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Thermodynamic modeling of microbial biomass in the subsurface and the impact of mixing zones

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The continental subsurface represents a significant yet largely unexplored reservoir of microbial biomass. To date, limited research has yielded reliable quantitative estimates, with the majority relying on the extrapolation of local cell abundance measurements [1]. In fractured subsurface environments, another source of uncertainty arises from the presence of microbial hotspots at fracture intersections where fluids of contrasting compositions mix [2]. However, the importance of these microbial hotspots is unknow, potentially introducing bias in estimates of deep microbial biomass.

The energy requirements of subsurface microorganisms are derived from chemical imbalances occurring at depth. It is hypothesized that element fluxes (e.g. dissolved O_2) limit the energy availability and thereby influence life in the deep subsurface. Here, we propose an innovative thermodynamic-based modelling approach to estimate the amount of energy in the subsurface, that can be used by microorganisms for growth or maintenance. We focus on iron-oxidizing bacteria, as they represent the most prevalent autotrophic bacteria found in the continental subsurface. Assuming $O_{2(aq)}$ as the limiting reactant, we estimate the microbial biomass that can be maintained by $O_{2(aq)}$ fluxes in groundwater. The modeling approach was first implemented by considering the recharge of oxic water along a flow path, and then by considering the mixing between oxic and anoxic fractures. This approach allows to compare how much energy is sustained by these two processes, both of which create habitable zones for microorganisms at depth. The estimates of potential biomass are then compared with field measurements of bacterial biomass taken at the Ploemeur-Guidel Critical Zone Observatory (SNO H+, OZCAR, Brittany, France). We simulate an energy front at depth that is consistent with the biomass estimates. Furthermore, we demonstrate that mixing zones increase significantly the bioavailable energy in the subsurface, but only under specific mixing conditions. With this study, we introduce a novel methodology to estimate microbial biomass in the subsurface, grounded in elemental fluxes and thermodynamic principles.

[1] Weighing the deep continental biosphere, McMahon & Parnell (2014), *FEMS* 87, 113-120

[2] Iron-oxidizer hotspots formed by intermittent oxic-anoxic fluid mixing in fractured rocks, Bochet et al. (2020), *Nature Geoscience* 13, 149-155