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A 200 million year delay in permanent atmospheric oxygenation

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The rise of atmospheric oxygen fundamentally changed the chemistry of surficial environments and the nature of Earth's habitability[1]. Early atmospheric oxygenation occurred over a protracted period of extreme climatic instability marked by multiple global glaciations[2,3], with the initial rise of oxygen to above 10^{-5} of the present atmospheric level (PAL) constrained to ~2.43 billion years ago (Ga)[4,5]. Subsequent fluctuations in atmospheric oxygen levels have, however, been reported until ~2.32 Ga[4], which currently denotes the perceived timing of irreversible oxygenation of the atmosphere[6,7]. Here, we report a high-resolution reconstruction of atmospheric and local oceanic redox conditions across the final two glaciations of the early Paleoproterozoic Era, as documented by marine sediments from the Transvaal Supergroup, South Africa. Based on multiple sulfur isotope and Fe-S-C systematics, we demonstrate continued oscillations in atmospheric oxygen levels after ~2.32 Ga, linked to major perturbations in ocean redox chemistry and climate. Oxygen levels thus fluctuated across the 10^{-5} PAL threshold for ~200 million years, with permanent atmospheric oxygenation finally arriving with the Lomagundi carbon isotope excursion at ~2.22 Ga, some 100 million years later than currently estimated.

The transition to a permanently oxygenated atmosphere underpins the concept of the Great Oxidation Event (GOE) and represents a pivotal period in Earth history that ultimately led to the Earth's current state of habitability[1,8]. While multiple independent lines of evidence document a significant rise in atmospheric oxygen during the GOE[1,8], the most definitive tool for tracking atmospheric oxygenation comes from the preservation (and subsequent loss) of mass-independent fractionation of multiple sulfur isotopes (MIF-S) in marine sedimentary rocks[9,10]. The onset of the GOE is constrained to ~2.43 Ga based on the initial loss of the MIF-S isotope signal[4,5]. However, the permanent loss of the MIF-S isotope signal, representing the transition from a functionally anoxic to an oxic atmosphere with pO$_2$ sustained above $>10^{-5}$ of the present atmospheric level (PAL)[11], has been timed to ~2.32 Ga[6,7].

The GOE is associated with extreme climatic instability, with the latest stratigraphic reconstructions advocating four distinct glaciations (Extended Data Fig. 1), where the oldest three are found at low-latitudes and thus represent possible 'Snowball' Earth intervals[3]. While atmospheric oxygenation has traditionally been viewed as a singular, irreversible event[12-14], emerging evidence supports significant instability in atmospheric oxygen concentrations across the GOE[2,4] (see Methods). Fluctuations in atmospheric composition are often linked to greenhouse gas concentrations and the development of glacial conditions, specifically via the oxidation of atmospheric methane[2,13,15-18]. Indeed, after the initial loss of the MIF-S signal in the lead up to the

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65   first Paleoproterozoic glaciation[5], there is compelling evidence for a return and subsequent loss of

66   MIF-S after the second glaciation in the Duitschland Formation of the Transvaal Supergroup, South

67   Africa[19]. This is followed by a return to MIF-S in the aftermath of the third glaciation in the Eastern

68   Transvaal Basin, before the signal is again lost in the upper Rooihoogte Formation[6,7] (Fig. 1),

69   representing the widely accepted permanent oxygenation of the atmosphere. The final

70   Paleoproterozoic glaciation occurs ~60-100 million years after this purported permanent rise of

71   atmospheric oxygen (Fig. 1), and thus currently stands alone in appearing to lack a direct link to

72   oxygen fluctuations across the 10^{-5} PAL threshold.

73   To test this history of Earth-system oxygenation and associated climate change, we provide a

74   high-resolution reconstruction of atmospheric and local oceanic redox conditions through the later

75   stage of the GOE, with analyses based on multiple sulfur isotope and Fe-S-C systematics (see

76   Methods for a full description of the geologic setting, analytical techniques and additional data

77   discussion, and Supplementary Information for all data). We focus on well-preserved drill cores

78   (EBA-1 and EBA-2) that intersected shallow-marine strata, with drill core EBA-1 likely

79   documenting a slightly shallower-water depth relative to EBA-2. The succession comprises the

80   Rooihoogte Formation (deposited shortly after the third Paleoproterozoic glaciation), two upward-

81   shallowing sequences (separated by a depositional break of ~40-50 million years) of the overlying

82   Timeball Hill Formation (which hosts the Reitfontein Diamictite at the top, representing the final

83   Paleoproterozoic glaciation), and the overlying Boshoek Formation (Fig. 1).

84   Our multiple sulfur isotope analyses document large Δ^{33}S anomalies indicative of a functionally

85   anoxic atmosphere ($<10^{-5}$ PAL) in interval A of the upper Rooihoogte Formation (Fig. 2; Extended

86   Data Fig. 2). Through interval B of the upper Rooihoogte Formation, the Δ^{33}S results are consistent

87   with existing data showing mass-dependent fractionation of multiple sulfur isotopes (MDF-S) and

88   hence higher atmospheric oxygen levels[7]. A return to MIF-S in interval C indicates a subsequent

89   drop in atmospheric oxygen levels[7], but the Δ^{33}S anomaly is then lost across the Rooihoogte-

90   Timeball Hill formation boundary (Fig. 2; Extended Data Fig. 2), marking the previously accepted

91   permanent rise of atmospheric oxygen to $>10^{-5}$ PAL[6,7] (Fig. 1). Our more extensive dataset,

92   however, captures a return to large MIF-S across several overlying intervals of the Timeball Hill

93   Formation, including the run-up and aftermath of the Reitfontein glaciation (Figs. 2 and 3).

94   While our data record a large range in MIF-S values (up to ~9‰ in the Rooihoogte Formation,

95   and up to ~3‰ through the Timeball Hill Formation and Reitfontein Diamictite), smaller MIF-S

96   anomalies observed in some early Paleoproterozoic sedimentary successions have been linked to

97   recycling of older Archean sulfides[5,26-28]. However, consistent with previous studies of the

98   Rooihoogte and Timeball Hill formations[6,7], we interpret our MIF-S data to dominantly reflect a

99   primary atmospheric signal. Specifically, an early diagenetic origin has been convincingly

100   demonstrated for sulfide minerals in the EBA drill cores[7] (see Extended Data Fig. 3 for additional

101   support), and there is no evidence for a hydrothermal contribution to the Δ^{33}S signal (see Methods).

102   Moreover, the MIF-S signals we document are consistent with expectations based on a dominant

103   primary, contemporaneous source of sulfide rather than recycling of weathered sedimentary pyrite.

104   Here, we note that following a return to low atmospheric oxygen ($<10^{-5}$ PAL), the expectation

105   would be for the sediments to record a dominant MIF-S signal, with a possible minor overprint

106   from remnant seawater sulfate carrying a mass-dependent sulfur isotope signal (MDF-S) from

107   earlier oxygenation. If we first adopt the conservative approach where Δ^{33}S values of 0.0 ± 0.3‰

108   document mass-dependent fractionation of sulfur isotopes (MDF-S), as is broadly consistent with

109   post-GOE variability (Fig. 3A), samples with Δ^{33}S >0.3‰, including those above the Rooihoogte-

110   Timeball Hill formation boundary, plot close to the Archean Reference Array (ARA) for Δ^{36}S/Δ^{33}S

111   (Fig. 3B; Extended Data Fig. 4) supporting a dominant, primary atmospheric signal[7,22,23]. These

112   MIF-S signals are fundamentally different to the deviation from both the ARA and the post-GOE

113   reference line evident for many early Paleoproterozoic samples for which MIF-S recycling has been

114   invoked[5,27,28] (Extended Data Fig. 5).

115   By contrast, our samples within the Δ^{33}S = 0.0 ± 0.3‰ range plot close to the Δ^{33}S/δ^{34}S and

116   Δ^{36}S/Δ^{33}S post-GOE reference lines representing sulfides deposited under an oxygenated

117   atmosphere (Fig. 3). Some of these samples plot in the field where the post-GOE reference lines and

118   the Archean Reference Array (ARA) overlap (Fig. 3), and hence could have been deposited under

119   anoxic atmospheric conditions. This may account for the shallower Δ^{36}S/Δ^{33}S slope (-3.2 ± 1.3;

120   Extended Data Fig. 5) evident for our MDF-S data, relative to the post-GOE reference slope (-7.2 ±

121   1.4). However, a contribution from recycled MIF-S, which would be an expectation following

122   atmospheric oxygenation and subsequent oxidation of pyrite, is also a possibility.

123   Our MDF-S data are also consistent with the presence of Earth's earliest seawater sulfate

124   evaporites in the Gordon Lake Formation of the Huronian Supergroup, and in the underlying upper

125   Duitschland Formation[29,30], which are coeval to the Rooihoogte and lower Timeball Hill formations.

126   These evaporite data record MDF-S signals[31], implying a well-mixed seawater sulfate inventory of

127   substantial size that did not carry a significant MIF-S signal (see Methods). Thus, while it is

128   theoretically possible for samples that plot along the ARA to have originated from recycling,

129   fluctuating levels of atmospheric oxygen provide the most parsimonious explanation for the distinct

130   S isotope trends evident in our data. Therefore, in contrast to previous inferences[6,7], oxygen

131   concentrations did not permanently cross the 10^{-5} PAL threshold after the third Paleoproterozoic

132   glaciation at ~2.32 Ga.

133   While multiple sulfur isotopes serve as a binary signal for atmospheric O_2 (MDF-S>10^{-5}

134   PAL<MIF-S), Fe-S-C systematics allow reconstruction of the local shallow-water redox response to

135   climate dynamics and changes in atmospheric oxygen levels, in a setting that is considered to have

136   been connected to the open ocean[25]. The synthesis of the $\Delta^{33}S$ and Fe-S-C datasets comprises a

137   timeline that begins with a likely substantial influx of glacially derived nutrients during deposition

138   of the Rooihoogte Formation, as also documented for the aftermath of Neoproterozoic 'Snowball'

139   Earth glaciations[32]. This nutrient input stimulated primary production, total organic carbon (TOC)

140   burial, and hence local oxygen production, leading to the development of oxic conditions (denoted

141   by Fe_{HR}/Fe_T* <0.38 and/or Fe/Al <0.66) in very shallow waters, as documented throughout the

142   Rooihoogte Formation in drill core EBA-1 (Fig. 2; Extended Data Fig. 2). However, consistent with

143   the perspective of shallow-ocean 'oxygen oases' in the run-up to permanent atmospheric

144   oxygenation[33-35], the flux of oxygen was not sufficient to fully oxygenate the atmosphere, and thus

145   MIF-S persists in interval A of the upper Rooihoogte Formation.

146   A very shallow oxycline is suggested by fluctuations between oxic, ferruginous (anoxic, Fe-

147   bearing; denoted by Fe_{HR}/Fe_T* >0.38 and/or Fe/Al >0.66, with Fe_{py}/Fe_{HR}* <0.7) and possibly even

148   euxinic (anoxic, sulfidic; denoted by Fe_{HR}/Fe_T* >0.38 and/or Fe/Al >0.66, with Fe_{py}/Fe_{HR}* >0.8)

149   conditions in the slightly deeper-water drill core EBA-2 through interval A and in the lower part of

150   interval B of the upper Rooihoogte Formation. Continued oxygen production then appears to have

151   transiently oxygenated the atmosphere during deposition of interval B of the upper Rooihoogte

152   Formation (Figs. 2 and 3). Oxidative weathering at this time would have increased the influx of

153   sulfate to the oceans, leading to the observed expansion in sedimentary $\delta^{34}S$ values. Elevated sulfate

154   levels, combined with abundant TOC, then led to the transient development of euxinia at the top of

155   interval B in the upper Rooihoogte Formation (drill core EBA-2, Fig. 2).

156   There was then a return to reducing atmospheric conditions during deposition of interval C of the

157   Rooihoogte Formation. This coincides with high $\delta^{34}S$ values at the base of interval C, consistent

158   with lower seawater sulfate concentrations due to a decrease in oxidative weathering, which

159   promoted a return to ferruginous water-column conditions (Fig. 2). Across the Rooihoogte-Timeball

160   Hill formation boundary, there was a second significant pulse of atmospheric oxygenation. This

161   pulse again increased the continental weathering flux of sulfate and biogenic pyrite formation (as

162   indicated by the major increase in the range of $\delta^{34}S$ values and elevated Fe_{py}/Fe_{HR}), before transiting

163   to oxic seawater conditions in the lower part of the Timeball Hill Formation (Fig. 2).

164 The water column redox dynamics through the Rooihoogte and basal Timeball Hill formations

165 thus reflect the combined influence of glacial melting and fluctuations in atmospheric oxygen

166 documented by our multiple sulfur isotope data. Longer timescale fluctuations in oxygenation are

167 nevertheless apparent throughout the remainder of the succession. While oxic shallow waters

168 persisted through the lower sequence of the Timeball Hill Formation (interval D; Fig. 2), oxygen

169 production was apparently not sufficient to maintain an oxygenated atmosphere, as the MIF-S

170 signature returns at this stratigraphic level (Fig. 3). This deoxygenation was relatively short-lived,

171 however, as a return to MDF-S indicates that the 10^{-5} PAL threshold was again exceeded in the

172 upper part of the lower Timeball Hill Formation sequence (Fig. 3). The upper Timeball Hill

173 Formation (interval E) initially shows evidence for fluctuations between oxic and ferruginous

174 depositional conditions in a relatively deeper-water setting, while the atmosphere transitioned from

175 a dominantly reducing to oxidizing state (Figs. 2 and 3). The oxycline then shallowed, resulting in

176 persistent shallow-water ferruginous anoxia and a reducing atmosphere towards the top of the upper

177 Timeball Hill Formation. This anoxic atmosphere-ocean state persisted through the Reitfontein

178 Diamictite and the lower part of the overlying Boshoek Formation (Fig. 3), with the exception of

179 terrestrial to shallow-marine quartzites that document oxic water-column conditions at the base of

180 the Boshoek Formation (Fig. 2).

181 While local marine redox data should be viewed with caution when making global inferences,

182 our data appear to support a long-lived, dynamic interplay between the biogeochemistry of shallow

183 waters and fluctuations in atmospheric oxygenation. We next explore links between shallow-water

184 redox conditions, and atmospheric O_2, CO_2 and CH_4, to assess potential drivers of the final

185 Paleoproterozoic glaciation. As with the Neoproterozoic 'Snowball' Earth glaciations, escape from

186 glaciation at the base of the Rooihoogte-Timeball Hill succession was likely initiated by a syn-

187 glacial volcanic build-up of CO_2, leading to intense greenhouse conditions[2,36]. Modelling suggests

188 that chemical weathering took $>10^7$ years to reduce atmospheric CO_2 concentrations to background

189 levels in the aftermath of Neoproterozoic glaciations[37]. Elevated CO_2 as a means to end the third

190 Paleoproterozoic glaciation is supported by the highly chemically weathered nature of the sediments

191 of the lower Timeball Hill Formation, as indicated by high values for the chemical index of

192 alteration (CIA) weathering proxy (Fig. 3c)[25].

193 In keeping with an atmospheric state prone to oscillations across the 10^{-5} PAL pO_2 level, our data

194 suggest that a shallow oxycline was typical of open-marine environments throughout this period,

195 and indeed, anoxia likely extended throughout the water column in certain (e.g., upwelling) regions

196 of the ocean[34], promoting a high global marine methane efflux to the atmosphere. In this context,

197 with the exception of the immediate aftermath of the second Paleoproterozoic glaciation, carbonate

198 C isotopes record fluctuating, but positive, values across this time period (Fig. 4). Thus, the low

199 organic carbon isotope values ($\delta^{13}C_{org}$) observed in relatively deeper-water parts of the succession[25]

200 are highly fractionated (Fig. 3), supporting a significant contribution from the oxidation of ^{13}C-

201 depleted methane. We thus propose that during deposition of the upper Timeball Hill Formation,

202 which began after a 40-50 million year hiatus[3], elevated post-glacial atmospheric CO_2 would have

203 long been drawn down through silicate weathering[37], and hence methane dynamics likely played a

204 more critical role than CO_2 in triggering the fourth Paleoproterozoic glaciation.

205 Indeed, during deposition of the upper part of interval E, less intense chemical weathering is

206 supported by a clear overall decrease in the CIA weathering proxy broadly coincident with

207 atmospheric oxygenation (Fig. 3), which is consistent with a cooling climate initiated by oxidation

208 of atmospheric methane. A decrease in chemical weathering would also have diminished the

209 oceanic influx of nutrients, thereby decreasing productivity, TOC burial, and hence oxygen

210 production, thus driving the atmosphere-ocean system back to an anoxic state prior to the onset of

211 the final Paleoproterozoic glaciation (Fig. 3). Low productivity then continued to limit organic

212 matter production and burial, thus maintaining atmospheric oxygen concentrations at a low level

213 throughout the glaciation.

214 Our new sulfur isotope data demonstrate that the Rooihoogte-Timeball Hill formation boundary

215 does not record the permanent crossover of the 10^{-5} PAL atmospheric oxygen level, and thus revises

216 the widely accepted ~2.32 Ga age for the permanent oxygenation of Earth's atmosphere. Indeed, a

217 compilation of sulfur isotope data (Fig. 4) demonstrates fluctuations in atmospheric oxygen levels

218 across the entire Paleoproterozoic glacial epoch (~2.43-2.22 Ga), and suggests that the Lomagundi

219 carbon isotope excursion at ~2.22 Ga was the tipping point for permanent atmospheric oxygenation,

220 which occurred more than 100 million years later than previously thought. Thus, we redefine the

221 GOE as the Great Oxidation Episode, a transitory period that lasted more than 200 million years,

222 during which atmospheric redox oscillations straddled the 10^{-5} PAL threshold, progressively

223 increasing beyond it to influence higher atmospheric oxygen threshold indicators such as oxidised

224 paleosols, continental red beds, a disappearance of redox-sensitive minerals in placer deposits, and

225 the first appearance of shallow-water sulfate evaporite deposits[8,39] (Fig. 4). The permanent

226 termination of this transitional period is supported by evidence for elevated atmospheric O_2 levels

227 during the Lomagundi Event, and possibly an 'overshoot' to oxygen concentrations not seen again

228 until the terminal Neoproterozoic[39].

229 The final Paleoproterozoic glaciation is not known in other key successions (see Methods), and

230 the latest paleogeographic reconstructions place the Transvaal Basin at mid-to-high latitudes during

emplacement of the Hekpoort Formation[40], which immediately overlies the Boshoek Formation (Fig. 1). This suggests that while the final Paleoproterozoic glaciation was driven by atmosphere-ocean redox dynamics, it may also represent a waning in the severity of the climatic response to oxygenation as the Earth became progressively poised at an overall higher oxygenation state across each glaciation of the GOE[2]. It thus appears that the delayed rise in permanent atmospheric oxygenation that we document ushered in a new period of climatic and biogeochemical stability that persisted until the 'Snowball' Earth glaciations of the late Neoproterozoic, some 1.5 billion years later.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at…

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341

342   **Main Figure Legends:**

343   **Fig. 1. Simplified Paleoproterozoic stratigraphy of the Eastern Transvaal Basin, South Africa,**
344   **showing the studied interval** (modified after ref [3]). Constraints on atmospheric oxygen are
345   provided by mass-independent (MIF-S) and mass-dependent (MDF-S) sulfur isotope
346   fractionations[6,7,19].

347   **Fig. 2. Geochemical and isotopic profiles for drill cores EBA-1 and EBA-2.** Note the scale
348   changes for stratigraphic depth (to aid clarity) for the Reitfontein Diamictite and Boshoek
349   Formation in drill core EBA-1, and for the Reitfontein Diamictite and Rooihoogte Formation in
350   drill core EBA-2. Dashed line on Fe/Al plots represents the boundary (0.66) for distinguishing oxic
351   and anoxic water-column conditions[20]. Dashed lines on Fe_{HR}/Fe_T* plots represent the boundaries for
352   distinguishing oxic (<0.22) and anoxic (>0.38) water column conditions[21]. Dashed lines on
353   Fe_{py}/Fe_{HR}* plots represent the boundaries for distinguishing euxinic (>0.8) and ferruginous (<0.7)
354   water column conditions, which are only applicable for samples deposited from an anoxic water
355   column (shown as closed circles). Fe_{HR}/Fe_T* and Fe_{py}/Fe_{HR}* ratios are corrected for transformation

of unsulfidized Fe_{HR} to Fe-rich clays during diagenesis (see Methods). Horizontal dashed lines divide the succession into informal intervals (A–E) to aid interpretational clarity. TOC = total organic carbon.

Fig. 3. Multiple-sulfur isotope systematics and summary of atmospheric and oceanic redox conditions. A. $\Delta^{33}S/\delta^{34}S$ plot showing the similarity between post-GOE data and samples with $\Delta^{33}S$ < 0.3‰ from the present study, as well as the clear distinction with respect to samples with $\Delta^{33}S$ > 0.3‰, which straddle the Archean Reference Array (ARA)[22,23]. Inset shows a magnification at the origin, where dashed lines represent the 95% confidence interval for MDF-S data. B. $\Delta^{36}S/\Delta^{33}S$ plot showing the close similarity between the data of the current study and the post-GOE[24] and Archean Reference Arrays. Inset shows a magnification at the origin for the data from the current study. C. Stratigraphic summary of $\Delta^{33}S$ composition, chemical index of alteration (CIA), organic C ($\delta^{13}C_{org}$) isotope composition[25], and atmospheric and oceanic redox evolution (normalized to the thickness of individual units in drill cores EBA-1 and EBA-2, after correction for thickness of diabase intrusions). Grey arrow on the CIA plot summarises the overall trajectory of the data.

Fig. 4. Compilation of $\Delta^{33}S$ data and simplified carbonate C isotope ($\delta^{13}C$) trends for the 2.5-2.0 Ga time interval. Red circles represent data from the current study; black circles represent a compilation of published data[7,24]. Blue bars represent the four glacial intervals. $\delta^{13}C$ curve is modified after ref [38], with the dashed line highlighting the portion that is poorly defined. Geological indicators for the redox state of the atmosphere-ocean system are from refs [30,39].

METHODS

Geological setting

Diamond drill cores EBA-1 (26.4669ºS, 27.5875ºE) and EBA-2 (26.4700ºS, 27.5883ºE) were drilled near Carltonville in South Africa (Kloof Goldfields Property, Eastern Boundary Area), and intersected well-preserved, early Paleoproterozoic strata of the Transvaal Supergoup. The studied interval comprises the Rooihoogte and Timeball Hill formations in both cores, in addition to the overlying Boshoek Formation in EBA-1 (Fig. 2). The two cores were drilled in close proximity (<1 km apart) on a paleohigh in the Potchefstroom Synclinorium (Extended Data Fig. 6), and the succession has been subjected to only lower greenschist facies regional metamorphism[49].

The Rooihoogte Formation is divided into upper and lower members, with the boundary at 1196.9 m in EBA-1 and 1345.6 m in EBA-2. The lower Rooihoogte Formation (not sampled for this study) comprises the Giant Chert Breccia Member, which is an *in situ* karst breccia, consisting of brecciated dolostone and chert with some black shale[7]. Above the Giant Chert Breccia Member is the Bevetts Member (not sampled for this study), which formed during transgression onto the erosional surface, resulting in deposition of a sheet-like basal conglomerate or poorly sorted reworked chert breccia with some black shale[7]. Elsewhere in the region, a glacial diamictite then occurs[49], but this lithology is not present in cores EBA-1 and EBA-2, likely due to erosion at the sequence boundary with the upper Rooihoogte Formation[7]. The upper Rooihoogte Formation in

394   drill cores EBA-1 and EBA-2 dominantly consists of mudstone and black shale that coarsen

395   upwards into siltstone, with a thin chert breccia at the top[49].

396   The Timeball Hill Formation comprises two upward coarsening sequences[25]. The lower part of

397   both sequences is composed of highly carbonaceous black mudstone[49]. The mudstones in each

398   sequence become less carbonaceous up-section, and are interbedded with dark-grey to grey

399   siltstone. The lower mudstone-siltstone unit grades upwards into the Galsrand Member, which is a

400   fine- to medium-grained quartzite succession with oolitic ironstone beds[49]. The mudstones and

401   black shales of the upper Rooihoogte and Timeball Hill formations were deposited in a prodelta

402   basin setting, which is generally thought to have had open connection to the ocean to the

403   southwest[49-52]. Based on the increased thickness and more oxygenated depositional setting (see

404   below) of the Rooihoogte Formation in core EBA-1 relative to core EBA-2, it is likely that the latter

405   was deposited in a somewhat deeper-water setting.

406   The lower sequence of the Timeball Hill Formation is interpreted to have formed by

407   progradation of deltaic complexes, ranging from deep-water, prodelta black mudstone at the base,

408   through delta front mudstone and siltstone, to shallow-water delta plain sands and oolitic ironstone

409   of the Galsrand Member[25]. The Galsrand Member is considered to have been deposited in a warm,

410   well-oxygenated setting, based on the occurrence of hematite and the highly chemically weathered

411   nature of the sediments, as indicated by the chemical index of alteration (CIA) weathering proxy[25].

412   In core EBA-1, a small zone of groundwater or hydrothermal alteration from ~624 to 639 m depth

413   has been documented[25,49]. We report two samples from this zone, one of which was measured for

414   multiple sulfur isotopes. However, this sample (at 637 m; Supplementary Information Table S3)

415   documents a mass dependent sulfur isotope fractionation (MDF-S), similar to surrounding samples,

416   suggesting minimal post-diagenetic alteration of its isotopic signal.

417   The upper sequence of the Timeball Hill Formation is capped by diamictite and conglomerate of

418   the Reitfontein Member. The Reitfontein diamictite is composed of angular to sub-rounded clasts in

419   a matrix of mudstone and siltstone[25]. In outcrop, faceted and bull-nosed glacially striated pebbles

420   have been observed, leading to its interpretation as a glacial deposit[49,50]. This glacial diamictite is

421   considered correlative to a diamictite in the Ditlhojana Shale in the Bushveld structural basin[53],

422   which is an extension of the Transvaal structural basin into Botswana to the west, as well as a

423   diamictite in the Tlaameng Formation in the Kanye structural basin ~50 km to the west of the

424   Bushveld structural basin[3]. However, based on current global correlations[41], this glacial horizon in

425   southern Africa is not recognized in other sedimentary successions (Extended Data Fig. 1). The

426   contact between the diamictite and the underlying shale/siltstone is gradational, and the matrix of

427   the diamictite reflects continued deposition of shale and siltstone[49]. For the present study, sampling

428   of the Reitfontein Member focussed on the matrix, rather than clasts. The Reitfontein Member is
429   overlain by the Boshoek Formation, the lower part of which occurs in core EBA-1. The lower
430   Boshoek Formation starts with a poorly sorted, chert-pebble conglomerate that fines upwards into
431   shale and siltstone[49]. The conglomerate is considered to represent reworking of the Reitfontein
432   diamictite during major regression, with the overlying black shale representing renewed marine
433   transgression[49].

434   U-Pb dating[3] of the lower Timeball Hill Formation gave ages of 2309 ± 10 Ma and 2307 ± 8 Ma
435   (Extended Data Fig. 1), which are consistent with a minimum isochron age of 2316 ± 7 Ma based
436   on Re–Os dating of syngenetic to early diagenetic pyrite across the Rooihoogte-Timeball Hill
437   formation boundary[54]. U-Pb dating[3] of two tuff beds at 709.5 m and 711.6 m in the upper sequence
438   of the Timeball Hill Formation in core EBA-1, gave ages of 2256 ± 6 Ma and 2266 ± 4 Ma,
439   suggesting a hiatus of about 40-50 million years between deposition of the lower and upper
440   sequences.

441

442   **Global correlations**
443   The global correlation of Paleoproterozoic glacial successions deposited during the Great Oxidation
444   Event (GOE) has undergone major revision over recent years, in part based on improved constraints
445   from radiometric dating. As shown in Extended Data Fig. 1, four discrete glaciations are currently
446   advocated between ~2.46 and 2.22 Ga[3,4,41]. The Duitschland Formation of the Transvaal sub-basin
447   has previously been correlated with the Rooihoogte Formation[49], a correlation that is still preferred
448   by some authors[7]. However, the glacial diamictite that occurs at the base of the lower Duitschland
449   Formation has also been correlated with the second glaciation of the Huronian succession in North
450   America, based on carbon isotope chemostratigraphy and lithostratigraphy[30,55]. This is supported by
451   the youngest detrital zircon age of 2424 ± 24 Ma for the Duitschland Formation[56], considerably pre-
452   dating age constraints for the Rooihoogte and lower Timeball Hill formations (Extended Data Fig.
453   1), which contains detrital and volcanic zircons with 2310 ± 9 and 2308 ± 8 Ma ages[3,56]. We stress
454   here, however, that the main conclusions of the current work are not contingent on the precise
455   nature of the correlation between the Duitschland Formation and the Rooihoote/Timeball
456   Hill/Boshoek formation succession.

457   The Makganyene Formation of the Griqualand West sub-basin also contains a glacial diamictite,
458   which was previously correlated with the Reitfontein diamictite[3,49]. However, improved age
459   constraints[4] confirm that the Makganyene Formation is instead correlative with the first glaciation
460   of the Huronian succession[41,57] (Extended Data Fig. 1). The Turee Creek Basin of the Pilbara
461   Craton, Western Australia, is another succession of critical importance for understanding the

13

462   dynamics of the GOE[27]. The succession has previously been regarded to span the entirety of the

463   GOE, from ~2.45 Ga to 2.2 Ga, incorporating two, or possibly three, glaciations[58,59]. However, in

464   addition to diagenetic pyrite, detrital pyrite is present in all sandstones, conglomerates and debrites

465   deposited throughout the succession[27,41], which implies low levels of atmospheric oxygen during

466   transport and deposition. Based on a detailed reappraisal of the sequence stratigraphy,

467   geochronology and carbon isotope chemostratigraphy of the Turee Creek Basin, it has been

468   suggested that the Turee Creek Group is more restricted in its time span, and instead broadly

469   correlates with the first glacial cycle of the Paleoproterozoic[41] (Extended Data Fig. 1).

470

471   **Existing constraints on atmospheric oxygenation**

472   Multiple sulfur isotope data have been reported[6] for a limited suite of samples from the Rooihoogte

473   Formation and base of the Timeball Hill Formation in core EBA-2. These data document a MDF-S

474   signal, which has widely been accepted as marking the onset of permanent atmospheric oxygenation

475   at ~2.32 Ga. More recently, higher resolution multiple sulfur isotope data through three drill cores

476   (including EBA-2) that intersected the Rooihoogte Formation and the base of the Timeball Hill

477   Formation have been reported[7]. These data show a rapid transition from a large mass-independent

478   sulfur isotope signal (MIF-S) of up to +8‰, through a thin transitional zone (3-4 m) where the

479   lower part records a small Δ^{33}S range (<0.5‰) and the upper part documents larger Δ^{33}S (0.5 to

480   2‰) shifts, to near-zero Δ^{33}S (<0.3‰) values in the uppermost Rooihoogte and lowermost Timeball

481   Hill formations. This implies that this episode of oxygenation occurred rapidly, over a 1 to 10

482   million-year time interval[7]. In contrast to the current study, no previous study has reported multiple

483   sulfur isotope data above the bottom 4 m of the >500-m-thick Timeball Hill Formation, and no

484   previous study has integrated oceanic redox data with multiple sulfur isotope systematics through

485   any part of the succession.

486   Multiple sulfur isotope data have previously been published for other successions recording the

487   Paleoproterozoic glacial period (as summarised in terms of MIF-S and MDF-S for four key areas in

488   Extended Data Fig. 1). In particular, the Duitschland Formation documents a loss of MIF-S[19],

489   which, based on updated correlations, suggests probable oscillations in atmospheric oxygen in rocks

490   older than those of the current study[4]. A record of possible fluctuations in atmospheric oxygen has

491   also been reported based on sedimentary sulfides deposited in the Turee Creek Basin (Hamersley

492   Province) of Western Australia[27]. In this case, small-magnitude MIF-S signals (generally <1.5‰)

493   persist throughout, punctuated by short intervals with sulfides having MDF-S. These signals have

494   been attributed to the weathering of sulfides with pre-existing MIF-S, combined with short time

495   intervals of atmospheric oxygenation[27]. This suggestion is supported by a combined sulfur and

496   oxygen isotope study of barite from the Kazput Formation[28], which was deposited in the upper part

497   of the Turee Creek Group (Fig. S2). However, as discussed above, the precise correlation of this

498   succession to other key GOE localities is controversial. Thus, the significance of the Turee Creek

499   Group in relation to our high resolution study of the final stages of the GOE, as recorded by the

500   Pretoria Group, is unclear.

501   Multiple S isotope data have also been reported for rocks deposited on the Fennoscandian Shield,

502   northwest Russia[5]. This places the onset of the GOE, as denoted by a MIF-S to MDF-S transition,

503   close to 2,434 ± 6.6 Ma in age. It has also been demonstrated that small, but resolvable, MIF-S

504   signals occur throughout the Huronian Supergroup of Canada, which potentially arises due to a

505   variety of reasons, including homogenization by regional metamorphism, sulfur recycling, or

506   dilution of MIF-S by magmatic fluids[43]. Given the uncertainties outlined above, the well-preserved

507   Paleoproterozoic succession of the Carltonville region of South Africa, as analysed in the present

508   study, offers a prime opportunity to explore the dynamics of the GOE as it approached its critical,

509   concluding stage.

510

511   **Geochemical analyses**

512   Total organic carbon (TOC) was determined on a C-S Leco CS230 Analyzer after treatment with

513   50% HCl to remove carbonate phases. Accuracy (>98%) was ensured relative to Leco certified

514   standards 501-506, and replicate extractions gave a RSD of <5%. Total Fe and Al were determined

515   on ashed samples via a $HF\text{-}HNO_3\text{-}HClO_4$ extraction, with HBO_3 used to ensure full solubilization

516   of Al. Solutions were analysed by ICP-OES with >98% recovery relative to international sediment

517   standard PACS-2, and with RSDs of <5%.

518   Iron speciation analysis was conducted via a well-established sequential extraction procedure[60],

519   with acid-volatile sulphides (Fe_{AVS}) and pyrite Fe (Fe_{py}) quantified gravimetrically via a two-step

520   chromous chloride distillation[61]. The sequential extraction protocol extracts different operationally

521   defined Fe pools, including carbonates such as siderite (Fe_{carb}), ferric oxides such as goethite and

522   hematite (Fe_{ox}), and magnetite (Fe_{mag}). These phases define an Fe pool which is considered 'highly

523   reactive' (Fe_{HR}) during sedimentation and diagenesis[62], determined as the sum of Fe_{carb} + Fe_{ox} +

524   Fe_{mag} + Fe_{py}. A boiling HCl extraction was also performed[63] to determine an Fe pool (consisting

525   largely of sheet-silicate Fe) that is poorly reactive during diagenesis[64] (Fe_{PRS}), but which includes

526   Fe-rich clay minerals that may have been formed from non-sulfidized Fe_{HR} during diagenesis[65,66].

527   Iron solutions were analysed via AAS, and replicate extractions gave RSDs of <5% for all Fe

528   phases[67]. Geochemical data are reported in Supplementary Information.

529   We report 261 new Δ^{33}S analyses (207 via SO_2, and 54 via SF_6, which also provides Δ^{36}S data).

530   Sulfur isotope measurements were performed on Ag_2S precipitates from the two-step chromous

531   chloride distillation, using three measurement techniques. Samples in Supplementary Information

532   Tables S3 and S4 that report only δ^{34}S compositions were determined via EA-IRMS by Iso-

533   Analytical Ltd, with repeat analyses of internal barium sulfate standards IA-R036 and IAEA-SO-5

534   giving values of 20.75 ± 0.20‰ (n = 34; accepted value = 20.74‰) and 0.37 ± 0.34‰ (n = 28;

535   accepted value = 0.50‰), respectively. Samples in Supplementary Information Tables S3 and S4

536   that report $\delta^{34}S_{SO2}$ and $\Delta^{33}S_{SO2}$ compositions were analysed using an EA attached to a

537   ThermoScientific Delta V Plus configured in continuous flow mode. Correction via SF_6 calibrated

538   standards[68] leads to an integrated δ^{34}S variability of 0.1‰, and Δ^{33}S variability of 0.07‰. Samples

539   in Supplementary Information Tables S3 and S4 that report $\delta^{34}S_{SF6}$, $\Delta^{33}S_{SF6}$ and $\Delta^{36}S_{SF6}$ were

540   analysed by conventional fluorination methods (SF_6). Compositions measured with the latter

541   method provide an additional check on $\Delta^{33}S_{SO2}$ data, but also allow for higher precision Δ^{33}S and

542   Δ^{36}S. Here, Ag_2S was fluorinated with F_2, and the product SF_6 was cleaned prior to measurement

543   on a dual-inlet MAT 253. The long-term precision is 0.2‰, 0.008‰ and 0.20‰ for δ^{34}S, Δ^{33}S and

544   Δ^{36}S, respectively.

545

Constraints on a primary versus secondary origin for MIF-S

547   A primary syngenetic or diagenetic origin for sulfide minerals in the EBA-1 and EBA-2 cores has

548   been extensively demonstrated[6,7]. A unique feature of our dataset is that we can place petrographic

549   observations of mineral morphology within the context of both the redox history of the samples and

550   their multiple sulfur isotope signature. In Extended Data Fig. 3, photomicrographs from scanning

551   electron microscopy (analyses were performed on a Tescan VEGA3 XM Scanning Electron

552   Microscope with an Oxford Instruments X-max 150 EDS detector) provide support for a syngenetic

553   or diagenetic origin for pyrite in all samples (Extended Data Figs. 3a, b, d, e, f), as well as support

554   for a syngenetic origin for a portion of the Fe-oxide minerals deposited under ferruginous

555   conditions (Extended Data Fig. 3c). We found no evidence for rounded or externally corroded (as a

556   result of weathering) detrital pyrite grains. Instead, pyrite is euhedral, occurring either in isolation

557   (Extended Data Fig. 3a), or as disseminated grains (Extended Data Fig. 3b) or clusters (Extended

558   Data Figs. 3b, f), and we stress that these morphologies were a feature of samples with significant

559   MIF-S. Pyrite-rich samples deposited under euxinic water column conditions (as determined via Fe

560   speciation) showed evidence of primary pyrite with secondary overgrowths (Extended Data Fig.

561   3e), which is entirely consistent with initial precipitation from a euxinic water column, followed by

overgrowths produced during early diagenesis. Similarly, in ferruginous samples we observed euhedral Fe-oxide clusters with clay laminations bending around the clusters, and with the clay matrix within the clusters (Extended Data Fig. 3c), supporting precipitation and settling of Fe-(oxyhydr)oxide minerals from a ferruginous water column.

While these observations suggest that our sulfur isotope data are not compromised by the inclusion of detrital pyrite carrying a remnant MIF-S signal, an additional factor concerns the possibility that the sulfate delivered to the basin originated from oxidative weathering of pyrite with a pre-existing MIF-S signal[26-28]. As previously argued[7], there are several lines of evidence against a significant remnant MIF-S signal. First, all euxinic samples carry a MDF-S signal, consistent with the requirement for a high influx of sulfate produced via oxidative weathering. This suggests that the rocks being weathered in the provenance area did not carry a strong MIF-S signal. In addition, the succession was deposited in a large open-marine basin, which was several hundred kilometres wide[41,69,70]. This suggests that, particularly if the atmosphere had remained oxic, the sulfate reservoir size should have been large enough to buffer against a weathering-derived MIF-S signal[7]. Indeed, the Gordon Lake Formation of the Huronian Supergroup, which hosts tuffs of the same age as the lower Timeball Hill Formation[3], contains shallow-marine sulfate evaporites[30,71,72]. This indicates that the seawater sulfate reservoir had reached a sufficient size to allow for the formation of such deposits, and these lack MIF-S and have moderately positive δ^{34}S values, reflecting the isotopic composition of seawater sulfate[30,71,72]. Similarly, sulfate evaporites in the underlying upper Duitschland Formation (Fig. S2) also lack MIF-S and have moderately positive δ^{34}S values[72]. Although it is likely that the seawater sulfate reservoir fluctuated in size during the early stage of the GOE, the critical point is that it was episodically large when the atmosphere was oxygenated, and at these times it did not carry a remnant MIF-S signal. Conversely, a smaller seawater sulfate reservoir is entirely consistent with intervals of atmospheric anoxia. In addition, we note that the occurrence of MIF-S signatures is not random, but instead occurs in clusters at similar stratigraphic levels in both cores (Fig. 3). Taken together, these considerations suggest that it is highly unlikely that the MIF-S signals document a random record of a remnant weathering signal derived from a poorly mixed marine sulfate reservoir under oxygenated atmospheric conditions.

Furthermore, as discussed in the main text, perhaps the most compelling evidence for a primary origin for the sulfur isotope composition lies in the observation that samples with mass-dependent and mass-independent fractionations fall close to the Δ^{33}S/δ^{34}S and Δ^{36}S/Δ^{33}S reference arrays (Fig. 3), which are considered to reflect an atmospheric composition for post-GOE and Archean sulfides, respectively[22-24]. In Extended Data Fig. 4, we use orthogonal data regression (ODR) to quantify the

595 uncertainty in the determination of $\Delta^{36}S/\Delta^{33}S$ slopes[5]. ORD incorporates uncertainties in both the

596 abscissa and ordinate, and is thus preferable to linear regression, which only takes into account

597 errors in the vertical coordinate when determining the best fit slope. This analysis clearly

598 documents the differing trajectories of the two data-sets, including for MIF-S-bearing samples

599 deposited above the Rooihoogte-Timeball Hill formation boundary, providing strong support for

600 primary fluctuations in atmospheric oxygen across the 10^{-5} PAL threshold.

601   By contrast, during oxidative weathering of older continental sulfides with a pre-existing MIF-S

602 signal, mixing of the resultant sulfate riverine influx with mass-dependent sulfate produced under

603 oxygenated atmospheric conditions, would produce isotopic signals that deviate considerably from

604 the post-GOE and Archean reference arrays. This is evident in published data[5,27,28] where a MIF-S

605 influence from recycling has been invoked (Extended Data Fig. 5). Pyrite and barite multiple S

606 isotope analyses from the Turee Creek Group, as well as pyrite analyses from Fennoscandia, show

607 considerable scatter, and while some of the data plot close to either the post-GOE $\Delta^{36}S/\Delta^{33}S$

608 reference line or the Archean Reference Array (ARA), a large proportion of samples straddle

609 between these reference lines. In particular, many of the Hamersley Province samples plot in the

610 slope range of -1.0 to -1.5. This range has been considered to represent perturbations to the standard

611 ARA due to temporally restricted episodes of either enhanced methane-derived organic haze[24,44,45]

612 or volcanic sulphur input[46]. Oxidative weathering of sulfide minerals carrying this specific signal

613 could therefore be responsible for data that fall in the -1.0 to -1.5 range[28]. However, this signal is

614 also consistent with mixing between recycled MIF-S and MDF-S produced in an oxygenated

615 atmosphere. Indeed, several samples plot outside this range, which may imply that such mixing was

616 a prominent factor in the generation of these isotopic signals. Nevertheless, regardless of the precise

617 pathway for generation of the sulfur isotope signals, deviation from the Archean and post-GOE

618 reference lines for a significant proportion of the Hamersley Province and Fennoscandia data

619 supports a recycled MIF-S component[5,27,28], or mixed MIF-S and MDF-S components, which might

620 be expected for samples deposited during the earlier stages of the GOE.

621   A final consideration concerns the possibility that the MIF-S signals above the Rooihoogte

622 Formation represent hydrothermal alteration by fluids carrying a MIF-S signal derived from older

623 rocks deeper in the succession. However, these drill cores are exceptionally well-preserved and

624 there is little evidence for fluid flow of this type[49]. We also note that such fluid flow would often be

625 expected to result in precipitation of a variety of metal monosulfides, in addition to pyrite. Indeed,

626 there are very low concentrations of metal monosulfides present in a very few samples (reported as

627 Fe_{AVS} in Supplementary Information Tables S1 and S2). However, where there was sufficient

628   sulfide extracted, these samples were analysed for their sulfur isotope compositions and in all cases,

629   the metal monosulfides carry a MDF-S signal, conclusively confirming that the MIF-S signals

630   originate from syngenetic or diagenetic pyrite.

631

632   **Assessment of ocean redox conditions**

633   Geochemical redox data for cores EBA-1 and EBA-2 are shown in Extended Data Figs. 7 and 8.

634   While local redox data may be affected by a variety of factors, including sea level fluctuations and

635   tectonics, the local response of the ocean to extreme perturbations induced by major periods of

636   global deglaciation can provide valuable insight into ocean-atmosphere interactions. To assess

637   whether samples were deposited from an oxic or anoxic water column we utilise Fe speciation and

638   Fe/Al ratios. In terms of Fe/Al ratios, the succession documents a complex history, with some

639   samples showing values above the upper threshold[20] for identifying water column anoxia (0.66),

640   and others having ratios below this value. In terms of Fe speciation, oxic conditions are indicated

641   when Fe_{HR}/Fe_T ratios are <0.22, while Fe_{HR}/Fe_T ratios exceeding 0.38 arise from additional water-

642   column precipitation of Fe_{HR} minerals under anoxic conditions. Fe_{HR}/Fe_T ratios between 0.22 and

643   0.38 are considered equivocal[21], and may be a consequence of either rapid background

644   sedimentation under anoxic conditions (e.g., turbidite deposition), or transfer of non-sulfidized Fe_{HR}

645   minerals to poorly reactive sheet-silicates (Fe_{PRS}) during diagenesis and burial metamorphism,

646   which is particularly common in anoxic, non-sulfidic sediments[65,66]. For samples deposited from

647   anoxic bottom waters, the Fe_{py}/Fe_{HR} ratio is used to discriminate between ferruginous ($Fe_{HR}/Fe_T >$

648   0.38; $Fe_{py}/Fe_{HR} < 0.7$) and euxinic water column conditions[21] ($Fe_{HR}/Fe_T > 0.38$; $Fe_{py}/Fe_{HR} > 0.8$).

649   Many of the samples with Fe/Al >0.66 (indicating deposition from an anoxic water-column)

650   have Fe_{HR}/Fe_T ratios that fall within the equivocal zone, and to assess potential loss of Fe_{HR} via

651   transfer to Fe-rich clay minerals, which does not affect Fe/Al ratios, we thus consider Fe_{PRS}

652   concentrations[65,66]. Phanerozoic sediments deposited from an oxic water column[48] have average

653   Fe_{PRS} concentrations of 1.80 ± 0.85 wt%, and average Fe_{PRS}/Al ratios of 0.21. Many of our samples

654   plot above this ratio (Extended Data Figs. 7 and 8), and also have significantly higher Fe_{PRS}

655   concentrations (Supplementary Information Tables S1 and S2), suggesting extensive transfer of

656   Fe_{HR} to Fe_{PRS}. We provide a conservative assessment of possible loss of Fe_{HR} (for samples with

657     $Fe_{PRS}/Al >0.21$) by calculating a corrected depositional Fe_{HR}^* concentration:

658     $$Fe_{HR}^*/Al = (Fe_{HR}/Al)_{meas} + ((Fe_{PRS}/Al)_{meas} - (Fe_{PRS}/Al)_{PAS})$$

659   where *meas* represents the measured ratio, and *PAS* represents average oxic Phanerozoic shale.

660   Based on these revised Fe_{HR}^* contents, we calculate corrected Fe_{HR}/Fe_T^* and Fe_{py}/Fe_{HR}^* ratios

661   (Extended Data Figs. 7 and 8). This approach provides a considerably improved assessment of

19

water-column redox conditions during deposition of cores EBA-1 and EBA-2, and while our approach does not alter our overall conclusions, it results in 80-90% consistency in terms of redox agreement between Fe_{HR}/Fe_T* and Fe/Al ratios for both cores, strongly supporting the use of these ratios as proxies for the redox state of the water column.

Data availability statement: All data generated or analysed during this study are included in this published article (and its Supplementary Information files).

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767   **Extended Data Figure Captions:**

768   **Extended Data Fig. 1 Generalised stratigraphic correlation between the**
769   **Transvaal/Griqualand (South Africa), Hamersley (Pilbara, Western Australia) and Huronian**
770   **(Ontario, Canada) successions** (modified after ref [41]). The exact stratigraphic position of the
771   loss of MIF-S in the Huronian Basin is uncertain[42,43] and hence not shown.

772   **Extended Data Fig. 2 Geochemical data for the lower part of the Pretoria Group.** Dashed lines
773   on Fe_{HR}/Fe_T* plots represent the boundaries for distinguishing oxic and anoxic deposition, and on
774   Fe_{py}/Fe_{HR}* plots represent the boundaries for distinguishing ferruginous and euxinic water-column
775   conditions[21]. Dashed lines on $\Delta^{33}S$ plots are at -0.3‰ and +0.3‰.

776   **Extended Data Fig. 3 Scanning electron microscope images of pyrite and Fe-oxide**
777   **morphologies.** A. EBA-2, Rooihoogte Fm., 1346.2 m. Sample deposited under oxic conditions,
778    $\Delta^{33}S$ = +2.16‰; B. EBA-1, Rooihoogte Fm., 1168 m. Sample deposited under oxic conditions, $\Delta^{33}S$
779   = +1.77‰; C. EBA-1, Timeball Hill Fm., 1137 m. Sample deposited under ferruginous conditions,
780    $\Delta^{33}S$ = +1.44‰; D. EBA-2, Rooihoogte Fm., 1335.6 m. Sample deposited under ferruginous
781   conditions, $\Delta^{33}S$ = +0.25‰; E. EBA-2, Rooihoogte Fm., 1338.3 m. Sample deposited under euxinic
782   conditions, $\Delta^{33}S$ = +0.17‰; F. EBA-1, Timeball Hill Fm., 706 m. Water column redox state not
783   analysed, $\Delta^{33}S$ = +1.61‰.

784   **Extended Data Fig. 4 Sulphur isotope trends for Rooihoogte-Timeball Hill formation samples.**
785   A. Orthogonal data regression for samples with MIF-S ($\Delta^{33}S$ >0.3‰), showing calculated $\Delta^{36}S/\Delta^{33}S$
786   slope (blue line) and 3σ confidence interval (shaded blue area). Samples from above the Rooihoogte
787   Formation are distinguished as open blue circles. B. Orthogonal data regression for MDF-S samples
788   ($\Delta^{33}S$ = 0 ± 0.3‰), showing calculated $\Delta^{36}S/\Delta^{33}S$ slope (red line) and 3σ confidence interval
789   (shaded red area).

790   **Extended Data Fig. 5 Sulphur isotope data from Fennoscandia[5] and Western Australia[27,28].**
791   ARA = Archean Reference Array[22,23]; R-TH = Rooihoogte/Timeball Hill/Boshoek formations. Blue
792   dashed lines represent the range for the ARA (-0.9 ± 0.1). Perturbed slope range represents

793 maximum deviation from the standard ARA due to the temporal effects of either enhanced
794 methane-derived organic haze[24,44,45] or volcanic sulphur input[46].

795 **Extended Data Fig. 6 Simplified geologic map of the Transvaal Supergroup outcrop area**
796 (modified after ref [47]).

797 **Extended Data Fig. 7 Ocean redox data for EBA-1.** Dashed line on Fe/Al plot represents the
798 upper boundary for distinguishing anoxia[20], and on the Fe_{PRS}/Al plot represents the Phanerozoic
799 average[48]. Dashed lines on Fe_{HR}/Fe_T and Fe_{HR}/Fe_T* plots distinguish oxic and anoxic deposition[21].
800 Dashed lines on Fe_{PRS} plot represent the average Phanerozoic range (1.80 ± 0.85 wt%; ref [48]).

801 **Extended Data Fig. 8 Ocean redox data for EBA-2.** Dashed line on Fe/Al plot represents the
802 upper boundary for distinguishing anoxia[20], and on the Fe_{PRS}/Al plot represents the Phanerozoic
803 average[48]. Dashed lines on Fe_{HR}/Fe_T and Fe_{HR}/Fe_T* plots distinguish oxic and anoxic deposition[21].
804 Dashed lines on Fe_{PRS} plot represent the average Phanerozoic range (1.80 ± 0.85 wt%; ref [48]).

Eastern Transvaal Basin

Formation	Details
Upper Pretoria Subgroup	2.06 Ga Bushveld Complex
Hekpoort Formation	oxidized palaeosol / ≤2240-2250 Ma
Boshoek Fm	
Rietfonteindam Dia.	2256 ± 6 Ma
Timeball Hill Fm	2266 ± 4 Ma
Rooihoogte Fm	? ↑MDF-S 2310 ± 9 Ma / 2316 ± 7 Ma / ↓MIF-S
Duitschland Formation	↑MDF-S ≤2424 ± 24 Ma / ↓MIF-S
Tongwane Fm	
Penge Iron Formation	2480 ± 6 Ma

study interval

Legend:
- Sandstone and conglomerate
- Shale and siltstone
- Carbonate
- Glacial deposits
- Mafic igneous rocks
- Banded iron formation
- Redbeds and ironstones
- Manganese-rich deposits
- Unconformity

EBA1

TOC (wt%) 0 2 4 6
Fe/Al 0 1 2
Fe_HR/Fe_T* 0.0 0.5 1.0
Fe_py/Fe_HR* 0.0 0.5 1.0
δ34S (‰) -30 0 30
Δ33S (‰) -2 0 2 4 6 8

545
575
600
(m)
700
800
900
1000
1100
1200

Rietfont. Bosh.
Rietont.
Timeball Hill Fm
Up. Rooi.

Fe   Fe

oxic / anoxic
oxic / anoxic
○ oxic ● anoxic
ferruginous / euxinic

EBA2

TOC (wt%) 0 2 4 6
Fe/Al 0 5 10 15
Fe_HR/Fe_T* 0.0 0.5 1.0
Fe_py/Fe_HR* 0.0 0.5 1.0
δ34S (‰) -35 0 35
Δ33S (‰) -2 0 2 4 6

794
800
(m)
900
1000
1100
1200
1300
1335
1345

Rietfont.
Timeball Hill Fm
Up. Rooi.

anoxic
oxic / anoxic
○ oxic ● anoxic
ferruginous / euxinic

E
D
C
B
A

shallower / deeper

KEY: chert breccia   black shale to siltstone   diamictite   quartzite   + + intrusion