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## **ENVIRONMENT**

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## DISRUPTING SOIL NITRIFICATION: AMMONIA-OXIDIZER PHYSIOLOGICAL RESPONSES TO SYNTHETIC AND BIOLOGICAL NITRIFICATION INHIBITORS

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Nitrification is a crucial process within the global nitrogen cycle but also a major contributor to nitrogen loss and environmental degradation in intensively managed agroecosystems. To mitigate these impacts, nitrification inhibitors (NIs) are employed to target ammonia-oxidizing microorganisms (AOM); however, the molecular mechanisms underlying their action remain poorly understood. Here, we investigate the physiological responses of key soil AOM groups, such as ammoniaoxidizing archaea (AOA) and bacteria (AOB), to both synthetic (SNIs) and biological (BNIs) nitrification inhibitors, with a particular focus on the model soil AOA Nitrososphaera viennensis. We developed and optimized dual RNA-protein extraction protocols and established species-specific time-of-harvest methods, based on the species generation time, to capture microbial responses at both transcriptional and translational levels. Time-series experiments revealed rapid gene expression shifts within 0.5 hours and delayed proteomic changes after 6 hours of NI exposure. Following this, we investigated the physiological and molecular responses of N. viennensis to seven NIs, including the SNIs DMPP and ethoxyquin and the BNIs zeanone, sakuranetin, MHPP, and 1,9-decanediol, along with allylthiourea

serving as a positive control. Our findings revealed that NIs triggered distinct physiological and molecular responses in N. viennensis, suggesting variable and potentially overlapping biochemical targets among AOM. By integrating transcriptomic and proteomic data, we provide a clearer understanding of how NIs influence microbial function and nitrifier resilience under chemical stress. This work advances our understanding of microbial nitrogen cycling and informs targeted strategies for managing soil nitrifying communities to enhance retention and nitrogen promote ecosystem sustainability.

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