1   A copper isotope investigation of methane cycling in Late Archaean sediments.

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11

13

Abstract

15

16   The rise of oxygenic photosynthesis arguably represents the most important

17   evolutionary step in Earth history. Recent studies, however, suggest that Earth's pre-

18   oxidative atmosphere was also heavily influenced by biological feedbacks. Most

19   notably, recent geochemical records propose the existence of a hydrocarbon haze

20   which periodically formed in response to enhanced biospheric methane fluxes.

21   Copper isotopes provide a potential proxy for biological methane cycling; Cu is a

22   bioessential trace metal and a key element in the aerobic oxidation of methane to

23   carbon dioxide (methanotrophy). In addition, Cu isotopes are fractionated during

24   biological uptake. Here, we present a high-resolution Cu isotope record measured in

25   a suite of shales and carbonates from core GKF01, through the ~2.6-2.5 Ga

26   Campbellrand-Malmani carbonate platform. Our data show a 0.85 ‰ range in Cu

27   isotope composition and a negative excursion that predates the onset of a haze

28 event. We interpret this excursion as representing a period of enhanced aerobic

29 methane oxidation before the onset of the Great Oxidation Event. This places

30 valuable time constraints on the evolution of this metabolism and firmly establishing

31 Cu isotopes as a biomarker in Late Archaean rocks.

32

33

34 **1. Introduction**

35

36 The Great Oxidation Event (GOE, c. 2.3 Ga) provides the first compelling evidence

37 that biology can play a significant role in altering the atmospheric composition and

38 Earth's surface environment. The rise of oxygenic photosynthesising cyanobacteria,

39 which triggered the pervasive oxygenation of Earth's oceans and atmosphere, is

40 considered to be one of the most important evolutionary steps in Earth's history,

41 eventually enabling the development of complex multicellular life (e.g. Canfield,

42 2005; Farquhar et al., 2010). More recently, the simple narrative of an irreversible

43 rise in atmospheric oxygen has been contested, with arguments for earlier

44 accumulation(s) of oxygen (e.g. Anbar et al., 2007; Garvin et al., 2009), as well as

45 proposals suggesting that Earth's pre-GOE atmosphere experienced periodic haze

46 events (PHEs), during which it was dominated by a methane-rich haze (Zerkle et al.,

47 2012).

48

49 The presence of S-MIF ($\Delta^{33}S$ and $\Delta^{36}S$; Farquhar and Wing, 2003) is a

50 characteristic signature in pre-GOE sedimentary rocks and provides the strongest

51 evidence for a reducing atmosphere with extremely low oxygen levels throughout the

52 Archaean, since low oxygen levels are required for the production and preservation

53   of S-MIF signals ($<10^{-5}$ present atmospheric levels; Pavlov and Kasting, 2002).

54   Variability in Δ^{33}S- Δ^{36}S dynamics have been forwarded as novel proxies that are

55   capable of resolving subtle changes in the chemical composition of the atmosphere

56   (e.g., Claire et al., 2014; Endo et al., 2016). Of relevance here, the broad correlations

57   between low Δ^{36}S/Δ^{33}S values and extremely negative organic carbon isotope

58   values ($\delta^{13}C_{org}$), termed C-S anomalies, have formed the primary evidence for the

59   development of PHEs during the Neoarchaean (Zerkle et al., 2012; Izon et al., 2015;

60   2017).

61

62   Highly negative $\delta^{13}C_{org}$ excursions are generally thought to represent extensive

63   assimilation of ^{13}C depleted substrates (i.e., methane) into sedimentary organic

64   matter by methane-oxidising bacteria (methanotrophs), potentially in response to an

65   increase in environmental methane production and availability (O'Leary, 1988; Zerkle

66   et al., 2005). The correlations between S-MIF and $\delta^{13}C_{org}$ trends thus suggest altered

67   atmospheric chemistry associated with enhanced methane in the environment.

68

69   Beyond speculation, the ultimate trigger for Neoarchaean PHE formation and the

70   extent to which these events were driven or influenced by biological processes

71   remains unknown. It has been posited that a methane haze could be generated

72   abiotically via tectonic perturbations (i.e. volcanism or serpentinisation) (Daines and

73   Lenton, 2016). Alternatively, recent work suggests that PHEs occurred as a result of

74   increased methanogenesis that was triggered by enhanced primary productivity and

75   organic carbon export to the sediments (e.g., Izon et al., 2017). Following this

76   scenario, the source of the associated negative $\delta^{13}C_{org}$ excursions would have been

77 enhanced methane cycling and incorporation into sediments, either via anaerobic

78 oxidation of methane (AOM) or aerobic methanotrophy.

79

80   Low temperature Cu isotope geochemistry is a relatively novel system (see Moynier

81 et al., 2017 for a recent review). Our current understanding of δ^{65}Cu variation indicates

82 limited variability in the δ^{65}Cu of igneous rocks, as well as sediments that have

83 undergone physical, but minimal chemical processes (i.e., clastic river sediments, lake

84 sediments, ocean sediment, aerosols, and mineral dust), and that these δ^{65}Cu

85 signatures (see section 2 for the definition of δ^{65}Cu) are broadly similar. Of specific

86 interest to this study are natural samples that have experienced a higher degree of

87 physical, chemical, and biological processing during environmental cycling and which

88 record a larger isotopic fractionation range of ~2.5‰. Fig. 1a plots Cu isotope

89 fractionations induced by biological uptake, sorption, organic complexation, and redox

90 (Moynier et al., 2017). The Cu isotope composition of modern marine sediments is

91 homogenous at ~+0.3‰ and isotopically light relative to seawater values, which range

92 between +0.6 and +0.9‰ (Little et al., 2017); fig. 1b shows the Cu isotope mass-

93 balance in the modern ocean (after Little et al., 2017). The isotopically light Cu output

94 fluxes are considered to be controlled by equilibrium isotope fractionation between (1)

95 isotopically heavy Cu complexed to organic ligands and the isotopically light reactive

96 Cu^{2+} species which is scavenged by particulates and delivered to sediment or (2) the

97 same ligand bound pool of Cu and isotopically light Cu sorbed to particulates, which

98 deliver Cu to the sediment (e.g., Little et al., 2017).

99

100 Copper is a bio-essential trace metal used by all forms of life for a multitude of

101 functions (Madigan et al., 2015) and biological uptake via plants, microbes, and

102   enzymes has been shown to impart a Cu isotope fractionation (Moynier et al., 2017;

103   Wang et al., 2017). In particular, Cu is a key element used to regulate the enzymatic

104   activity of certain aerobic methanotrophs. Methanotrophs use methane

105   monooxygenase (MMO) to catalyse the oxidation of methane to methanol. Methane

106   monooxygenase exists in two forms, iron (Fe)-dependent cytoplasmic or soluble

107   methane monooxygenase (sMMO) and Cu-dependent membrane bound or particulate

108   methane monooxygenase (pMMO; e.g., Hakemian et al., 2008; Semrau et al., 2010;

109   2018). Given that Cu is used by certain aerobic methanotrophs in order to regulate

110   enzyme activity (e.g. Madigan et al., 2015; Semrau et al., 2010), and that biological

111   uptake of Cu by other Cu-using metabolisms imparts a resolvable isotope effect

112   (Naverrete et al., 2011), Cu isotopes could provide a window into ancient methane

113   cycling.

114

115   Testing this hypothesis, we explore the potential of Cu isotopes as a proxy for

116   aerobic methanotrophy in the Late Archaean via the generation of a high-resolution

117   δ^{65}Cu record through the youngest of three identified PHEs from the ~2.6-2.5 Ga

118   Campbellrand-Malmani carbonate platform (Izon et al., 2017). These data, when

119   examined alongside previously published C and S isotope data, establish an

120   important role for aerobic methane oxidation in the incorporation of methane into

121   Late Archaean sediments.

122

123   **2. Materials and Methods**

124

125   *2.1. Samples*

126

127   Core GKF01 was drilled by the Agouron Institute through the carbonate-dominated

128   Campbellrand Subgroup (Transvaal Supergroup) in the Griqualand West Basin,

129   South Africa (SI figure S1). GKF01 intersects ~1075 m of dolomitic facies, including

130   microbialite, micritic, and gravity flow units, that are intercalated with siliciclastic

131   mudstones. All facies were deposited in a slope setting, mostly below storm weather

132   wave base though some facies were deposited below fair-weather wave base

133   (Schröder et al., 2006; 2009). The units preserved in GKF01 have been logged and

134   interpreted by Schröder et al. (2006). The Campbellrand Subgroup was deposited

135   between ~2583 and 2521 Ma (Martin et al., 1998; Sumner and Beukes, 2006).

136

137   This study targeted an interval between 800 – 890 m which was selected as it

138   records the youngest known putative PHE. Further rationale for selecting this

139   anomaly for high resolution study is based on its proximity to the Kamden iron

140   formation, a pervasive stratigraphic marker that enables the tracing of the anomaly to

141   other cores (Izon et al., 2017). A schematic log of the 790-890 m section analysed in

142   this study is shown in Fig. 2

143

144   Scanning electron microscope (SEM-EDS) analysis of hand specimens was

145   undertaken to determine the dominant Cu-containing phase. Samples were analysed

146   at the Analysis and Characterisation facility of the ACEMAC Facility at the University

147   of Aberdeen, using a Carl Zeiss GeminiSEM 300 high resolution Field Emission

148   Scanning Electron Microscope with secondary electron, backscattered electron, and

149   cathodoluminescence detectors. Measurable Cu concentrations could only be

150   detected in pyrites; no other Cu-bearing phases above the detection limit could be

151   identified.

152

2.2. Major and trace element analysis

154

Major elemental abundances were determined by X-Ray Fluorescence (XRF) at the University of St Andrews. Bulk sample powders were fused using a mixed lithium tetraborate (20%) and lithium metaborate (80%) flux, with ammonium iodide as a releasing agent. Fused samples were analysed on a Spectro Xepos HE instrument with a 50 W end-window X-ray tube to excite the samples and a 30 mm2 Peltier cooled silicon-drift detector, which provides a spectral resolution (full-width at half-maximum) of ≤155 eV at Mn Kα.

162

 Trace element analyses were performed at the University of St. Andrews via fusion-enhanced dissolution followed by inductively-coupled mass spectrometry (ICP MS). Approximately 0.25g of bulk powdered sample was weighed into platinum crucibles and roasted at 1000oC, overnight, to remove the volatile component. These pre-combusted residues were then then mixed and fused with 1.25g of a 50:50 mixture of lithium metaborate and lithium tetraborate flux, using a trace amount of ammonium iodide to produce a free homogeneous glass-bead. The resultant glass-bead, after dissolution in 5 % (vol/vol) ultra-pure nitric acid, was manipulated and prepared for analysis using a Thermo X series2 ICP-MS. Standardisation was achieved via matrix-matched synthetic standards, while drift was corrected by internal normalisation following Re, Ge and Rh doping. Alongside unknowns, various international standards (GSR1–6, SBC-1, OU-8, OU-6 and SD0-1) were processed in an identical manner, producing data typically better than 10 % of certified values. Rare earth element and yttrium values (REY_{SN}) were normalized to Post-Archean Australian

177   Shale (PAAS; Taylor and McLennan, 1985). REY_{SN} anomalies (namely Eu_{SN}) were

178   calculated using the equations of Lawrence et al. (2006) which are commonly used to

179   assess the preservation and redox history of Archean and Proterozoic carbonates

180   (Tostevin et al., 2016; Bellefroid et al., 2018; Warke et al., 2018; 2019)

181

182   *2.3. Cu isotope analysis*

183

184   All sample processing for Cu isotope analyses was conducted within trace metal

185   clean (ISO 5 hoods) laboratory conditions at the University of St Andrews. Sample

186   digestions were performed via ammonium bifluoride (NH_4HF_2) attack following the

187   method described by Zhang et al. (2012). Briefly, 50-100 mg of bulk sample rock

188   powder was weighed into a pre-cleaned 7ml Savillex PTFE vials, followed by

189   approximately 200 mg of NH_4HF_2 (400mg for 100mg sample aliquots). Samples were

190   then heated in an oven at 230 °C for 3 hours, after which 2 ml of concentrated,

191   distilled HNO_3 was added. The digests were then dried down on a hot plate

192   overnight at ~160 °C. Once dry, the samples were redissolved in 2 ml of

193   concentrated HCl, fluxed for 24 hours and evaporated at 120 ºC. Final residues were

194   taken up in 1ml 7 M HCl + 0.001 % H_2O_2 (trace analysis grade) for ion-exchange

195   chromatography.

196

197   For isotope analyses, Cu was concentrated and purified using a double-pass

198   single-column anion exchange chromatographic technique. Bespoke shrink fit teflon

199   columns with a 15 ml reservoir and a 1.8 ml resin bed (6 mm diameter) were loaded

200   with Bio-Rad AG MP-1 anion resin (after Marechal et al., 1999). The columns were

201   cleaned with alternating loads of MQ water and 0.5 M HNO_3 and the resin

202 conditioned with 6 ml of 7 M HCl + 0.001 % H_2O_2. Next, the sample aliquot,

203 dissolved in 1 ml of 7 M HCl + 0.001% H_2O_2, was loaded on to the resin, followed by

204 7ml of 7 M HCl + 0.001 % H_2O_2 to elute matrix elements. A further 20 ml of 7 M HCl

205 + 0.001 % H_2O_2 was added to elute Cu. The isolated Cu was then dried down

206 overnight, and the column chemistry repeated to further purify the Cu. After

207 chemistry, the final purified sample was dissolved in 0.1 M HNO_3 for MC-ICP-MS

208 analysis. The total procedural blank was ~16 ng, equating to less than a maximum of

209 2 %; typically averaging at 0.5 % of the sample-derived Cu which is not a significant

210 contribution.

211

212 Copper isotope analyses were carried out on a Thermo Neptune Plus MC-ICP-MS

213 at the University of St Andrews. The instrument was operated in low resolution mode

214 (ion currents ranging from 15-36 pA), with wet sample introduction via an ESI PFA

215 microflow nebuliser (75 µl min^{-1} flow rate) attached to a Thermo SIS glass spray

216 chamber. Prior to isotope analysis, an aliquot of each sample was analysed for Cu,

217 relative to concentrations of Mg, Na, and Ti, as their argides or oxides can form

218 molecular interferences on Cu masses, and so care must be taken to ensure that the

219 sample aliquot is purified of these elements. Ion chromatography chemistry was

220 repeated if these elements were present in significant quantities in the aliquot (ratios

221 of Mg, Ca, Fe, Na, Mn, to Cu > 0.001; Ti/Cu > 0.03) (e.g. Liu et al, 2014a). Ion

222 beams were collected in Faraday cups connected to amplifiers using 10^{11} Ω

223 resistors. The collector array was configured to collect the two Cu isotopes (L1: ^{62}Ni;

224 H1: ^{64}Ni; H2: ^{65}Cu; H3: ^{66}Zn and C collecting ^{63}Cu). Each measurement of samples

225 and standards consisted of 22 cycles of 8.389 second integrations. Samples were

226 bracketed by standard measurements and repeated 3 times.

227

228 For samples with high Cu concentrations (e.g. >20 ppm Cu) the instrument was

229 operated in low resolution mode with an ion beam voltage of 3.6 V, analysing Cu

230 concentrations of 75 ppb. For samples with low Cu concentrations (<20 ppm Cu), the

231 instrument ran in low resolution mode with an ion beam voltage of 1.5 V analysing

232 Cu concentrations of 25 ppb. These lower concentration runs did not result in poorer

233 precision; 2SD of high Cu (>20 ppm) averages at 0.05 ‰, while 2SD for low Cu (<20

234 ppm) averages at 0.06 ‰.

235

236 Cu isotope ratios were calculated using sample-standard bracketing by comparison

237 to ERM AE633 (calibrated as 0 ‰ to NIST SRM 976; Moeller et al., 2012) and are

238 reported relative to this standard in delta (per mil, ‰) notation:

239

240
$$\delta^{65}Cu = \left[\frac{\left({}^{65}Cu / {}^{63}Cu \right)_{sample}}{\left({}^{65}Cu / {}^{63}Cu \right)_{NIST976}} - 1 \right] x\ 1000$$

241

242

243 In order to examine analytical accuracy and internal precision, 1 in every 12

244 samples was repeated as a total procedural repeat, i.e., from digestion through to

245 isotope measurement. Further to this, isotope measurements were also made

246 alongside reference material (BHVO-2a; 0.08 ‰ ± 0.05 ‰ (2SD; n = 9), this study).

247 Our measurements of various USGS reference standards (BIR-1, BHVO-2, SCO-1,

248 SDC-1, and SDO-1) are statistically indistinguishable from published values (SI table

249 S2).

250

251   Based on replicate analyses of natural samples and synthetic solutions, the long

252   term reproducibility for δ^{65}Cu measurements was conservatively estimated at 0.002

253   ‰ ± 0.11 ‰ (2SD; n = 75). This is based on a grand dataset created by normalising

254   every measurement and subtracting the mean from each sample measurement to

255   redistribute them around zero (after Steele et al., 2011; Kenney and Keeping, 1951).

256

257

258   **3. Results**

259

260   Copper isotope and abundance data from core GKF01 are provided in Table S1 and

261   presented in Fig. 2. In this figure, we plot our δ^{65}Cu data against stratigraphic height;

262   also shown are Cu concentrations vs. stratigraphic height. There is no significant

263   correlation between Cu concentrations and δ^{65}Cu (R^2 = 0.17), Cu concentrations and

264   sulphur (S) (R^2 = 0.11), or δ^{65}Cu and S (R^2 = 0.002) (Fig. S4).

265

266   A negative δ^{65}Cu excursion exists between 838.6 m – 850.8 m. Here values deviate

267   from an average pre-excursion background ratio of 0.05 ‰ ± 0.08 ‰ (close to

268   current estimates of BSE of 0.06‰; Moynier et al., 2017) to -0.66 ‰ over

269   approximately 12 m of stratigraphy. Up-section from this interval, the δ^{65}Cu values

270   return to similar background values of 0.05‰ ± 0.08‰ over 4m of stratigraphy and

271   persist for the majority of the measured section (37m). When integrated with

272   previously published S-MIF and $\delta^{13}C_{org}$ data (Fig. 2; Izon et al., 2017), there is a clear

273   offset, with the δ^{65}Cu excursion occurring below the S-MIF and $\delta^{13}C_{org}$ excursions.

274   This pattern is independent of lithological variations, as the background remains

275   consistent across multiple facies changes. In addition to this, the excursion itself

276   transects a calcareous mudstone and a mudstone, which appears to have little

277   impact on the data.

278

279   **4. Discussion**

280

281   *4.1. Syn-and post-depositional processes that can affect Cu isotope systematics*

282

283   *4.1.1 Diagenesis and metamorphism*

284

285   The potential for post-depositional alteration to affect $\delta^{65}Cu$ values must first be

286   considered, particularly given the age of these rocks. The Campbellrand Subgroup

287   has undergone sub-greenschist grade regional metamorphism (Button, 1973). The

288   peak metamorphic temperatures recorded by Campbellrand carbonates are only ~250

289   °C (Eroglu et al., 2017). Nonetheless, lower temperature diagenetic processes can

290   reset or alter carbonate trace metal and isotopic inventories. The carbonate

291   geochemistry of the Campbellrand Subgroup, including core GKF01, has been well

292   studied in relation to primary seawater *vs* secondary alteration signatures (Schröder

293   et al., 2006; 2009; Fischer et al., 2009; Eroglu et al., 2015; 2017; 2018; Warke et al.,

294   2019). Here, we consider diagenetic processes (dolomitisation styles, fluid interaction)

295   that may have altered the upper Nauga Formation and factors that may affect Cu

296   systematics.

297

298   As outlined above, SEM-EDS analysis of our samples confirms that sulphides

299   constitute the dominant Cu-bearing phase in GKF01. Copper is, however, also noted

300   within the Upper Nauga Formation ferroan dolostones that host Cu-bearing sulphides

301   and hence dolomite could represent an additional pool of Cu in our samples.

302   Synchrotron X-Ray Fluorescence has shown that microbial fabric-retentive ferroan

303   dolomite in GKF01 can contain up to 20 ppm Cu, although generally concentrations in

304   the ferroan dolomite are lower than detection (~1 ppm; Warke et al., 2019). This form

305   of dolomitisation, driven by seawater derived fluids (Warke et al., 2019), is common in

306   the studied interval and is therefore unlikely to account for the higher Cu

307   concentrations measured in this study.

308

309   Fabric destructive dolomitsation, driven by interaction of precursor carbonate phases

310   with a burial brine, is also known to affect the Campbellrand Subgroup (Sumner,

311   1996). Dolomite precipitating from such a fluid could enrich Cu host dolostones up to

312   concentrations of 150 - 1500 ppm (Warke et al., 2019). As we do not observe

313   concentrations above 126 ppm in this study, and this portion of GKF01 retains

314   discernible microbial fabrics (Schröder et al., 2006; 2009), we discount this

315   mechanism as exerting a significant control on Cu concentrations in our samples.

316   Further, burial brines and other fluids (discussed below) would significantly alter

317   carbonate isotope ($\delta^{18}O_{carb}$ and $\delta^{13}C_{carb}$) systematics. Significant interaction with post-

318   depositional fluids can be expected to progressively lower $\delta^{18}O_{carb}$ values from

319   Archean seawater values of around -7 to -8 ‰ to values as low as -17 ‰ (Eroglu et

320   al., 2017). In GKF01 both $\delta^{18}O_{carb}$ and $\delta^{13}C_{carb}$ values are consistent with Neoarchean

321   seawater values and show no evidence of disturbance over the interval we have

322   examined, and over the $\delta^{65}Cu$ excursion interval (Fig. S2 Fischer et al., 2009; Eroglu

323   et al., 2017).

324

325   Post-depositional hydrothermal fluids with high fluid to rock ratios – capable of

326   corroding Cu-sulphide minerals - can severely disturb carbonate systematics. In

327   addition to causing pronounced and coupled lowering of $\delta^{18}O_{carb}$ and $\delta^{13}C_{carb}$ values,

328   as discussed above, they can strongly alter carbonate-bound, shale-normalised, Rare

329   Earth Element and Yttrium (REY_{SN}) trends. Hydrothermal alteration of this manner

330   leads to characteristic REY_{SN} arrays that are depleted in the heavier lanthanides, lack

331   Y/Ho anomalies, and carry a strong positive Eu anomaly (Tostevin et al., 2016; Warke

332   et al., 2018). Dolomitic facies in the upper Nauga Formation, however, show no

333   evidence of such disturbance and preserve robust seawater REY_{SN} arrays (Eroglu et

334   al., 2017; Warke et al., 2019) albeit with the small, positive, shale-normalised Eu

335   anomaly (Eu_{SN}) that is characteristic of Neoarchean seawater which we discuss below.

336   Therefore, there is no evidence - either from considering the sulphides themselves or

337   from considering the susceptible host sediments - to suggest that Cu-sulphides have

338   been significantly altered.

339

340   Finally, we note that the Transvaal Supergroup was affected by the Kheis orogenic

341   event (c. 1.92 Ga) which promoted epigenetic Zn and Pb mineralisation and

342   pyrrhotitisation of Fe-bearing minerals (De Kock et al., 2009). A previous S-isotope

343   study found no acid volatile sulphur (AVS; Izon et al., 2017). In Precambrian rocks

344   AVS commonly occurs in later diagenetic sulphide phases (e.g., Paiste et al., 2018);

345   the absence of AVS precludes pervasive pyrrhotitisation, demonstrating that the Kheis

346   orogeny had little impact on the sulphide inventory over the examined interval.

347

348   *4.1.2 Possible primary (hydrothermal) controls*

349

350   Hydrothermal vent materials display a relatively large range of $\delta^{65}Cu$ attributed to

351   multiple mineralisation events or remobilisation of Cu (over 9 ‰; e.g. Larson et al.,

352 2003; Mason et al., 2005). Thus, an unrecognised hydrothermal influence has the

353 potential to obscure, or even overprint, primary sedimentary $\delta^{65}Cu$ values. We see no

354 evidence of enhanced hydrothermal flux in core GKF01, with no significant

355 enrichments in common hydrothermal elements such as Ba, Mn, and Fe (e.g. German

356 and Von Damm, 2003) (Fig. S2). Importantly, there is no significant relationship

357 between these hydrothermal proxies and their corresponding $\delta^{65}Cu$ compositions (R^2

358 values for $\delta^{65}Cu$ v Mn: 0.16; $\delta^{65}Cu$ v Fe: 0.05; $\delta^{65}Cu$ v Ba: 0.18).

359

360 During the Neoarchean, primary seawater signals carry a hydrothermal signal

361 inherited from the stronger influence of hydrothermal processes on seawater metal

362 inventories (Beukes and Gutzmer, 2008). Hydrothermal input imparts positive shale-

363 normalised Eu anomalies (Eu_{SN}) in Neoarchean platform carbonates and have been

364 previously noted in the Campbellrand Subgroup (Eroglu et al., 2017; Warke et al.,

365 2019) as well as in this study (Fig. S2). Fluctuations in the Fe and Mn content of

366 Campellrand carbonates is also thought to follow the changing hydrothermal influence

367 on seawater at the time of deposition, rather than significant modification during

368 dolomitisation as limestone and dolostone Fe and Mn concentration ranges often

369 overlap (Eroglu et al., 2015; 2017; 2018).

370

371 In Fig. S3.1-3, we note a weak positive relationship exists between Cu and Eu

372 concentrations (R^2: 0.19; n=91) but no relationship exists between Cu concentration

373 and Eu_{SN} anomalies (R^2: 0.02; n=91). This strongly suggests that the Cu hosted within

374 the Lower Nauga units is not directly controlled by hydrothermal seawater input. Only

375 a weak positive relationship exists between Eu concentration and $\delta^{65}Cu$ values (R^2:

376 0.15; n=28).

377

378 The δ^{65}Cu signal of hydrothermal inputs are still unconstrained but hypothesised to be

379 a source of isotopically light Cu relative to seawater (Fig. 1b; Little et al., 2017). Hence,

380 if periods of higher hydrothermal input significantly lowered δ^{65}Cu values, a co-

381 relationship with higher Eu$_{SN}$ anomalies may be evident. We note only a weak negative

382 relationship between these two variables (R^2: 0.25; n=28; Fig. S3.4). This relationship

383 weakens further when only negative δ^{65}Cu values are considered (R^2: 0.15; n=12).

384

385 Therefore, while we cannot totally preclude that increased hydrothermal influence may

386 partly cause a negative δ^{65}Cu excursion, given the size of the dataset and the

387 weakness of the trends observed, it does not appear to be the dominant driver of the

388 observed δ^{65}Cu trends. We consider other, biological, drivers below.

389

390 *4.2. Primary Cu isotope fractionation mechanisms*

391

392 Copper isotope ratios exhibit a range of 2.5 ‰ in "low temperature" environments; Fig.

393 1a summarises the measured constraints on known Cu isotope fractionations. The

394 dominant low-temperature processes that account for negative δ^{65}Cu signals in

395 mineral phases and biological products are redox reactions and biological uptake; in

396 the modern oxidative ocean environment there is a significant range in Cu isotope

397 fractionation associated with Cu-sulphides, for example: Mathur et al. (2009). As such,

398 we focus on these possible mechanisms for producing the negative δ^{65}Cu excursion

399 we observe.

400

401

402

403

4.2.1. Redox

405

Redox reactions are an important control on the chemical speciation of Cu, which exists as native Cu(0), "reduced" Cu(I) and "oxidised" Cu(II) in Earth materials (Moynier et al., 2017). Importantly, the reduction of aqueous Cu(II) has been demonstrated to produce measurable and relatively large isotopic fractionations. The reduction of Cu(II) to an iodide precipitate (Zhu et al., 2002) and Cu(I)S (covellite; Ehrlich et al., 2004) enriches the reduced phase in ^{63}Cu, with respective fractionation factors of +1.004 ‰ and +3.06 ‰. Additionally, via abiotic oxidative leach experiments, Mathur et al (2005) demonstrated that Cu(I) in chalcocite (Cu_2S) and chalcopyrite ($CuFeS_2$) was 1.3 ‰ and 2.7 ‰ lighter than aqueous Cu(II). Copper sulphides in dolomites further indicate the association between Cu(I) and ^{63}Cu, again implying a negative fractionation during the reduction of aqueous Cu(II) (Asael et al., 2007).

417

Due to their apparent susceptibility to oxygen availability, Cu isotopes have been investigated as a potential palaeoredox proxy. Chi-Fru et al. (2016) observed a transition from predominantly shale-hosted negative $δ^{65}Cu$ compositions in Late Archaean shales to more positive ratios in the wake of the GOE. Assuming that banded iron formations (BIFs) preferentially sequester ^{65}Cu, leaving residual seawater (and thus shales) depleted in ^{65}Cu, the authors interpreted the temporal increase in $δ^{65}Cu$ values to record the waning deposition of BIFs through the Late Archaean-Proterozoic transition. The post-GOE loss of the BIF ^{65}Cu-sink, along with a heightened oxidative weathering flux, increased the ocean inventory of ^{65}Cu, promoting the observed post-GOE shale-hosted ^{65}Cu enrichments. For the Late

428   Archaean, Chi-Fru et al. (2016) present four data points, with $\delta^{65}Cu$ values ranging

429   from ~-0.6‰ to +0.2‰. While these values bracket the GKF01 dataset (Fig. 2), $\delta^{65}Cu$

430   in GKF01 are not consistently depleted as would be predicted by their model, and

431   there is limited evidence of contemporaneous BIF formation in the measured section

432   of GKF01. Moreover, recent Cu isotope analyses of BIFs from the Transvaal

433   Supergroup (Thibon et al., 2019) have determined an average $\delta^{65}Cu$ range of ~0 ‰,

434   suggesting that BIF Cu isotope compositions are not related to oceanic reservoir

435   effects. Instead, BIF $\delta^{65}Cu$ values from the Transvaal reflect variation in Cu

436   provenance (volcanic ash deposits in this instance) and, this considered, the authors

437   posit that Cu has limited use as a redox proxy during BIF formation. It therefore seems

438   unlikely that, in this instance, changes in BIF deposition were responsible for the

439   observed short-term variability seen in GKF01 $\delta^{65}Cu$, and alternative mechanisms

440   should be sought.

441

442   *4.2.2. Biological uptake: Aerobic methanotrophy*

443

444   While there are currently no direct experimental studies of Cu isotope fractionation

445   during aerobic methanotrophy, there is evidence for a microbially induced fractionation

446   of Cu isotopes during Cu uptake by other types of organisms such as *Pseudomonas*

447   *aeruginosa* and yeast (Zhu et al,. 2002), *Escherichia coli* (Zhu et al., 2002; Navarrete

448   et al., 2011), *Bacillus subtilis* (Navarrete et al., 2011) and *Thiobacillus ferooxidans*

449   (e.g. Mathur et al., 2005; Kimball et al., 2009). Both Zhu et al. (2002) and Navarrete et

450   al. (2011) found that live bacterial and yeast cells preferentially incorporate ^{63}Cu,

451   resulting in an isotopic fractionation relative to the starting media of -1.0‰ – -2.3‰.

452   While speculative, these fractionations are hypothesised to involve the reduction of

453   Cu(II) to Cu(I) within the cell membrane. In contrast, studies involving *Thiobacillus*

454   *feroox idans,* an acidophilic autotroph commonly found in acid mine drainage, show a

455   preferential association of ^{65}Cu with *T. ferooxidans.* This is due to the formation of

456   isotopically heavy Cu nanoparticles around the cell and is thought to develop as a

457   cellular response to high environmental metal concentrations (Mathur et al., 2005;

458   Kimball et al. 2009). Thus, to recapitulate: cellular uptake of Cu likely involves a redox

459   state change and leads to preferential incorporation of light Cu, and sorption of Cu to

460   cells leads to the preferential incorporation of heavy Cu.

461   We speculate that, given the importance of Cu to pMMO, it is likely that biological Cu

462   uptake by organisms utilising this enzymatic pathway would result in similar

463   fractionation of Cu isotopes to that observed in the studies of Zhu et al. (2002) and

464   Navarrete et al. (2011).

465   It has been previously demonstrated that Ni, an element of enzymatic importance in

466   methanogenesis, is similarly fractionated during uptake by methanogens (Cameron et

467   al., 2009) and uptake of Mo during N_2 fixation by cyanobacteria has been shown to

468   induce a large fractionation in Mo isotopes (Zerkle et al., 2010). While Cu is used by

469   a wide variety of enzymes and proteins in the modern environment, many of these are

470   involved in oxidase reactions that likely evolved after the GOE (Zerkle et al., 2005).

471   Moreover, molecular clock analyses show that the Cu-based methane oxidation

472   metabolism may have emerged as early as *c.*2.7 Ga (Moore et al., 2017). As most

473   'modern' Cu enzymes and proteins relate to more widespread metabolic functions

474   rather than specific metabolisms (i.e., aerobic methanotrophy), we also hypothesise

475   that any significant 'generic' uptake of Cu could be evidenced by a strong correlation

476   between $\delta^{65}Cu$ and TOC, which is not indicated by our data (R^2 of $\delta^{65}Cu$ v TOC =

477   0.006; Fig. S4).

478

4.3. Cu isotopes in context

480

In order to consider the roles of redox and biological uptake on the δ^{65}Cu signal measured in core GKF01, the prevailing Late Archaean conditions must also be considered. We adopt the Late Archaean reducing weathering model presented by Hao et al. (2017) as the background state; we see no evidence of high O_2 levels in this interval of core GKF01 and therefore discount the possibility of redox processes as drivers of the δ^{65}Cu excursion. Specifically, Fe-speciation data from this section (Izon et al., 2017) suggest an anoxic, ferruginous water column during deposition of the δ^{65}Cu excursion, with evidence of local water-column oxygenation in the overlying interval. Furthermore, the persistence of S-MIF throughout the entire succession necessitates an oxygen-free atmosphere (Izon et al., 2017). Finally, the absence of a correlation between δ^{65}Cu and total S (Fig. S4) argues that the extent of reduction and sulphidisation associated with MSR was not the dominant control on sedimentary δ^{65}Cu values.

494

Given the arguments presented above, we focus the remainder of this discussion on the two most plausible mechanisms via which marine Cu could be incorporated into shales under reducing conditions (Fig. 3). For both mechanisms, we consider the measured Cu to be derived from organic matter that, under microbial sulphate reducing (MSR) conditions, has been either incorporated into pyrite or has formed its own sulphide phases, such as CuS or CuS_2 (e.g. Tribovillard et al., 2006).

501

502   Under reducing atmospheric conditions ($pCO_{2,g} = 10^{-1.5}$ bars; $pH_{2,g} = 10^{-5.0}$ bars),

503   chalcopyrite ($CuFeS_2$) is weathered from olivine basalt crust, forming chalcocite

504   (Cu_2S) in surface waters, leading to further precipitation of bornite (Cu_5FeS_4) in deeper

505   sections of the water column (Hao et al., 2017). These processes would lead to low

506   overall riverine and oceanic Cu concentrations which are reflected in the low Cu

507   concentrations measured in core GKF01 (Fig. 2). While model predictions indicate that

508   Cu(II) could be present within the chalcopyrite crystal lattice (i.e. Klekovkina et al.,

509   2014), the state of Cu within chalcopyrite is generally believed to be Cu(I) (Goh et al.,

510   2006), which implies that no redox fractionation would occur between weathering and

511   transport to ocean sediments.

512

513   In scenario *(1)* (Fig. 3b.1; *Abiogenic*), we assume that marine Cu would be

514   isotopically light, relative to modern seawater, as it is transferred directly from basalt-

515   hosted sulphide to the oceans without oxidative breakdown. As such, the isotopic

516   composition of marine Cu should be very similar to average basalt Cu isotope

517   composition (as sulphides represent the major Cu host in these rocks; Moynier et al.,

518   2017). This prediction agrees well with our isotopically light $\delta^{65}Cu$ background values

519   of 0.06 ‰ (relative to modern seawater values of 0.5 ‰ – 0.9 ‰; e.g., Little et al.,

520   2017), which are almost identical to estimates for bulk silicate Earth (BSE, 0.08 ‰;

521   Moynier et al., 2017). This is also shown in more modern Devonian black shales which

522   formed in a reducing basin and which possess overlapping $\delta^{65}Cu$ values of 0.04 ±

523   0.16‰ (Mathur et al., 2012).

524   In this scenario, Cu isotope fractionation occurs due to enhanced assimilation of

525   reduced (and therefore light) Cu into sulphides. Given that we observe no correlation

526   between $\delta^{65}Cu$ and total S (fig. S2), we consider this scenario to be unlikely.

528   Under scenario *(2) (*Fig. 3b.2*; Biogenic)*, Cu(I) is taken up via aerobic methanotrophs,

529   and the negative δ^{65}Cu excursion largely reflects enhanced uptake of ^{65}Cu-depleted

530   Cu during aerobic methanotrophy. While experimental studies of Cu isotope

531   fractionation during aerobic methanotrophy are lacking, enhanced methanotrophy is

532   supported by the extremely depleted δ^{13}C$_{\text{org}}$ values ($<$ -38‰) in the overlying PHE interval

533   (Fig. 2). Carbon isotope values <-38‰ represent extensive assimilation of organic matter into

534   sediment via methanotrophy (Hayes et al., 1999). The established presence of enhanced

535   methane cycling coupled with an excursion in δ^{65}Cu, a bioessential trace metal for aerobic

536   methanotrophic bacteria, provides evidence for a biological mechanism for the

537   negative δ^{65}Cu excursion. We therefore consider the implications for this interpretation

538   in the remainder of our discussion.

539

540   *4.4. A biological origin for the Cu isotope excursion*

541

542   *4.4.1 Anaerobic oxidation of methane*

543

544   Given the low availability of oxygen in the Late Archaean, the negative δ^{13}C$_{\text{org}}$

545   excursion within the PHE has previously been attributed to incorporation of methane

546   into sediments via anaerobic oxidation of methane (AOM) coupled to microbial

547   sulphate reduction (MSR; Izon et al., 2017). The metabolic pathway utilised by

548   anaerobic methanotrophs is generally considered to be a reversal of the

549   methanogenesis metabolic pathway and therefore reliant on Ni (e.g. Hallam et al.,

550   2004). Thus, while AOM could have been the dominant metabolism accounting for

551   enhanced incorporation of methane into sediments during the PHE, it fails to account

552 for the $\delta^{65}Cu$ excursion. Nevertheless, AOM remains a significant metabolism

553 throughout the late Archaean and must be considered in any discussion surrounding

554 methane cycling.

555

556 If AOM was the dominant form of methanotrophy operating throughout the PHE

557 section analysed in this study, the preceding negative $\delta^{65}Cu$ excursion could represent

558 a pulse of aerobic methanotrophy occurring before or in the early stages of the

559 purported PHE. This pulse could have been triggered by a perturbation in nutrient

560 availability driven by hydrothermal input, which we preclude as the dominant cause for

561 the $\delta^{65}Cu$ excursion but acknowledge as potentially occurring at this time (section

562 4.1.2). Any Cu provided by hydrothermal input could have been rapidly consumed by

563 aerobic methanotrophs, allowing Ni-dependent AOM to become the dominant form of

564 methanotrophy for the remainder of the PHE. As AOM is most commonly coupled to

565 sulphate reduction (e.g., Moran et al., 2007), and accounting for the low sulphate

566 conditions that persisted throughout the Late Archaean (Izon et al., 2017), it is likely

567 that AOM would also have been supressed, allowing methane to accumulate in the

568 atmosphere and leading to haze formation. While possible, we consider this case to

569 be unlikely. We do not see any significant evidence for an increased flux in Cu before

570 the PHE and therefore consider alternative interpretations.

571

572 *4.4.2 Aerobic methane oxidation*

573

574 Alternatively, aerobic methane oxidation could have remained the dominant pathway

575 for methanotrophy throughout the duration of the PHE. This is supported by Fe-

576 speciation data from Izon et al (2017) which suggest an oxic environment of deposition

577   during the PHE. However, given that previously published Fe-speciation data (Izon et

578   al., 2017) suggest anoxic conditions before the PHE, there are three significant points

579   to consider for this interpretation, specifically: the bioavailability of Cu in a reducing

580   environment, the impact of oxygen limitation on aerobic metabolisms in a reducing

581   environment, and the stratigraphic occurrence of the δ^{65}Cu excursion before the onset

582   of the $\delta^{13}C_{org}$ excursion.

583

584   Methanotrophs have a specific Cu uptake system, whereby an extracellular Cu-

585   complexing agent (a chalkophore) is excreted by the methanotroph in order to extract

586   Cu from the surrounding environment (Yoon et al., 2010; Semrau et al., 2010). For

587   example, methanobactin (MB), which was first identified in pMMO-expressing bacteria

588   (Kim et al., 2004), binds either Cu(I) without an oxidation state change, or Cu(II), which

589   is subsequently reduced to Cu(I) (Choi et al., 2006). In the reduced Late Archaean

590   environment modelled by Hao et al (2017), most, if not all Cu, was bound as Cu(I)

591   sulphides. This would have decreased Cu bioavailability, creating a challenging

592   environment from which microbes could extract Cu. It has been suggested that, in

593   similar modern environments, some methanotrophs are capable of expressing specific

594   chalkophores to extract Cu from mineral-bound Cu complexes, giving them a

595   competitive advantage over cells that do not (Semrau et al., 2010). Chalkophore

596   production appears to be widespread in both *gammaproteobacteria* methanotrophs

597   and *alphaproteobacteria* methanotrophs (Choi et al., 2008; Semrau et al., 2010). The

598   presence of chalkophores also brings forth another potential Cu isotope fractionation

599   mechanism; the transport of Fe by siderophore-producing bacteria have been shown

600   to fractionate Fe isotopes, with evidence to suggest that the dissolution of Fe in

601   minerals by the siderophore causes this fractionation (Brantley et al., 2001).

602

Aerobic methanotrophic bacteria use CH_4 as their sole source of carbon and are also obligate aerobes, making them dependent on O_2 and limited by O_2 availability (e.g., Roslev and King, 1994). As above, previously published Fe speciation data for this section indicate anoxic, ferruginous depositional conditions during the Cu isotope excursion, with localized oxygenation during the PHE. However, the extent of this oxygenation was likely limited to the local water column or sediment-water interface, with the persistence of S-MIF throughout this section indicating low atmospheric O_2 (Izon et al., 2017). In addition, even local oxygen availability would have been limited due to the widespread occurrence of Fe(II) and other reduced compounds that would have quickly consumed O_2 in the sediments (Zerkle et al., 2012). It has been shown that some aerobic methanotrophs are able to survive under both carbon and O_2 starved conditions, suggesting that methane oxidation can occur in environments that do not continuously support aerobic methanotrophic growth (Roslev and King, 1994). Further investigation has since demonstrated that methanotrophs which live at the low end of the O_2 gradient in natural environments have established mechanisms for survival in such environments (Knief, 2015). For example, experiments have demonstrated that under low O_2 conditions and under prolonged O_2 starvation, the model *gammaproteobacteria* methanotroph *Methylomicrobium buryatense* 5GB1 maintained a metabolic state that combined fermentation and respiration, secreting acetate and formate (Gilman et al., 2017). Given the adaptive abilities of aerobic methanotrophs (both in terms of accessing reduced Cu and surviving under O_2 starved conditions) and in the absence of evidence to the contrary, we consider the possibility of aerobic methanotrophy as a relevant consideration when interpreting geochemical data from sediments of this age.

627

628   The sequence of changes captured by the examination of our multi-proxy

629   geochemical datasets at high resolution further supports this interpretation. Through

630   863 m – 817 m, $\delta^{65}Cu$ values becomes more negative over approximately 7 m of

631   stratigraphy, reaching a minimum value of -0.66 ‰ at 841 m (Fig. 2, dashed line (a));

632   the most negative $\delta^{65}Cu$ value measured coincides with the onset of the large $\delta^{13}C_{org}$

633   excursion. From this point (Fig. 2, dashed line (b)), the $\delta^{65}Cu$ values return to baseline

634   rapidly, over ~4m of stratigraphy, and remain in this state for the majority of the $\delta^{13}C_{org}$

635   excursion.

636

637   Significant changes in $\Delta^{33}S$/ $\Delta^{36}S$ over this same section (Fig. 2) have been

638   interpreted to represent changes in global atmospheric chemistry at high atmospheric

639   CH_4 (Izon et al., 2017), whereas low $\delta^{13}C_{org}$ values have been interpreted to reflect

640   local production and consumption of methane. Lower $\delta^{13}C_{org}$ values can therefore

641   imply locally high methane concentrations, as it has been demonstrated that higher

642   volumes of methane lead to much faster aerobic methane oxidation (Sherry et al.,

643   2015). The less depleted background $\delta^{13}C_{org}$ values throughout the majority of this

644   section could thus be representative of lower levels of aerobic methane oxidation and,

645   by extension, lower methane concentrations.

646

647   In low methane environments, type I methanotrophs that express pMMO have the

648   competitive advantage over type II methanotrophs, which thrive under high CH_4 low

649   O_2 settings (Hanson and Hanson, 1996). When methane concentrations increase,

650   methanotrophs that express sMMO (soluble methane monooxygenase, containing an

651   Fe metal centre rather than Cu) take over as dominant methane oxidisers (Semrau et

652   al., 2010). If this was similarly the case in the Neoarchaean, we interpret the negative

653    $\delta^{65}Cu$ excursion to represent pMMO-expressing methanotrophs thriving in the

654   relatively low methane conditions indicated by the relatively high $\delta^{13}C_{org}$ values. The

655   fast return of the $\delta^{65}Cu$ to background values coincides with the sudden decrease in

656    $\delta^{13}C_{org}$, suggesting the takeover of Fe-utilising sMMO-expressing methanotrophs in

657   response to higher methane availability (Fig. 4). This ecological change is strongly

658   supported by the fact that we continue to observe extensive assimilation of organic

659   matter into sediment via methanotrophy, yet this is not visible in the $\delta^{65}Cu$ record.

660   Alternatively, the disappearance of the Cu isotope excursion during the PHE could be

661   the result of pMMO-expressing methanotrophs using Cu to completion, such that no

662   isotope effect is expressed. In this instance, the observed C isotope excursion (Fig. 2)

663   is the result of Cu-dependent aerobic methanotrophy rather than Fe-dependent

664   aerobic methanotrophy.

665

666   *5. Conclusions*

667

668   Examining a ~2.5 Ga sedimentary succession that is associated with an episode of

669   organic haze formation, we present a new high-resolution $\delta^{65}Cu$ record that reveals a

670   pronounced negative $\delta^{65}Cu$ excursion. Precluding post-depositional alteration and

671   abiotic redox reactions we interpret this record as reflecting aerobic methanotrophy

672   under low methane conditions, however we stress that further work is required in order

673   to draw a firmly definitive conclusion.

674

675   While most Cu-dependent metabolisms are assumed to have evolved after the GOE

676   (Zerkle et al., 2005), estimates for the origin of aerobic methanotrophy range between

677 2.91 – 2.15 Ga. Due to the disappearance of S-MIF at 2.4-2.3 Ga and the subsequent

678 implications for environmental oxygen availability, the younger end of this age range

679 is often favoured (e.g. Battistuzzi et al., 2004). However, given the ever-emerging

680 evidence for oxygenic photosynthetic life prior to the disappearance of S-MIF (e.g.

681 Crowe et al., 2013; Lyons et al., 2014), along with evidence suggesting the presence

682 of oxygenic oases before the GOE (e.g., Olson et al., 2013; Planavsky et al., 2014;

683 Yang et al., 2019), there is scope for this assumption to be reconsidered.

684

685 Our new $\delta^{65}Cu$ data may support an earlier emergence of aerobic methanotrophy,

686 arguing for the presence of Cu-dependent aerobic methanotrophy by ~2.5 Ga,

687 approximately 200 Myr before the disappearance of S-MIF (Luo et al., 2016; Fig. 4).

688 This is consistent with models predicting the existence of aerobic ecosystems before

689 the GOE, notably aerobic methanotrophy, which is postulated to have suppressed

690 methane and oxygen fluxes to the atmosphere (i.e., Daines and Lenton, 2016).

691

692 Besides demonstrating the potential utility of $\delta^{65}Cu$ as an isotopic biomarker of

693 aerobic methanotrophy in Late Archaean rocks, the presence of an established pre-

694 GOE aerobic methane sink has important implications for models resolving the role of

695 methane cycling in modulating climate and atmospheric redox transitions on the early

696 Earth, including the Great Oxidation Event (e.g., Catling et al., 2007; Daines and

697 Lenton, 2016). Moving forward, these data illustrate the importance of continuing to

698 develop $\delta^{65}Cu$ as an isotopic biomarker capable of resolving early-Earth's early

699 methane cycle, thus providing important constraints for climate and biogeochemical

700 models alike.

701

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