miocene (~10 million years ago to present). (*a*) δ^2H values from the C_{31}

1275 *n*-alkane (Karp et al. 2021) (*black*) and δ^2H values corrected for vegetation fractionation

1276 to generate estimates of environmental water δ^2H (*blue*), following Polissar et al. (2021).

1277 Colored shaded areas represent 1-sigma errors. (*b*) $\delta^{13}C$ values from pyrene (*dark red*)

1278 and C_{31} *n*-alkane (*orange*), both corrected for $\delta^{13}C$ changes in CO_2 (Karp et al. 2021).

1279 (*c*) Total pyrogenic PAH concentrations normalized to tetra aromatic β-amyrin

1280 derivative, following the method of Karp et al. (2021b). Abbreviation: PAH, polycyclic

1281 aromatic hydrocarbons.

1282 Figure 4 Carbon and hydrogen isotopic compositions of plant wax biomarkers capture

1283   rapid climate changes. (*a*) Long-chain *n*-alkanoic acid δ^2H data from the Gulf of Aden

1284   (*blue*) track northern hemisphere temperature anomaly (*gray*), indicating regional aridity

1285   accompanies twentieth-century warming, and *n*-alkanoic acid δ^{13}C data (*orange*)

1286   capture the Suess effect, as represented by δ^{13}C of atmospheric CO_2 (Tierney et al.

1287   2015). (*b*) Long-chain *n*-alkanoic acid δ^2H values from Lake Titicaca capture millennial-

1288   scale climate shifts during the last deglaciation (Fornace et al. 2014) that are as rapid—

1289   if not more—than the record of δ^{18}O from the Huascarán ice core in Peru (Thompson et

1290   al. 1995).

1291   Figure 5 Relationships between lipid ^{14}C and δ^2H$_{wax}$ values since the last deglaciation

1292   (~17 thousand years ago to present). Results show a general negative relationship

1293   between δ^2H$_{wax}$ and plant-wax mean transit time [reported as the ratio of ^{14}C activity

1294   between plant waxes and the contemporaneous atmosphere at the time of deposition

1295   (F^{14}R)] and indicate shorter terrestrial residence times under wetter climates. Data from

1296   Fornace (2016) (Lake Titicaca), Hein et al. (2020) (Bay of Bengal), and Schefuß et al.

1297   (2016) (Congo Fan). Uncertainty for Lake Titicaca is taken as either the propagated

1298   analytical uncertainty for combined chain lengths or the maximum-minimum difference

1299   between values for different chain lengths, whichever is greater. Uncertainty for other

1300   records is taken as that propagated in the original publications.

1301   Figure 6 Enhanced terrestrial methane cycling in Otaio River, New Zealand, during the

1302   onset of the Paleocene-Eocene Thermal Maximum (~56 million years ago). (*a*) Bulk

1303   organic matter δ^{13}C values. (*b*) C_{30} hop-17(21)-ene δ^{13}C values. (*c*) brGDGT-inferred

1304   MAAT estimates in marine interbeds only. The light blue shaded region in panel *c*

1305   indicates 1σ error. Data from Inglis et al. (2021). Abbreviations: MAAT, mean annual air

1306   temperature; brGDGT, branched glycerol dialkyl glycerol tetraether.

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Temperature
e.g., brGDGTs

Methane cycling
e.g., diploptene

Hydrology
e.g., C$_{29}$ n-alkane

Fire
e.g., pyrene

Vegetation
e.g., lignin monomers

Soil residence time
e.g., C$_{28}$ n-alkanoic acid

Prydz Bay,
Antarctica

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