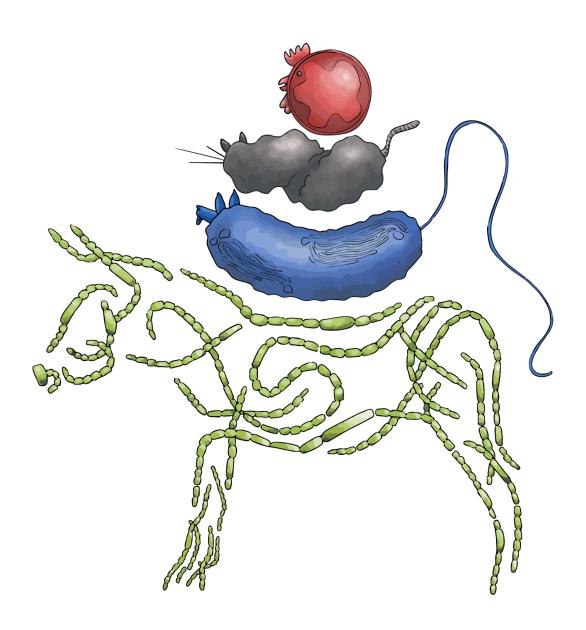
ICON9

JUNE 22-26 2025 BREMEN, GERMANY



ABSTRACT BOOKLET

Dissecting the Physiological Effect of Nitrification Inhibitors in *Nitrososphaera* viennensis via Time-Resolved Omics Approaches

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Nitrification, a key process in the global nitrogen cycle, contributes to substantial nitrogen losses and environmental pollution in high-yield agricultural systems. To mitigate these effects and enhance nitrogen use efficiency, nitrification inhibitors (NIs) are employed to suppress the activity of ammonia-oxidizing microorganisms (AOM). However, the biochemical modes of action of widespread synthetic (SNIs) and emerging biological (BNIs) nitrification inhibitors remain poorly understood. This study aims to elucidate the physiological responses and molecular mechanisms of selected NIs on ammonia-oxidizing archaea (AOA) and bacteria (AOB), with a particular focus on Nitrososphaera viennensis. We optimized dual RNAprotein extraction protocols and established time-of-harvest methods tailored to AOA growth dynamics. Time-series experiments revealed rapid transcriptional changes within 0.5 hours and proteomic shifts after 6 hours of NI exposure. We further compared the in vitro effects of DMPP to those of allylthiourea, a well-characterized inhibitor of ammonia oxidation, and observed similar inhibitory outcomes, suggesting possible varying analogous biochemical targets. Through integrated omics approaches, we link gene and protein expression patterns to physiological responses, deepening our understanding of NI mechanisms. As both DMPP and allylthiourea are presumed to be involved in copper chelation, we also interpret our findings in the context of previous copper limitation studies. This work introduces novel tools for evaluating agrochemical impacts on soil microbial communities and nitrogen fluxes, supporting the development of improved N-fertilizers with broader efficacy.

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