

Nitrogen Transformation in Groundwater: N₂O Isotopocules and Nitrate Isotope Studies for Evaluating Natural Fertilizer Efficiency

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In our study site, yeast factory wastewater was used as a sustainable alternative to synthetic fertilizers. However, nitrate levels in groundwater exceeded 80 mg/L, raising concerns. Laboratory incubation studies of these groundwater were conducted to explore natural optimal conditions for denitrification. With a slight addition of ¹⁵N-NO₃⁻ tracer and glucose to boost microbial activity, samples were incubated at 16°C for 3 weeks/90 rpm. Headspace samples were measured for N₂O isotope and mineral nitrogen levels and analyzed through bacterial denitrification by *Pseudomonas aureofaciens* and *Stenotrophomonas nitritireducens*.

Gas sample analysis under anoxic conditions shows a strong positive correlation between N₂O-δ¹⁵N, i.e., higher δ¹⁵N with active denitrification and conversion of ¹⁵N-NO₃⁻ into N₂O. Post-glucose, correlations for N₂O-δ¹⁵N, SP-δ¹⁵N and SP-δ¹⁸O weaken, suggesting diverse microbial processes influencing isotopic signatures. Increased N₂O and strong SP-δ¹⁵N correlation indicates enhanced denitrification and microbial activity stimulated by glucose.

N₂O isotope data was analysed using FRAME (isotope FRActionation and Mixing Evaluation) model [1]. Initially, bacterial (bD) & fungal denitrification (fD) were low, with bD up to 30% and nitrification up to 71%. After 1 day, intensive NO₃⁻ consumption mostly due to bD surged to 73%, with increase in fD. N₂O residual fraction varied from 10% to 26%, reflecting effective N₂O reduction. All piezometers display similar trends in N₂O pathway contributions, transitioning from nitrification to denitrification.

DNA extraction and qPCR for microbial analysis [2] showed significant post-incubation increases in denitrification genes (*nirK*, *nirS*, *nosZI*) and nitrogen fixation (*nifH*). Notable rises were observed in *nirS* and *nosZI*, with moderate increases in *nirK* and *nosZI*, which is in agreement with isotope results, confirming enhanced microbial activity in nitrogen transformation pathways.

References:

- [1] Lewicki et.al., FRAME—Monte Carlo model for evaluation of the stable isotope mixing and fractionation. PLoS One. 2022;17(11):e0277204.
- [2] Deb et.al., Microbial nitrogen transformations tracked by natural abundance isotope studies and microbiological methods: A review. Science of the Total Environment. 2024:172073.