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Coupled Sulfur and Nitrogen Cycling at a Catchment Scale: Isotopic and Molecular Insights

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The biogeochemical cycles of nitrogen (N) and sulfur (S) underpin the ecosystem functionality on Earth. However, their potential coupling process and underlying mechanisms in the nature remain unclear. Through joint applications of river water's isotopic compositions, isotope-pairing experiments, and molecular techniques, this study revealed the coupled N-S cycling processes at a catchment scale from both geochemical and biological perspectives. The river water's natural abundance isotopic compositions indicated that sulfide oxidation was an important source (67.0 ± 5.5 % in summer and 72.0 ± 5.5 % in winter) of riverine sulfate. In addition, sulfide oxidation and NOx reduction (especially denitrification) could be tightly coupled in summer but less significantly so in winter. However, the coupling of sulfide oxidation and dissimilatory nitrate reduction to ammonium (DNRA) could not be overlooked in winter. The ^{15}N pairing experiments quantitatively showed that the high sulfide oxidation rates in summer were significantly associated with the denitrification (Fig. 1). Metagenomic sequencing, annotated using NcycDB and ScycDB, identified that microbes involved in N and S cycling predominantly belonged to the Proteobacteria, Actinobacteria, Bacteroidetes, and Chloroflexi phyla. Co-occurrence networks show there were 149 and 53 genera involved in NOx reduction and S oxidation in summer and winter, respectively (Fig. 2). Metabolic pathway analysis revealed 103 species with genes for both dissimilatory NOx reductases and sulfur oxidase or dehydrogenase, indicating year-round activity. Notably, known nitrate reducing-sulfide oxidizing bacteria such as *Thiobacillus denitrificans*, *Thiobacillus thioparus*, *Thiobacillus delicatus*, and *Rhodococcus erythropolis* were identified (Fig. 2). Strong Spearman correlations ($p \leq 0.05$) between *narGH*, *nirK*, *nirBD*, and *sqr*, *soxYZ*, *TST*, *SUOX* illustrated the coupling between NOx reduction and S^{2-} oxidation, particularly denitrification, within the catchment. The higher gene abundance and stronger correlations also highlighted marked seasonal shifts of this coupling. This study provides compelling evidence that S oxidation and NOx reduction are intricately connected at the catchment scale, enhancing our understanding of N-S cycling and its seasonal dynamics. This insight adds a new dimension to our understanding of biogeochemical interactions in natural ecosystems.

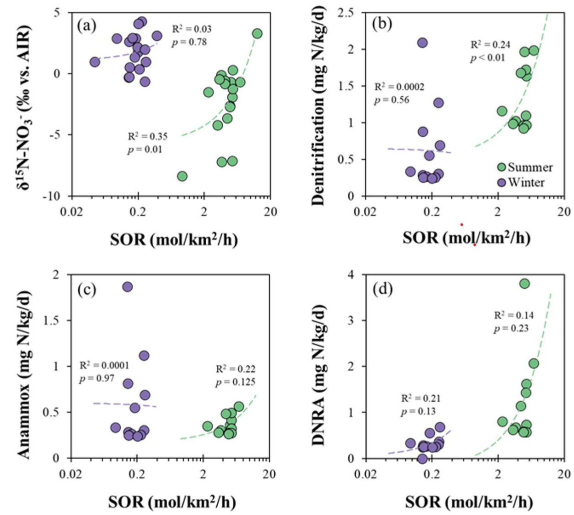

Figure 1. The relationships between the sulfide oxidation rate (SOR) and (a) $\delta^{15}N\text{-}NO_3^-$, (b) denitrification rates, (c) anammox rates, and (d) DNRA rates in the two seasons.

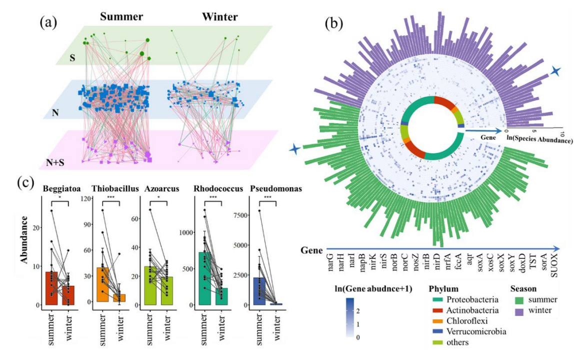

Figure 2. (a) Microbial co-occurrence network at the genus level; (b) Species containing genes for both dissimilatory NO_x reduction and S oxidation. (c) Abundances of key genera of NO_3^- reducing-sulfide oxidizing bacteria. *, $p < 0.05$; ***, $p < 0.001$.