



## **Redox buffering by natural organic matter in microbial reduction and O<sub>2</sub> re-oxidation cycles: Assessment of reversibility and sustainability, and implications for carbon cycling in temporarily anoxic environments**

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Natural organic matter contains redox-active functional moieties, including quinone/hydroquinone groups, that play an important role as redox mediators and buffers in many biogeochemical and pollutant electron transfer reactions. In temporarily anoxic environments, such as paddy and wetland soils, NOM may serve as terminal electron acceptor in anaerobic microbial respiration. Reduction of NOM may competitively suppress electron transfer to inorganic electron acceptors, including to CO<sub>2</sub> in hydrogenotrophic methanogenesis. Upon re-aeration, reduced NOM moieties may become re-oxidized by reacting with O<sub>2</sub>. Previous studies independently demonstrated microbial NOM reduction and partial to complete O<sub>2</sub> re-oxidation of (electro-)chemically reduced NOM. Changes in NOM redox state over successive microbial reduction and O<sub>2</sub> oxidation cycles have, however, not been explicitly investigated, despite the importance of such NOM redox cycling in temporarily anoxic systems. The objectives of this work were to assess (i) electron transfer reversibility to/from NOM over microbial reduction and O<sub>2</sub> re-oxidation cycles, (ii) the sustainability of electron transfer over repeated redox cycles, and (iii) to elucidate the thermodynamics of microbial NOM reduction. To this end, we quantified the changes in redox states of four humic acids (HAs) over successive cycles of reduction by NOM respiring bacteria, the facultative anaerobe *Shewanella oneidensis* MR-1, and subsequent re-oxidation by O<sub>2</sub>. The HAs were chosen as models for NOM and were isolated from terrestrial and aquatic sources. Changes in the redox states of HAs were quantified by analytical electrochemistry, which included the electron accepting and donating capacities of HA (EAC and EDC; i.e. the number of electrons accepted and donated by a given mass of HA) and the redox potentials, Eh, of HAs. Anoxic incubations of HAs with *S. oneidensis* and lactate as electron donor resulted in extensive microbial HA reduction, as evidenced from decreasing EACs and increasing EDC. Aeration of reduced HAs resulted in HA oxidation by O<sub>2</sub>, as shown by increasing EACs and decreasing EDCs. Comparing EAC and EDC values during redox cycling revealed largely reversible electron transfer to and from HA in a single redox cycle and fully sustainable HA redox buffering over consecutive redox cycles. Thermodynamics controlled the extent of HA reduction, as suggested by the finding that all four HAs were reduced to comparable redox potentials Eh between -0.18V and -0.2V. This Eh range was narrow compared to the redox potentials over which the HAs accepted electrons, including denitrifying, iron reducing, sulfur reducing and methanogenic conditions. Reversible and sustainable redox buffering by HA over wide Eh ranges has important implications for element cycling in temporarily anoxic environments. We estimate that the electron transfer to NOM under anoxic conditions and subsequently to O<sub>2</sub> under oxic conditions may lead to a suppression of methane fluxes of 190'000 mol CH<sub>4</sub> per km<sup>2</sup> a<sup>-1</sup>, which corresponds to between 10 and 166% of reported average CH<sub>4</sub> fluxes from northern peatlands.