

## **NO<sub>3</sub><sup>-</sup> reduction is Fe-dependent in a ferruginous chemocline**

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In oxygen poor regions of the modern ocean, fixed nitrogen is lost as N<sub>2</sub> through heterotrophic and sulfide dependent denitrification and anammox. The low oxygen conditions of the Precambrian, then, may have exacerbated fixed N-loss, possibly limiting global biological production [1]. Unlike the modern oceans, where denitrification is linked to the sulphur cycle, Precambrian seas were generally ferruginous (iron-rich and anoxic), and the S-cycle was likely restricted in magnitude compared to today—the Fe cycle would have dominated. Though Fe-dependent N cycling has been demonstrated in laboratory cultures [2], its ecological significance remained untested in modern ferruginous basins. N cycling in Kabuno Bay, a ferruginous freshwater basin situated in East Africa, is characterized by high rates of denitrification and dissimilatory nitrate reduction to ammonium (DNRA), whereas anammox, if present, operates below our detection limits. DNRA indeed constitutes up to 33 % of the total NO<sub>3</sub><sup>-</sup> reduction, indicating extensive fixed N recycling. The addition of Fe(II) enhances rates of both denitrification and DNRA, implicating Fe(II) as an electron donor in both pathways. Together, denitrification and DNRA limit the accumulation of NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> in Kabuno Bay to sub-micromolar concentrations. Our results suggest that ferruginous chemoclines support intensive NO<sub>3</sub><sup>-</sup> reduction, but that much of this reduction leads to N retention as NH<sub>4</sub><sup>+</sup> rather than loss as N<sub>2</sub>. This implies that Fe-dependent N-recycling may have played a role in regulating the nutrient status of the Precambrian oceans.

[1] Canfield *et al* (2010) *Science* **330**, 192-196 [2] Weber *et al* (2006) *Nat Rev Microbiol* **4**, 752-764