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Stable isotope probing of hypoxic toluene degradation at the Siklós aquifer reveals prominent role of *Rhodocyclaceae*

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Abstract:

The availability of oxygen is often a limiting factor for the degradation of aromatic hydrocarbons in subsurface environments. However, while both aerobic and anaerobic degraders have been intensively studied, degradation betwixt, under micro- or hypoxic conditions has rarely been addressed. It is speculated that in environments with limited, but sustained oxygen supply, such as in the vicinity of groundwater monitoring wells, hypoxic degradation may take place. A large diversity of subfamily I.2.C extradiol dioxygenase genes has been previously detected in a BTEX-contaminated aquifer in Hungary. Older literature suggests that such catabolic potentials could be associated to hypoxic degradation. Bacterial communities dominated by members of the *Rhodocyclaceae* were found, but the majority of the detected C230 genotypes could not be affiliated to any known bacterial degrader lineages. To address this, a stable isotope probing (SIP) incubation of site sediments with $^{13}C_7$-toluene was performed under microoxic conditions. A combination of 16S rRNA gene amplicon sequencing and T-RFLP fingerprinting of C230 genes from SIP gradient fractions revealed the central role of degraders within the *Rhodocyclaceae* in hypoxic toluene degradation. The main assimilators of ^{13}C were identified as members of the genera *Quatrionicoccus* and *Zoogloea*, and a yet uncultured group of the *Rhodocyclaceae*.

Keywords: biodegradation, oxygen limitation, DNA-stable isotope probing, subfamily I.2.C extradiol dioxygenase (C230), groundwater, *Rhodocyclaceae*