



## **Quantifying microbial metabolic activity by the Resazurin/Resorufin smart tracer system from plot to catchment scales**

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Biogeochemical cycling, and in particular the turnover of nutrients in aquatic and terrestrial ecosystems is directly controlled by microbial metabolic activity (MMA). MMA, however, varies strongly in space and time, depending on physical (temperature) and biogeochemical (oxygen content, access to bioavailable organic carbon) controls. Hence, monitoring the spatial and temporal dynamics in complex and dynamic environments such as aquatic-terrestrial interfaces represents an experimental challenge.

We present results of the application of the Resazurin/Resorufin (Raz/Rru) smart tracer system using in-situ fluorometry for the detection of tracer breakthrough curves in lab-based incubation experiments, artificial river (flume) and in-situ field experiments to quantify the spatiotemporal dynamics of microbial metabolic activity at aquatic-terrestrial interfaces. Our results indicate a strong impact of substrate temperature on MMA rates, exceeding the impacts of organic matter availability as microbial food source. Manipulation experiments exposing streambed sediments to variable densities of Chironomids reveal that the bioturbating invertebrates can significantly enhance hyporheic sediment respiration. Push-pull tracer injections into streambed porewater highlight the importance of hotspots of microbial and thus, biogeochemical activity in streambed environments and in-stream injections during variable discharge conditions reveal the event-based activation and de-activation of metabolically active storage zones that substantially affect stream metabolism and biogeochemical turnover. Tracer injections into systems exposed to multiple stressor impacts (engineered nanoparticle contamination in conjunction with temperature increase) and potential restoration mechanisms (aquatic macrophyte induced enhancement of transient storage and fine sediment trapping) demonstrate the potential of the Raz/Rru system to quantify the ecohydrological and biogeochemical response of aquatic ecosystems to environmental change.

Overall, the Raz/Rru applications across this wide range of ecosystems, spatial and temporal scales reveal both capabilities as well as limitations in the application of the smart tracer system for in-situ quantification of microbial metabolic activity, which are discussed alongside technological innovations in in-situ fluorometry and data interpretation.