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Modified Isotope Pairing Technique (MIPT) to study nitrogen transformations in polluted aquatic systems: theory

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In aquatic systems that suffer from anthropogenic pollution (e.g., treated or untreated sewage, agricultural return flows, aquaculture discharge, etc.), sediment denitrification has the capability to remove surplus nitrate (NO_3^-) by converting it into gaseous N species (mainly dinitrogen (N_2) and nitrous oxide (N_2O)) and consequently assisting in a recovery of the water body. Recent studies provide evidence that in heavily polluted water bodies the production of N_2O (which is a greenhouse gas) may be significant. Another major process responsible for N_2O formation is nitrification.

The main sources of NO_3^- deemed to be responsible for denitrification are: (i) NO_3^- diffusing directly from the overlying water, (ii) NO_3^- being produced by nitrification in the oxic layer, and (iii) NO_3^- entering the water body through the hyporheic zone (e.g., NO_3^--polluted groundwater). The latter source of NO_3^- may predominate over the other sources when NO_3^- levels in the groundwater are high enough. For instance, the findings of a joint Israeli-Jordanian-Palestinian study of the salinity sources along the Lower Jordan River (LJR) implied that a significant mass of NO_3^- is being added to the LJR through such groundwater influx. These findings, however, were only qualitative. The best quantitative insight into the sources and transformations of nitrogenous pollutants may be obtained using ^{15}N as tracer, with the isotope pairing technique (IPT) being the leading method. The traditional method is to add ^{15}N-labeled nitrate to the water column and to subsequently monitor the formation of ^{15}N-labeled N_2 molecules. The main shortcomings of the IPT are that the formation of N_2O is ignored (resulting in an underestimation of denitrification) and it does not account for a possible NO_3^- influx through the hyporheic zone. Our objectives were to overcome

these shortcomings.

We have further developed and analyzed the theoretical basis of the original IPT to specifically adapt it to systems with high nitrogenous and organic loads. The two important factors that we took into account are the isotopic composition of N_2O and the input of an external source of nitrate. We also examined the option of adding ^{15}N-labeled ammonium to the water column to specifically adjust the technique to field studies.

The presented modified technique (MIPT) allows to: (i) improve the estimation of sediment denitrification capacity, (ii) gain an insight into the N_2O formation mechanism(s) and fluxes, and (iii) assess a possible input of nitrate-polluted water through the hyporheic zone. These modifications may be utilized both in laboratory- and in field-scale studies.

Preliminary experiments show that the N_2O emission rates from the LJR sediments are comparable to the rates from a Mediterranean soil irrigated with secondary effluent. The formation of N_2O is an essential prerequisite for a successful implementation of the MIPT, which will eventually enable *in situ* assessment of NO_3^--polluted groundwater inputs.