

Joint International Conference of

mikroBIO kosmos & CEESME

Central and Eastern Europe
Symposium on Microbial Ecology

22-24 September 2025

Concert Hall of Thessaloniki
Greece



ABSTRACT BOOK



Funded by
the European Union



Under the auspices:
ARISTOTLE UNIVERSITY OF THESSALONIKI
FACULTY OF AGRICULTURE, FORESTRY
AND NATURAL ENVIRONMENT

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LET THE INHIBITOR OUT OF THE FLASK: CULTURE–SOIL DISPARITIES IN BNI EFFICACY

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Biological nitrification inhibition (BNI) has emerged as a promising strategy to enhance nitrogen use efficiency and reduce nitrogen losses via leaching and nitrous oxide emissions. While various plant-derived BNIs have shown potential, their efficacy under field-relevant conditions remains poorly characterized. In this study, we assessed the inhibitory activity of six BNI compounds —zeanone, 2-methoxy-1,4-naphthoquinone, sakuranetin, MHPP, 1,9-decanediol, and MBOA — against a mixture of two synthetic nitrification inhibitors (SNIs), ethoxyquin and DMPP. Experiments were conducted using three distinct experimental systems: (1) single-strain cultures of ammonia-oxidizing archaea (AOA) and bacteria (AOB), (2) short-term potential nitrification assays in soil slurries, and (3) soil microcosm incubations using two agricultural soils. Our results indicate that BNI efficacy is strongly influenced by the targeted ammonia oxidizers (AOA vs AOB) and the experimental context. Although robust inhibition was

observed in culture systems, these effects did not consistently carry over to soil-based assays. Soil microcosms showed the lowest levels of inhibition, likely due to rapid compound degradation, as suggested by previous findings. Notably, even the most effective BNIs — MHPP and MBOA — were significantly less potent than the SNI mixture. These findings highlight the critical role of biotic and abiotic soil factors in shaping BNI performance and underscore a potential disconnect between laboratory efficacy and field relevance. Our results, emphasize the need to evaluate BNI strategies under conditions that closely mimic natural soil environments.

Acknowledgments

This work is part of the project ACTIONr that has received funding from the European Union's Horizon 2021-2027 research and innovation programme under grant agreement No 101079299.

