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4 **Title: Limited oxygen production in the Mesoarchean ocean**

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26 analyzed data; F.OO. wrote the paper with significant input from all authors.

27 **Abstract:** The Archean Eon was a time of predominantly anoxic Earth surface conditions,
28 where anaerobic processes controlled bio-essential element cycles. In contrast to oxygen oases
29 well documented for the Neoproterozoic (2.8-2.5 billion years ago; Ga), the magnitude, spatial
30 extent, and underlying causes of possible Mesoproterozoic (3.2-2.8 Ga) surface ocean oxygenation
31 remain controversial. Here, we report $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values coupled with local seawater redox
32 data for Mesoproterozoic shales of the Mozaan Group (Pongola Supergroup, South Africa), which
33 were deposited during an episode of enhanced Mn (oxyhydr)oxide precipitation between ~2.95
34 and 2.85 Ga. Iron and Mn redox systematics are consistent with an oxygen oasis in the
35 Mesoproterozoic anoxic ocean, but $\delta^{15}\text{N}$ data indicate a Mo-based diazotrophic biosphere with no
36 compelling evidence for a significant aerobic nitrogen cycle. We propose that in contrast to the
37 Neoproterozoic, dissolved O_2 levels were either too low or too limited in extent to develop a large
38 and stable nitrate reservoir in the Mesoproterozoic ocean. Since biological N_2 fixation was
39 evidently active in this environment, the growth and proliferation of O_2 -producing organisms
40 were likely suppressed by nutrients other than nitrogen (e.g., phosphorus), which would have
41 limited the expansion of oxygenated conditions during the Mesoproterozoic.

42
43 **Keywords:** oxygen oasis; nitrogen isotopes; nutrient limitation; oxygenic photosynthesis;
44 Mesoproterozoic

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46 **Significance Statement:** Episodic development of “oxygen oases” during the Archean Eon
47 characterizes the hundreds of millions of years transition to permanent oxygenation in the
48 atmosphere-hydrosphere system at the Great Oxidation Event (~2.4-2.3 Ga). One of these well-
49 characterized “oxygen oases” is recorded in Mesoproterozoic (~2.95-2.85 Ga) sediments of the
50 Pongola Supergroup. We show that in contrast to the Neoproterozoic (2.8-2.5 Ga), biological
51 oxygen production in a shallow ocean having Mo-based nitrogen fixation was not sufficient to
52 result in a dissolved nitrogen reservoir that would carry the isotopic effects of an aerobic
53 nitrogen cycle. Nevertheless, it appears that low concentrations of bioavailable phosphorus,
54 rather than nitrogen, suppressed the growth and expansion of oxygenic photosynthesizers, and
55 may explain why pervasive and permanent oxygenation was delayed during the Archean Eon.

56
57 **body**

58 A dramatic rise in atmospheric oxygen level during the Great Oxidation Event (GOE) at ~2.4
59 Ga is marked by the disappearance of mass-independent fractionation of sulfur isotopes,
60 oxidation of detrital pyrite and uraninite, and the appearance of red beds, reflecting the
61 irreversible transition from an anoxic to an oxic world (1-2). While it is widely accepted that
62 oxygenic photosynthesis was a first-order control on the GOE (3), Archean shallow-marine
63 “oxygen oases” and “whiffs” of atmospheric oxygen (O_2) have been proposed to have occurred
64 up to several hundred million years prior to the GOE (4-18). However, while processes that
65 drove oxygen production during transient and localized oxygenation events in the Neoproterozoic
66 (2.8-2.5 Ga) are supported by a wide range of geochemical proxies (e.g., 4-6, 13-18), those
67 from the Mesoproterozoic (3.2-2.8 Ga) are constrained by only a limited number of studies (7-12).

68 The nitrogen (N) cycle from the early Archean up to ~2.7 Ga is widely considered to have been
69 dominated by bioavailable ammonia (NH_4^+) under anoxic water column conditions (15, 16).
70 Oxidation of NH_4^+ would have been suppressed in an early Archean ocean characterized by
71 extremely low O_2 concentrations (15-17). Free O_2 is produced through oxygenic
72 photosynthesis, the rate of which is mainly controlled by the concentrations of bioavailable N
73 and phosphorus (P) (19-24). While the sedimentary $\delta^{15}\text{N}$ record suggests that N was
74 bioavailable and that diazotrophic Mo-based nitrogenase dominated N_2 fixation in the
75 Mesoarchean, the record also places a robust minimum age for the occurrence of aerobic N
76 cycling at ~2.72 Ga in the Neoproterozoic (e.g., 15, 16 and references therein). Indeed, prominent
77 N isotope excursions in the Neoproterozoic provide evidence for temporary NH_4^+ oxidation, and
78 thus the $\delta^{15}\text{N}$ record has been used to infer the development of locally oxygenated surface
79 ocean environments after ~2.7 Ga (5, 15, 17, 18, 25).

80 Independently, stable isotope systematics of redox-sensitive elements such as Fe, Mo, U and
81 S, as well as locally enhanced Mn (oxyhydr)oxide precipitation, support an earlier emergence
82 of oxygenic photosynthesis and episodic development of “oxygen oases” in the Mesoarchean
83 surface ocean (7-9, 11), well before currently accepted evidence for oxidative nitrogen cycling.
84 Furthermore, phylogenomic estimates based on molecular clocks also suggest that
85 cyanobacterial stems capable of oxygenic photosynthesis might find their roots in the Archean,
86 with a development and progressive diversification starting as early as ~3.5 Ga (26-28).
87 However, the factors that caused a delay in pervasive oxygenation of the atmosphere-
88 hydrosphere system after the establishment of oxygenic photosynthesis in the early Archean
89 remain poorly constrained, particularly with regard to the role of their two main bio-limiting
90 nutrients, N and P (19-23). Modelling studies have demonstrated that low dissolved P
91 concentrations would severely suppress the rate of oxygenic photosynthesis and ultimately the
92 spatial extent of Archean oxygen oases (29). However, there is currently no consensus on
93 dissolved P concentrations in the Archean ocean (21-23, 30-32).

94 In order to assess controls on the spatial development and intensity of Earth’s first oxygen
95 oases, we measured nitrogen ($\delta^{15}\text{N}$) and organic carbon ($\delta^{13}\text{C}_{\text{org}}$) isotopes, local water column
96 redox proxies (Fe speciation and Mn concentrations), and elemental data for shales of the
97 ~2.95-2.85 Ga Mozaan Group, Pongola Supergroup, South Africa (see Supplementary
98 Information for geologic setting and all data). Our aim is to clarify the factors that controlled
99 the nature and development of oxygen oases in the Mesoarchean.

100

101 **Results and discussion**

102 **Water column redox reconstruction.** Our samples span a shallow-marine (above wave base)
103 depositional setting in the White Mfolozi Inlier, to a deeper-water (below wave base)
104 equivalent in the Nongoma area, and comprise three sequences deposited at different water
105 depths (Figs. 1, S1, S2; Table S1). In the White Mfolozi Inlier, sequence I, deposited in the
106 most proximal, intertidal to shallow subtidal setting, is characterized by high Mn contents and
107 Mn/Fe ratios compared to average values for shales of the Pongola Supergroup (33; Table S1),
108 mostly high ratios of highly-reactive Fe to total Fe (FeHR/FeT) and high Fe/Al ratios (see
109 Methods for detailed analytical techniques). Sequence II was deposited in a deep subtidal, but
110 above fair-weather wave base setting, and shows a progressive decrease in Mn, Mn/Fe,
111 FeHR/FeT and Fe/Al, while Mn and Fe contents are higher than in average Pongola shales (33;
112 Table S1). The uppermost sequence III represents deepening to between fair-weather and storm
113 wave base, and is characterized by persistently low Mn, Mn/Fe, FeHR/FeT and Fe/Al, with Mn
114 and Fe contents similar to those in average Pongola shales (33; Table S1). In the more distal,
115 deeper-water setting of the Nongoma area, where distinct compositional trends were not
116 observed, Fe/Al ratios tend to be high, but Mn contents remain low and Mn/Fe ratios shift to
117 values lower than the average for shales of the Pongola Supergroup (33; Table S1).

118 To explain these data, we invoke upwelling of anoxic waters that were rich in Fe²⁺ and Mn²⁺
119 into oxic shallow waters. Precipitation of Fe as (oxyhydr)oxide minerals may have started
120 under low oxygen or anoxic conditions, potentially via photoferrotrophy in shallower waters
121 directly overlying deeper anoxic waters (34), and this likely explains the observed Fe
122 enrichments in the distal Nongoma setting (Fig. 1b). Fe(II) oxidation would have been
123 progressive during upwelling, leading to increased FeHR/FeT and Fe/Al enrichments (Fig. 1a)
124 as water depth shallowed through a redoxcline (as captured by sequence II in the White Mfolozi
125 Inlier) into the shallow and locally oxygenated waters of sequence I, where Mn(IV)
126 (oxyhydr)oxides precipitated (Fig. 1a; Fig 2). Increased Mn/Fe ratios in shallower waters thus
127 reflect progressive removal of dissolved Fe(II) and/or enhanced precipitation of Mn
128 (oxyhydr)oxides as upwelling anoxic waters reached the redox threshold for Mn(II) oxidation.
129 However, sequence III in the White Mfolozi Inlier has Mn/Fe ratios similar to the average value
130 for shales of the Pongola Supergroup, with no evidence for FeHR enrichment, likely reflecting
131 deeper anoxic waters where there was limited oxidant availability to promote Fe- or Mn-

132 (oxyhydr)oxide precipitation in the water column. At first glance, Fe enrichments in shallower
133 waters and their absence in deeper waters of the White Mfolozi Inlier may appear contradictory,
134 since Fe enrichments are commonly taken to denote water column anoxia (35). However, our
135 data are entirely consistent with current understanding of how Fe enrichment may be enhanced
136 under anoxic ferruginous conditions, whereby one prominent pathway for developing high
137 Fe_{HR}/Fe_T and Fe/Al ratios is via upwelling of deep, anoxic waters into shallower oxic settings
138 (35, 36).

139 Once Mn and Fe (oxyhydr)oxides had formed and became deposited, they were then largely
140 converted to carbonate minerals through microbial respiration during early diagenesis, as
141 indicated by high Fe_{carb} concentrations in sediments of sequence I and II (Table S1). In support
142 of this, highly negative $\delta^{13}\text{C}$ (between -22 and -13% , VPDB) and $\delta^{18}\text{O}$ values (between -21
143 and -8% , VPDB) indicate carbonate precipitation through organic carbon remineralization
144 during diagenesis (7, 37). This happened below the sediment-water interface in sediments
145 deposited below a water column characterized by relatively high rates of organic carbon (OC)
146 burial (high productivity) (7). In contrast, Fe_{carb} is scarce in the deeper water sequence III and
147 the more distal Nongoma setting (Table S1), where instead most of the Fe is associated with
148 chlorite and stilpnomelane (7). It is thus likely that Fe (oxyhydr)oxides were converted to Fe-
149 rich clay minerals during diagenesis in this setting, probably via reverse weathering (38).
150 Another possibility involves conversion of Fe (oxyhydr)oxides into mixed ferrous/ferric phases
151 such as green rust during settling (39, 40), before their final transformation to stilpnomelane
152 and chlorite during diagenesis and metamorphism. Thermodynamic estimates based on the
153 chemical composition of Fe-chlorite showed that Fe- and Mn-rich clay minerals of the Mozaan
154 Group formed during diagenesis and metamorphism (37). Regardless of the precise nature of
155 precursor Fe minerals, Fe/Al ratios much higher than those in average Pongola shales (33;
156 Table S1) indicate that their precipitation gave rise to significant Fe enrichments in the deep-
157 water sediments, and during upwelling of deep ferruginous waters into shallower oxic settings.

158 The $\delta^{13}\text{C}_{\text{org}}$ values average -27.6% in the shallow-water sequence I samples, reflecting isotopic
159 fractionations expected during autotrophic CO₂ fixation (41). During deposition of sequence
160 II, $\delta^{13}\text{C}_{\text{org}}$ values progressively decrease to the average value of -30% , and down to -38% in
161 the deep-water settings of the White Mfolozi Inlier and Nongoma areas (Fig. 1). The highly
162 negative $\delta^{13}\text{C}_{\text{org}}$ values in these deeper-water, ferruginous settings likely reflect biological
163 carbon cycling with a significant contribution from methanogens and methanotrophs (42). The
164 variability in biological processes with water-depth might be linked to the water column redox

165 gradient, where (1) high Mn/Fe ratios and Mn(II) oxidation (which requires free O₂) are
166 consistent with photoautotrophic CO₂ fixation and oxygenic photosynthesis in the shallow-
167 water settings, and (2) Fe enrichments without Mn(II) oxidation (Mn/Fe ratios lower than the
168 average Pongola shale values) are consistent with methanotrophs utilizing dissolved O₂ or
169 Fe(III) compounds to oxidize methane at the redoxcline or chemocline, respectively, under
170 hypoxic or anoxic conditions in deeper-water settings. In view of this, the water column
171 appears to have been both redox and ecologically stratified.

172

173 **N isotope systematics and preservation of primary isotopic signals.** Our geochemical data
174 suggest a shallow-water oxygen oasis in the Mesoproterozoic Pongola sea at ~2.9 Ga. If these
175 conditions were stable and extensive enough to support oxic nitrogen metabolism, then this
176 should be reflected in nitrogen isotope systematics, as observed in younger Neoproterozoic
177 sedimentary successions (5, 15, 17, 18, 25). Large N isotope heterogeneity revealed by the
178 NanoSIMS technique in isolated microfossils from the ~3.0 Ga Farrel Quartzite (Western
179 Australia) has been linked to biological aerobic nitrification (43), indicating the emergence of
180 this metabolic pathway even before deposition of the Mozaan Group. In ancient marine
181 sediments, $\delta^{15}\text{N}$ values between -4 and $+2\text{‰}$ (Air-N₂) are usually attributed to isotopic
182 fractionation imparted during biological N₂ fixation using the Mo-nitrogenase enzyme (Nif; 5,
183 15-18, 25). The use of V- (Vnf) and Fe-based (Anf) alternative nitrogenase enzymes produces
184 more depleted $\delta^{15}\text{N}$ values, between -6 and -8‰ (15, 44). $\delta^{15}\text{N}$ values above $+4\text{‰}$ would
185 provide compelling evidence for an aerobic N cycle coupling nitrification and
186 denitrification/anammox processes (*e.g.*, 5, 15, 17, 18, 25). Nitrogen isotope values for 18 out
187 of 22 samples fall in the range of -5 to $+3\text{‰}$ (Air-N₂), and reflect isotopic fractionation driven
188 by Mo-based diazotrophy (Fig. 1; Table S2). Positive values above $+4\text{‰}$ are limited to 4
189 samples, including 2 from the White Mfolozi Inlier and 2 others from the more distal Nongoma
190 area.

191 Here, we exclude abiotic sources for bioavailable nitrogen, because they were probably too
192 small in magnitude and should have otherwise dominated the early Precambrian $\delta^{15}\text{N}$ record,
193 counter to what is observed (15). However, several mechanisms can alter the original $\delta^{15}\text{N}$
194 values of marine biomass, ranging from early diagenesis to the thermal degradation of organic
195 matter (OM) during deeper burial diagenesis and metamorphism (15, 45-48). The redox state
196 of the water column, sedimentation rate, and OM accumulation can also impart different N

197 isotope fractionations between sinking organic particles and surficial marine sediments. NH_4^+
198 release during OM remineralization below the sediment-water interface and partial oxidation
199 in pore waters can increase bulk sediment $\delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{bulk}}$) values by $\sim 4\%$ under oxic diagenetic
200 conditions, while this effect tends to be minimal during anoxic diagenesis, with an isotopic
201 fractionation $< 1\%$ (15, 45). The predominance of Fe- and Mn-carbonate minerals derived from
202 the reduction of Fe- and Mn-(oxyhydr)oxides indicate anoxic diagenetic conditions (7, 37) and
203 thus likely a minimal effect of early diagenetic processes on primary $\delta^{15}\text{N}$ values. Importantly,
204 oxic diagenesis would result in isotopic compositions that reflect an aerobic nitrogen cycle,
205 which is not seen in our dataset.

206 Organic-bound NH_4^+ can also be released through thermal devolatilization of organic matter
207 during burial diagenesis and metamorphism, resulting in a maximum increase in $\delta^{15}\text{N}_{\text{bulk}}$ values
208 of 1–2‰ at greenschist facies, 3–4‰ at lower amphibolite facies, and up to 6–12‰ at upper
209 amphibolite facies; even larger offsets can occur in sedimentary rocks affected by circulating
210 fluids (46). The Mozaan Group experienced lower greenschist facies metamorphism (37),
211 suggesting a maximum increase in $\delta^{15}\text{N}_{\text{bulk}}$ values of less than 2‰. In order to alleviate
212 potential effects caused by mechanisms described above on $\delta^{15}\text{N}$ values, N isotope data were
213 also measured on extracted kerogen ($\delta^{15}\text{N}_{\text{ker}}$) to compare with bulk sample data ($\delta^{15}\text{N}_{\text{bulk}}$). The
214 offset between $\delta^{15}\text{N}_{\text{bulk}}$ and $\delta^{15}\text{N}_{\text{ker}}$ values allows evaluation of the extent of preservation of the
215 N isotope signature imparted by the initially deposited biomass. Two samples from the
216 Nongoma area showing evidence for hydrothermal processes are characterized by large offsets
217 between $\delta^{15}\text{N}_{\text{bulk}}$ and $\delta^{15}\text{N}_{\text{ker}}$ values and very positive N isotope values (Fig. 1; Table S2), which
218 likely supports post-depositional alteration by circulating fluids. Therefore, these 2 samples
219 will not be further considered in this study. In contrast, the minimal offset in most of the studied
220 samples supports good preservation of the primary isotopic signature (Fig. 1; Table S2). The
221 samples with minimal offset between $\delta^{15}\text{N}_{\text{bulk}}$ and $\delta^{15}\text{N}_{\text{ker}}$ values also lack evidence of
222 secondary alteration by later circulating fluids or hydrothermal processes (37). Furthermore, a
223 minimal effect of post-depositional processes on the isotope composition of biomass is also
224 indicated by the absence of co-variation among $\delta^{15}\text{N}$ and TN, $\delta^{15}\text{N}$ and C/N, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, as
225 well as between $\delta^{13}\text{C}_{\text{TOC}}$ and TOC for bulk sediments (Fig. 3). A weak negative co-variation
226 between $\Delta^{15}\text{N}_{\text{ker-bulk}}$ (the difference between $\delta^{15}\text{N}_{\text{ker}}$ and $\delta^{15}\text{N}_{\text{bulk}}$) and total K further supports
227 a minimal contribution of ammoniated phyllosilicates (*e.g.*, NH_4^+ substituted for K^+) with a
228 distinct isotopic composition (see Fig. S4).

229

230 **A lack of evidence for aerobic nitrogen cycling in the Mesoarchean Pongola basin oxygen**
231 **oasis.** In the modern ocean, where the main processes intrinsic to the aerobic N cycle, including
232 N₂ fixation, nitrification, and denitrification/anammox, are at play, $\delta^{15}\text{N}$ values of +5 to +7‰
233 in sedimentary organic matter reflect ¹⁴N loss to the atmosphere through denitrification and
234 anammox in oxygen-minimum zones (48). Buried biomass is an indirect archive of this
235 process, because organisms assimilate the isotopically heavy nitrate as a nutrient. Therefore,
236 $\delta^{15}\text{N}$ values $\geq +4\%$ found in Neoproterozoic marine sediments are interpreted to reflect temporary
237 aerobic N cycling (*e.g.*, 5, 15, 17, 18, 25). In contrast, the nitrogen isotope data of the Mozaan
238 Group are inconsistent with the establishment of a significant aerobic nitrogen cycle. Assuming
239 that Mn(II) oxidation occurs at higher redox potential than NH₄⁺ oxidation, redox conditions
240 may have been sufficient for evolved nitrifying bacteria in the Pongola basin, because high Mn
241 concentrations in sequence I indicate the precipitation of Mn(IV) oxyhydroxide minerals (7),
242 which required O₂. Photochemical oxidation of Mn is inhibited under Fe-saturated conditions
243 (49) and, unlike Fe(II), significant oxidation of Mn requires oxygen and a catalyst (50). It is
244 thus likely that free O₂ was locally available in the water column during deposition of sequence
245 I (7) and that the subtle increase in $\delta^{15}\text{N}_{\text{ker}}$ values (up to +5.2‰) measured in 2 samples from
246 the upper part of sequence I (deposited at the redoxcline) may reflect
247 nitrification/denitrification and uptake of residual nitrate in the water column (Figs. 2).
248 However, the absence of a more compelling isotopic shift in $\delta^{15}\text{N}$ values over the extended
249 stratigraphic interval indicates that NO₃⁻ (*i.e.* the residual nitrogen species that carries the
250 isotopic information in the modern ocean) did not build up to high enough concentrations to be
251 an important nitrogen source to the biosphere.

252 While the iron and manganese proxies have the capacity to promptly respond to redox
253 perturbations on a local scale, the nitrogen isotope proxy requires the build-up of a dissolved
254 nitrogen reservoir that carries the isotopic effects of redox reactions at the ecosystem scale. A
255 good modern analog illustrating such a discrepancy between Mn (higher redox potential) and
256 N (lower redox potential) cycles can be found in the Black Sea. Here, rapid oxygen-dependent
257 microbial Mn(II) oxidation is observed at low micromolar (< 3-5 μM) dissolved oxygen
258 contents in the suboxic zone of the Black Sea (51), where nitrification produces a maximum
259 nitrate concentration of only 3.5 μM (52). However, this maximum nitrate level appears to be
260 too low to leave an isotopic signature of aerobic N cycling (53) (in contrast to open ocean
261 nitrate concentrations of up to ~35 μM , which leave an average $\delta^{15}\text{N}$ signal of around +5‰;
262 *ref 15 and references therein*). Estimates of O₂ content based on $\delta^{56}\text{Fe}$ variations in

263 Mesoarchean oxygen oases suggest a maximum concentration of 10 μM (7, 11). Such dissolved
264 O_2 levels are thus consistent with the potential activity of both Mn(II) oxidizing and nitrifying
265 bacteria during deposition of the Mozaan Group.

266 It appears that the geographical extent of oxygen oases was likely too restricted in the
267 Mesoarchean ocean to develop a nitrate reservoir that was large enough to leave an isotopic
268 signature, which contrasts with their Neoproterozoic equivalents (assuming water-column O_2
269 concentrations were similar in the Mesoarchean and Neoproterozoic oxygen oases; 7, 11, 29).
270 Overwhelming supply of reducing inputs (e.g., Fe(II) and Mn(II)) from submarine volcanism
271 to the Mesoarchean ocean could have suppressed more widespread oxygenation and thus
272 limited nitrification. However, iron formation (IF) secular records (e.g., 54, 55) indicate that
273 volumetrically, the Neoproterozoic IFs are much larger than their Mesoarchean analogs, and yet
274 the Neoproterozoic to early Paleoproterozoic IFs are characterized by compelling evidence for
275 aerobic N cycling (5, 25, 56). It therefore appears that the reducing sinks from submarine
276 volcanism were not the main driving factor that suppressed the expression of aerobic N cycle
277 in the Mesoarchean ocean.

278

279 **Implications for oxygenic photosynthesis in the Mesoarchean ocean.** Overall, our data
280 reveal an ecosystem that was dominated by Mo-based diazotrophy, in an oxygen oasis where
281 a combination of the restricted spatial extent and low dissolved O_2 concentration likely limited
282 the build-up of a sufficient nitrate reservoir to impart an isotopic expression of aerobic N
283 cycling. In the modern ocean, cyanobacteria are the main N_2 fixers (57), and our data suggest
284 that this relationship may extend back to the Mesoarchean. Mo-based diazotrophy requires
285 soluble MoO_4^{2-} availability in the ocean. In the modern ocean, Mo is mainly delivered via
286 riverine inputs following oxidative continental weathering, with a minor contribution from
287 submarine hydrothermal systems (see ref. 16 and references therein). The mild Mo enrichment
288 (relative to average concentration for the upper crust) recorded by the Mozaan Group (Table
289 S1) suggests that dissolved Mo was available in this oxygen oasis (8). However, it has been
290 shown that a very low Mo content (down to 1 nM, which is ca. 1% of modern seawater
291 concentrations) can sustain Mo-based diazotrophy in modern environments (58). In view of
292 this, submarine hydrothermal Mo inputs could have been sufficient to sustain Mo-based N_2
293 fixation in the Archean ocean (see ref. 16 and references therein). Moreover, it has also been
294 demonstrated that continental Mo could have been mobilized and delivered to the ocean even

295 under Archean anoxic atmospheric conditions (O_2 concentration $< 10^{-5}$ PAL; 59, 60 and
296 references therein).

297 Free O_2 produced by oxygenic photosynthesis had an impact on the water-column redox and
298 ecological gradients of the Pongola basin, as observed in stable isotope data confirming aerobic
299 Fe and Mn cycling in shallow-water settings (7). Bioavailable N and P are the main nutrients
300 that control marine productivity over time (*e.g.*, 19-23). Their scarcity may have limited
301 biological O_2 production, resulting in delayed pervasive and permanent oxygenation of the
302 atmosphere-hydrosphere system after the emergence of oxygenic photosynthesis in the
303 Mesoarchean. Since our $\delta^{15}N$ data indicate that N was bioavailable in the Mesoarchean marine
304 oasis, P scarcity could have been the main limiting factor in biological O_2 production,
305 consistent with previous biogeochemical modelling (29). Indeed, we observe very low P
306 contents in the Pongola sediments (Fig. 1; Table S1), which would be entirely consistent with
307 the suggestion of widespread P limitation under global ferruginous conditions (21), prior to
308 more extensive anoxic P recycling linked to the build-up of seawater sulfate following more
309 expansive environmental oxygenation (22).

310 It is possible that positive $\delta^{15}N$ values in the ca. 3.2 Ga riverine deposits of the Moodies Group,
311 South Africa, interpreted as evidence for denitrification (12), and a weakly oxidizing U cycle
312 in the Mesoarchean ocean (11, 61), may reflect episodes of mild, local oxidizing conditions in
313 the atmosphere-hydrosphere system (ref. 13 and references therein). However, estimates based
314 on preserved Mesoarchean detrital uraninite in the Witwatersrand Supergroup (South Africa)
315 deposited contemporaneous with the Pongola Supergroup suggest that atmospheric O_2
316 concentrations were lower than 3.2×10^{-5} atm (62). Furthermore, the general absence of $\delta^{15}N$
317 values above +4‰ in most Mesoarchean marine and continental deposits around the world (15,
318 16, 63; this study), is consistent with the view that low rates of biological O_2 production limited
319 the geographical extent of oases and, ultimately, controlled the size of the seawater nitrate
320 reservoir, which did not reach the level necessary to leave a more widespread and persistent
321 isotopic signature of an aerobic N cycle. Regardless of the mechanism/s that controlled
322 dissolved P concentrations in Archean oceans, oxygenation of Earth's early biosphere was
323 apparently limited by a low supply of bio-available P, rather than N, under anoxic to very low-
324 oxygen surface conditions.

325

326 **Methods**

327 **Major and trace elements.** Powdered samples were analysed for major element
328 concentrations by X-ray fluorescence spectroscopy. Analysis was carried out on fusion beads,
329 using a PANalytical MagiX Pro PW2540 spectrometer at the University of Johannesburg.
330 Accuracy was checked with certified reference materials and was better than 1%. Elemental
331 concentrations are reported in wt.% with a detection limit of 0.04 wt.%. Trace elements were
332 measured at the Isotope Geochemistry Lab, University of Tuebingen (Germany) according to
333 the analytical procedure previously described (10, 64). Around 30 mg of ashed powdered
334 samples (heated to 600°C for 12 hours to ash organic compounds) were dissolved using a mix
335 of concentrated and distilled HF (2 mL) and HNO₃ (0.3 mL) in screw-top 15 mL Savillex®
336 PFA beakers at 120°C for 4 days. After evaporation at 80°C, samples were taken up in 1.5 mL
337 6 M HCl and re-dissolved in closed beakers at 130°C for 1 day. The samples were evaporated
338 to incipient dryness at 90°C and reacted twice with 0.3 mL aliquots of concentrated HNO₃ with
339 evaporation at 90°C in between to remove excess F and Cl. An aliquot of 5 M HNO₃ (1 mL)
340 was added to the sample residues and heated at 80°C for ~1 hour for dissolving the samples.
341 Analyses were performed in the iCap-Qc ICP-MS coupled to an ESI SC-2 DX auto-sampler
342 with an ESI Fast uptake system equipped with a 4 mL sample loop. For analysis, solution
343 samples in 1 mL 5 M HNO₃ were diluted twice; first in MQ water (dilution factor 1000) and
344 second in an internal standard solution made of 0.3 M HNO₃ (dilution factor 10,000) containing
345 a spike mixture of ⁶Li (~3 ppb), In (~1 ppb), Re (~1 ppb), and Bi (~1 ppb). Analytical accuracy,
346 estimated from the 1 r.s.d. of the mean, varied between 3 and 15% and was monitored by
347 repeated measurements of reference materials OU-6, QS-1, W-2a, and AGV-2. Enrichment
348 factors were calculated as $(\text{element}/\text{Al})_{\text{sample}}/(\text{element}/\text{Al})_{\text{reference}}$ using the average
349 concentrations for the upper crust as reference (65).

350 **Iron speciation analysis.** Iron speciation analysis was performed at the University of Leeds,
351 UK using a calibrated sequential extraction protocol followed by Fe analysis via AAS (66).
352 This method is designed to quantify four different pools of Fe considered to be highly-reactive
353 (FeHR) towards H₂S in surface and near-surface environments: (1) pyrite S extracted via Cr-
354 reduction followed by trapping as Ag₂S, with Fe calculated assuming an FeS₂ stoichiometry
355 (Fe_{Py}); (2) carbonate-associated iron extracted with a sodium acetate solution (Fe_{Carb}); (3) ferric
356 oxides extracted with a dithionite solution (Fe_{Ox}); and (4) mixed-valence iron oxides,
357 principally magnetite, extracted using ammonium oxalate (Fe_{Mag}). The total Fe content in
358 ancient marine shales (Fe_T) represents the sum of FeHR and Fe bound in silicates (66, 67). It
359 has been established that marine shales deposited under oxic water column conditions are

360 characterized by low ratios of FeHR/FeT < 0.22 driven by the lack of Fe mineral precipitation
361 following transport of Fe(II) under anoxic water column conditions. Under anoxic water
362 column conditions, FeHR/FeT ratios tend to be higher (above 0.38), whereas values between
363 0.22 and 0.38 are considered equivocal due to additional processes (*e.g.*, rapid sedimentation)
364 that may obscure Fe enrichments under anoxic water column conditions (35). Furthermore, for
365 anoxic water column conditions with FeHR/FeT > 0.38, FePy/FeHR < 0.7 represents
366 ferruginous conditions, whereas FePy/FeHR values above 0.7–0.8 characterize euxinic
367 conditions (35, 67). In some cases, FeHR can be converted to poorly reactive silicates (FePRS)
368 during diagenesis resulting in apparently low FeHR/FeT ratios, which can be wrongly
369 interpreted to reflect oxic water column conditions (35, 67). Therefore, it is required to pair
370 FeT/Al ratios and FePRS concentrations in order to distinguish samples deposited under oxic
371 from those deposited under anoxic water column conditions, where FeT/Al ≤ 0.55 ± 0.11 would
372 indicate oxic water column conditions (67). FeT/Al > 0.66 would reflect local Fe enrichments
373 either under anoxic water column conditions, or due to input of anoxic hydrothermal fluids into
374 oxic seawater (67).

375 **Carbon and nitrogen isotope analyses.** The nitrogen isotope composition of bulk rock
376 ($\delta^{15}\text{N}_{\text{bulk}}$) and the carbon and nitrogen isotope compositions of the extracted kerogen ($\delta^{13}\text{C}_{\text{org}}$,
377 $\delta^{15}\text{N}_{\text{ker}}$) were determined by elemental analysis/isotope ratio mass spectrometry (EA/IRMS) at
378 the Institute of Earth Surface Dynamics of the University of Lausanne (Switzerland), using a
379 Carlo Erba 1108 (Fisons Instruments, Milan, Italy) elemental analyzer connected to a Delta V
380 Plus isotope ratio mass spectrometer via a ConFlo III open split interface (both of Thermo
381 Fisher Scientific, Bremen, Germany) operated under continuous helium flow (68, 69). The
382 kerogen, the fraction of organic matter insoluble in organic solvents, non-oxidizing acids and
383 bases, dispersed in lithified sediments, was isolated through several steps. These steps are based
384 on the dichloromethane-extraction procedure followed by HF–HCl treatment at the Institute of
385 Earth Surface Dynamics, University of Lausanne. The procedure modified from Durand and
386 Nicaise (70) involved Soxhlet extraction with a mixture of methanol and dichloromethane for
387 removal of the soluble organic fraction (bitumen), removal of carbonates (as well as sulfides,
388 sulfates, oxides and hydroxides) by treatment with 6N HCl, removal of silicates by treatment
389 with a mixture of 40% HF and 6N HCl. The acid treatments were done at 65–70°C while stirring
390 with a PTFE coated magnetic stirrer. The solid residue was thoroughly washed with warm
391 deionized water and water purified with a Direct-Q UV3 Millipore® System, and then dried at
392 40°C. Mineralogical analysis of the dried solid residues was conducted at the Institute of Earth

393 Sciences of the University of Lausanne using a Thermo Scientific ARL X-TRA Diffractometer,
394 and confirmed the complete elimination of silicates (mainly clay minerals), which constituted
395 up to 35% of the bulk rock samples before the HCl-HF treatment.

396 The stable isotope compositions are reported in the delta (δ) notation defined as

$$397 \quad \delta^i E_{sample/standard} = \frac{R(^i E / ^j E)_{sample}}{R(^i E / ^j E)_{standard}} - 1$$

398 where R is the molar ratio of the heavy ($^i E$) to light ($^j E$) isotope of chemical element E (*e.g.*,
399 $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$). The standard for carbon isotope ratios ($\delta^{13}\text{C}$) is Vienna Pee Dee
400 Belemnite limestone (VPDB), for nitrogen isotope ratios ($\delta^{15}\text{N}$) is molecular nitrogen in air
401 (Air- N_2). For calibration and normalization of the measured isotopic ratios to the
402 international scales, a 3- or 4-point calibration was used with international reference materials
403 (RMs) and UNIL (University of Lausanne) in-house standards. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of
404 the in-house standards were normalized with the RMs USGS64, USGS65 and USGS66. The
405 RMs used together with the UNIL-standards for normalization and quality control of the
406 measured $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were USGS-24, USGS-40, USGS-41 and IAEA-600.
407 Average $\delta^{13}\text{C}$ value obtained for USGS-40 was $-26.4 \pm 0.1\%$ ($n = 6$), which is in good
408 agreement with accepted value of -26.39% (71). The accuracy of the analyses was checked
409 periodically through the analysis of international reference materials. For $\delta^{15}\text{N}$, we obtained –
410 $4.5 \pm 0.2\%$ ($n = 6$) for USGS-40 and $+1.0 \pm 0.2\%$ for IAEA-600, also in good agreement
411 with accepted values of -4.52% and $+1.0\%$, respectively (71). The carbon and nitrogen
412 concentrations (TOC and TN) were determined from the peak areas of the major isotopes
413 using the calibrations for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. The repeatability was better than 0.2 wt.% for
414 carbon and nitrogen contents.

415 Additional nitrogen isotope analyses of bulk rock samples were carried out at the University
416 of St. Andrews with an EA Isolink coupled via a Conflo IV to a MAT 253 IRMS (Thermo
417 Fisher Scientific, Bremen, Germany). Untreated rock powders were weighed into tin capsules
418 and combusted in a helium stream at 1020°C with a 5-sec pulse of O_2 gas (with a flow rate of
419 250 ml/min). Chromium oxide granules were used as an additional combustion aid in the
420 reactor. SO_2 was trapped with silvered cobaltic cobaltous oxide in the reactor, while CO_2 and
421 H_2O were trapped at room temperature with carbosorb and magnesium perchlorate,
422 respectively. Nitrogen oxides were reduced to N_2 with a Cu column at 650°C , which also
423 trapped excess of O_2 gas. N_2 was further purified with a GC column at 50°C . A blank was run

424 after each sample, and a set of standards was included before and after every set of five samples.
425 USGS40 and USGS41 were used for isotopic calibration while SGR-1 was used as a quality
426 control standard. We obtained a value of $+17.4 \pm 0.5\%$ ($n = 8$) for SGR-1, which is in good
427 agreement with previous measurements ($+17.4 \pm 0.4\%$, ref. 72).

428

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442

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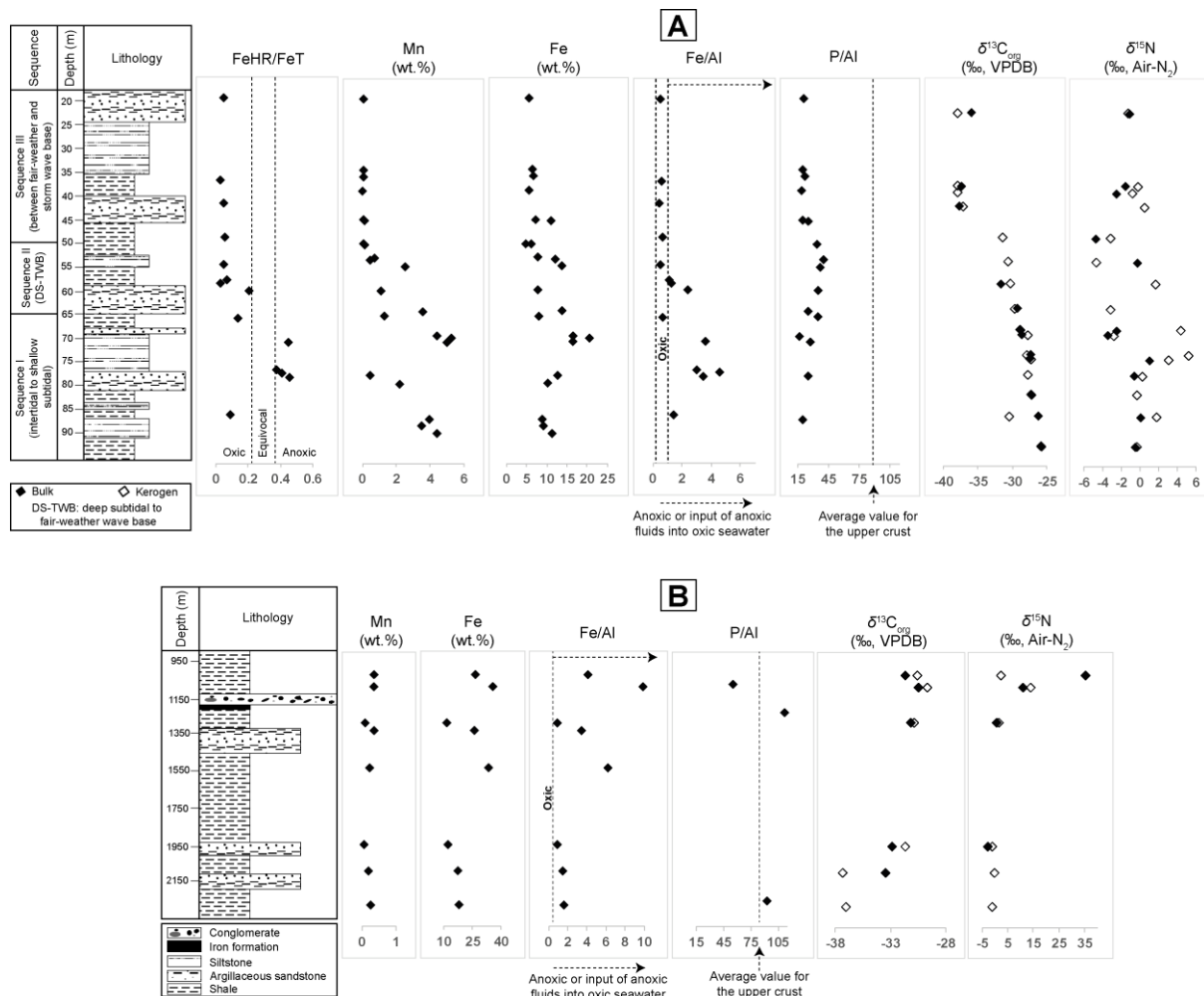
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622 **Figures and Legends**

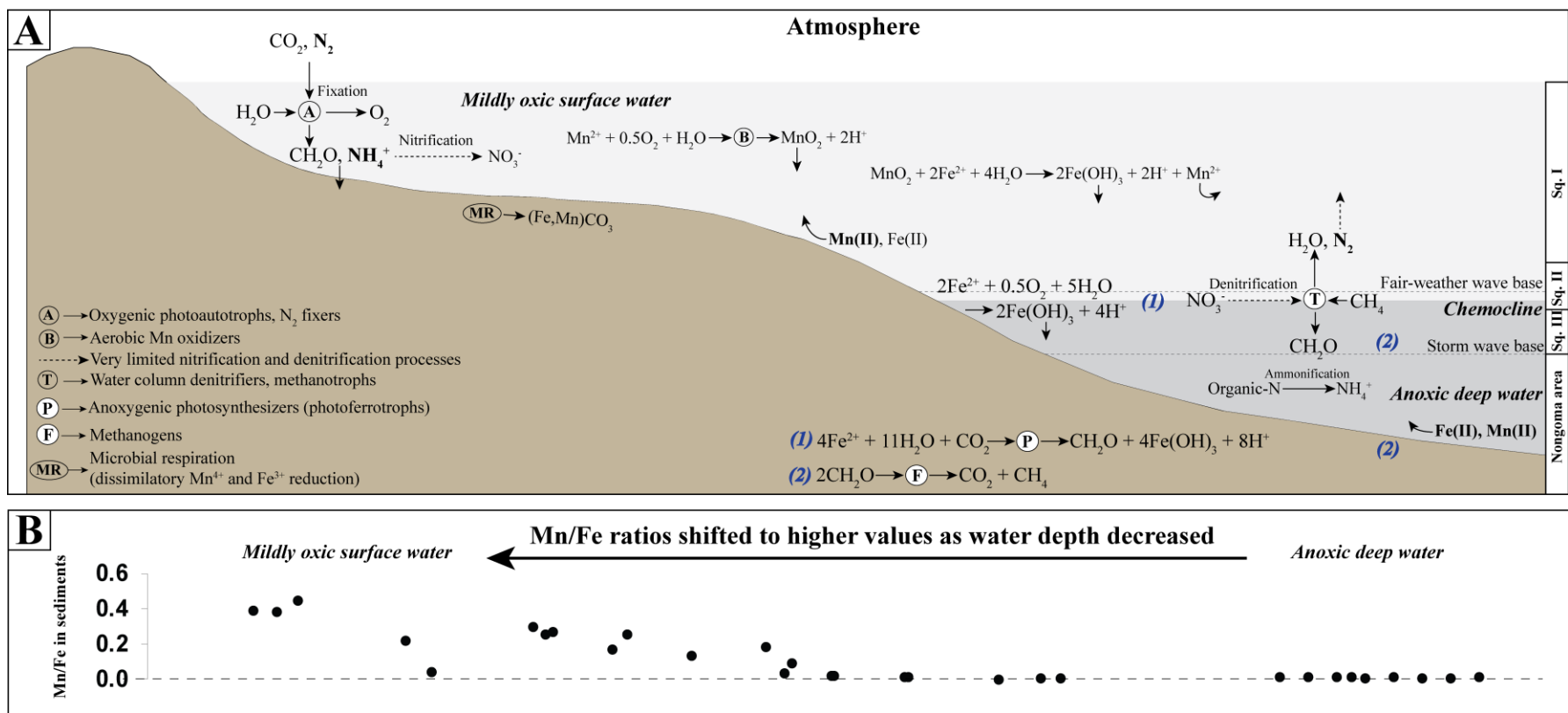


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625 **Fig. 1.** Geochemical data for shale samples plotted along the lithostratigraphic columns of the
 626 studied sections of the Mozaan Group (**A**) from the shallow part of the Ntombe Formation in
 627 the White Mfolozi Inlier (Pongola basin), and (**B**) its deeper-water equivalent in the
 628 Nongoma area (see Fig. S2 for details). Sequences are defined based on water depth
 629 indicators and chemostratigraphic data. Vertical lines and horizontal arrows on Fe/Al plots
 630 are based on the description provided in analytical methods (ref. 67), whereas the average
 631 value for the upper crust on P/Al plots is from ref. 65.

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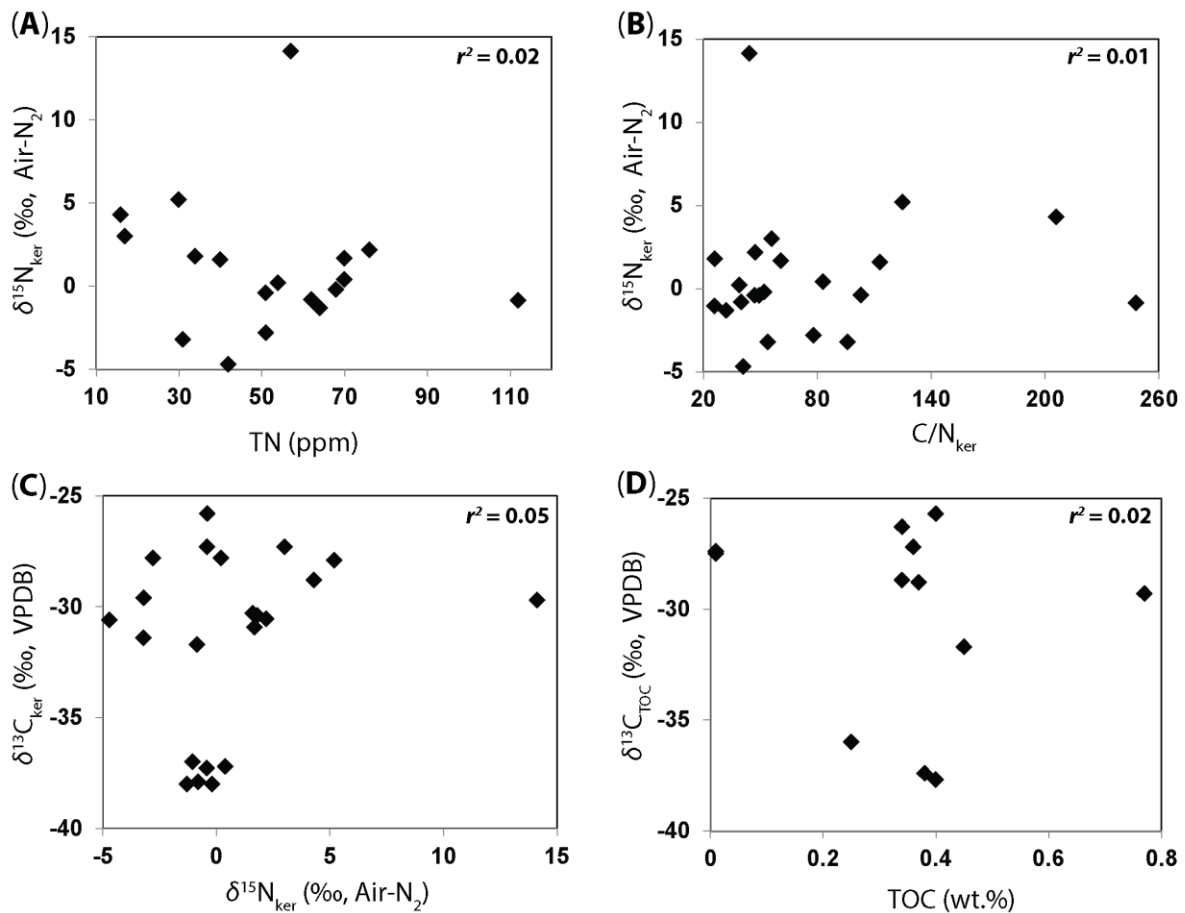
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Fig. 2. Proposed paleoenvironmental reconstruction of the Mesoarchean Pongola basin during deposition of the Ntombe Formation, Mozaan Group (modified from ref. 7). **(A)** Water column chemistry and biogeochemical cycles developed in the localized oxygenated surface waters (recorded by the sequences I and II), overlying anoxic deep-waters (recorded by the sequence III and the sedimentary succession in the Nongoma area). Low biological O_2 production in shallow-marine environments likely limited expression of nitrification and denitrification signals in sediments deposited in the Pongola basin **(B)** Mn/Fe ratios in sediments reflective of seawater redox increase towards the shoreline as ferruginous waters upwelled from anoxic, deep settings to mildly oxygenated, shallow-marine environments. Sq. I (sequence I); Sq. II (sequence II); Sq. III (sequence III).



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643 **Fig. 3.** Cross-plots showing relationships among C and N elemental and isotopic data, with
 644 no obvious co-variation among these parameters suggesting a minimal impact of post-
 645 depositional processes on the original C and N isotopic composition of the marine biomass.
 646 (A) TN vs. $\delta^{15}\text{N}$ of kerogen ($\delta^{15}\text{N}_{\text{ker}}$); (B) Atomic C/N ratios in kerogen ($\text{C}/\text{N}_{\text{ker}}$) vs. $\delta^{15}\text{N}_{\text{ker}}$;
 647 (C) $\delta^{15}\text{N}_{\text{ker}}$ vs. $\delta^{13}\text{C}_{\text{org}}$ of kerogen; (D) TOC vs. $\delta^{13}\text{C}_{\text{org}}$ of bulk sediment.