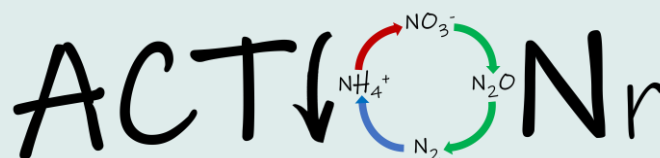


ACTIONr NEWSLETTER



Issue 5/ July 2025

ACTIONr

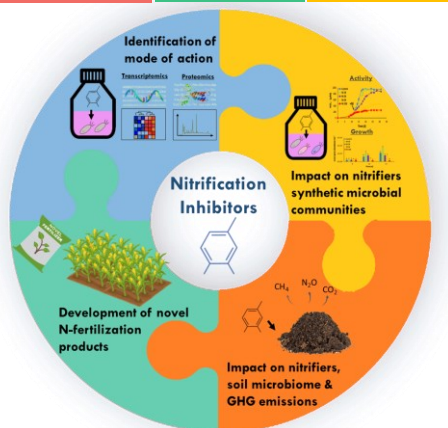
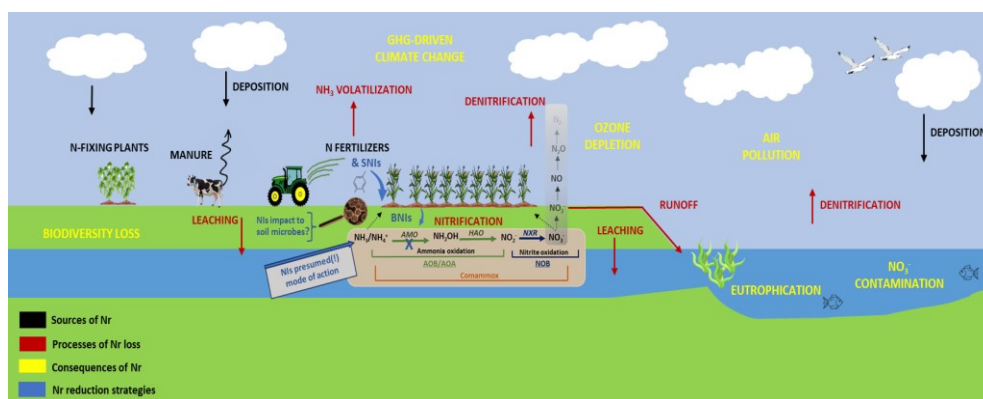
Research Action Network
for Reducing Reactive
Nitrogen Losses from
Agricultural Ecosystems

It is a Horizon 2021-2027
Research and Innovation
program funded by the
European Commission under
Grant Agreement
No 101079299.



ABOUT ACTIONr

More than half of the nitrogen (N) fertilizer used in agriculture is lost as nitrate and N-oxides. A promising solution to increase N use efficiency (NUE) is the mitigation of reactive nitrogen (Nr) loss via nitrification inhibitors (NIs), synthetic and biological. Thessaly (Greece) is a suitable model for relevant research. However, local capacity is not yet fully explored. The EU-funded ACTIONr project will unravel the scientific excellence and innovation potential of the University of Thessaly (UTH) by establishing new tools and pathways to optimize NUE, decelerate the N cycle, and decrease the environmental footprint of Nr. To achieve this, UTH will twin with two internationally leading partners in the Ecogenomics (University of Vienna-UNIVIE) and Microbial Ecology (Université Claude Bernard Lyon 1) of the soil N cycle.



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The ACTIONr project is exploring novel strategies to mitigate reactive nitrogen losses from agricultural ecosystems.

By Dr. Chiara Perruchon

“The Importance of Rotating Nitrification Inhibitors to Preserve Effectiveness”

Nitrogen (N) is a vital nutrient for plant growth that supports crop development, promoting healthy foliage, root systems and higher yields. However, although it is the most abundant element on Earth, most N exists in the atmosphere, with only a small fraction present in the soil. To ensure successful crop production, N applications to agricultural soils are considered essential and have been steadily increasing. For example, global consumption of synthetic N fertilizers rose from 11.8 Teragrams (Tg) in 1961 to 108 Tg in 2017. Accordingly, global N use per unit area of cropland increased from 8.46 kg ha⁻¹ in 1961 to 69.71 kg ha⁻¹ in 2017¹.

Unfortunately, depending on soil and climate conditions, up to 50% of the N applied through fertilization may not be taken up by crops and is instead lost to the environment. This results in economic losses for farmers and contributes to environmental problems such as water pollution, air pollution, and greenhouse gas emissions. Reactive nitrogen from cropland is primarily lost through three pathways: volatilization of ammonia (NH₃), leaching of nitrate (NO₃⁻) beyond the root zone, and denitrification, which converts nitrate into gaseous forms of nitrogen. After application, nitrogen fertilizers typically convert to ammonium (NH₄⁺), a form readily absorbed by plants. However, when applied as urea, an intermediate step in the conversion process produces ammonia, which can easily volatilize, especially in warm, alkaline soils. Soil pH strongly influences the balance between ammonia gas and ammonium in the soil solution, with higher pH favoring ammonia loss to the atmosphere. Ammonium can also undergo nitrification, a microbial process that converts it into nitrate. Although nitrate is also readily taken up by crops, its high mobility in soil—due to its negative charge—makes it prone to leaching. Additionally, nitrates can be transformed by specific soil microorganisms through denitrification into nitrogen gases, including dinitrogen (N₂) and nitrous oxide (N₂O), which escape into the atmosphere. The outcome of nitrogen fertilization depends on many interacting factors, making it difficult to precisely predict how much nitrogen will actually be used by plants versus lost to the environment.

Different technologies have been developed to reduce N losses and increase N use efficiency, with a positive effect both on farmers' pockets and on the environment. Among these is the incorporation of nitrification inhibitors (NIs) into ammonium-based fertilizer, molecules able to retard the nitrification process which is the microbial conversion of ammonium to nitrate, thereby delaying N leaching and emissions associated with nitrate formation. NIs extend the time the active N component of the fertilizer remains in the soil for plant uptake from 6-8 weeks to 8-16 weeks, indirectly improving the yield of horticultural and cereal crops by 2 to 4.5%². The global NI market was valued at 1.625 million USD in 2023 and is expected to reach a market size of 2.627 million USD by the end of 2030³. A growing market, indeed, indicating a widespread worldwide use which, however, essentially relies on only three molecules: dicyandiamide (DCD), 3,4-dimethylpyrazole phosphate (DMPP), and nitrapyrin. For a visual overview of NIs function and associated risks, see Figures 1 and 2.

Glossary

Volatilization:

The process by which ammonia (NH₃) gas escapes from the soil surface into the atmosphere, leading to nitrogen loss from the soil. This usually occurs when ammonium-containing fertilizers (like urea) are applied to the soil surface and are not incorporated into the soil.

Leaching:

The downward movement of water through soil that can carry dissolved nutrients like nitrate beyond the reach of plant roots.

Denitrification:

A microbial process that converts nitrate (NO₃⁻) into gaseous nitrogen (N₂), releasing it into the atmosphere and reducing nitrogen availability in soil.

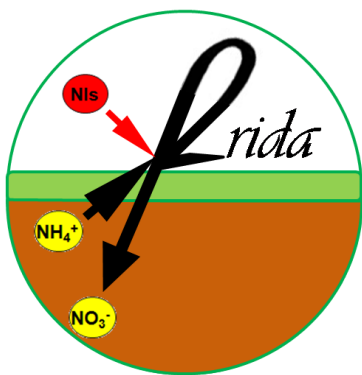
Nitrogen Use Efficiency (NUE):

A measure of how effectively plants use the nitrogen applied to soils; improving NUE means higher crop yields with less environmental loss.

Nitrification Inhibitors (NIs):

Synthetic or biological compounds that delay the conversion of ammonium to nitrate, reducing nitrogen losses and increasing fertilizer efficiency.

The continuous application of the same molecule can, over time, decrease its effectiveness. This applies to pesticides, antibiotics, and, of course, NIs. The decline in efficacy mainly depends on the possibility of biodegradation occurring, but also on the development of resistance in the target microorganisms. Biodegradation is the process by which microorganisms such as bacteria and fungi break down organic pollutants into simpler substances, using them as sources of carbon, nitrogen, or energy. This eliminates the compounds from the environment into which they were introduced, ultimately rendering their application ineffective and leading to a significant impact on agricultural practices and costs. Unlike natural products, which are more readily decomposed, man-made compounds are often resistant to degradation because they are not recognized by naturally occurring enzymes and therefore do not enter common metabolic pathways. However, especially when applied in the same environment over a long period, their presence can select for a microbial fraction that coincidentally possesses the catabolic traits needed to degrade them. Similarly, long-term application of the same molecule can select for resistant microorganisms that are no longer sensitive due to spontaneous modification of the target site.



Within this framework, the project called “FRIDA”, currently underway in the Lab of Environmental Microbiology and Virology at the Department of Environmental Sciences of the University of Thessaly, aims, among other objectives, to shed light on the potential for NI biodegradation. FRIDA stands for “FRom Inhibition to aDaptation: exploring the interplay between nitrification inhibitors and the soil microbiome towards a sustainable agriculture”, and it is a two-year project funded by the Hellenic Foundation

for Research & Innovation (H.F.R.I.) (Project Number: 7840).

As part of a long-term laboratory experiment, different soils were collected from agricultural fields with contrasting NI-use histories - some with prior NI application, and others with no exposure. These soils were treated repeatedly with a representative NI at field-relevant concentrations, over seven application cycles spanning 3.5 years (September 2022 – March 2025). Intermittent nutrient deprivation periods were introduced between treatments to promote microbial adaptation and potential enrichment of degradation traits. It was hypothesized that microbial degradation of NIs would be more likely in soils with a history of NI use, due to potential prior selection of capable microorganisms. Surprisingly, in a general attempt to isolate NI-degrading microorganisms, significant degradation of DMPP was observed, with complete disappearance occurring within 28 to 35 days in liquid cultures inoculated with soil collected from a rice field with no previous history of NI exposure. The soil had been enriched as described above and was then added to a minimal medium containing the NI as the sole carbon source. These results indicate that, under laboratory conditions, even a limited number of repeated treatments can select for degradative microbial populations and highlight the potential for microbial adaptation, emphasizing the need for greater attention to NI persistence and fate in agricultural environments. This is especially relevant given the widespread and repeated use of NIs in many cropping systems, and the limited availability of alternative compounds.

Biodegradation:

The breakdown of substances by microorganisms such as bacteria or fungi into simpler compounds, which may remove synthetic chemicals from the environment.

Catabolic Traits:

The metabolic abilities of microorganisms to break down complex chemical compounds such as nitrification inhibitors into simpler substances for use as energy or nutrient sources. These traits enable microbes to degrade synthetic compounds, potentially affecting the persistence and effectiveness of such chemicals in soil environments.

FRIDA:

A research project funded by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under Project #07840. FRIDA investigates the interactions between nitrification inhibitors (NIs) and soil microbial communities, focusing on the mode of action, potential microbial adaptation or degradation of NIs, and their broader ecological impacts.

<https://frida.env.uth.gr>

Recalcitrant:

A term describing substances (such as certain synthetic chemicals) that are resistant to biodegradation and persist in the environment.

Research is also stepping in to help. In recent years, significant efforts have been made to discover new NIs, particularly biological ones that may have a lower environmental impact. At the same time, other studies—such as those conducted by FRIDA—are focusing on existing NIs, which still have many poorly understood aspects. These include their precise mode of action on target microorganisms and their broader impact on the soil microbial community. The ultimate goal is to make these compounds more effective and more selective.

But what can already be done in practice? Prevention remains the best strategy. Increasing the dose—especially when reduced efficacy is already observed—is strongly discouraged. Doing so risks intensifying selective pressure, potentially worsening the problem while increasing environmental impact. The most effective approach is to periodically alternate N-based fertilizers containing different NIs. While this may seem inconvenient—different fertilizers may require varying doses, application methods, timing, and costs—rotation, should become a standard agricultural practice, as with pesticides. Indeed, it helps maintain long-term effectiveness and prevent resistance. In turn, this strategy supports sustained nitrogen use efficiency, leading to consistently high crop yields, reduced fertilizer costs, and lower environmental nitrogen pollution. It may be a bit challenging, but it's well worth the effort.

Visual Summary: How Nitrification Inhibitors Work and Why It Matters

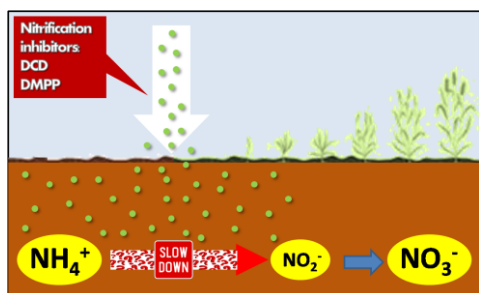


Figure 1. Nitrification inhibitors like DCD and DMPP slow the microbial conversion of ammonium (NH_4^+) to nitrate (NO_3^-), reducing nitrogen losses from soil.

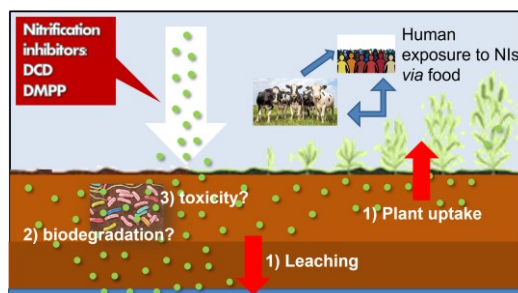


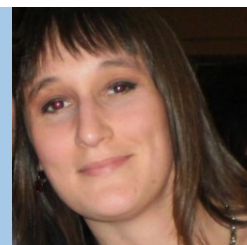
Figure 2. Potential risks from repeated NI use include leaching into groundwater, microbial degradation, toxicity to soil communities, and entry into the food chain.

References

1. [Tufail et al., 2022, Fuel, 324\(C\): 124725.](#)
2. [Klimczyk et al., 2021, Sci Total Environ, 771:145483](#)
3. [Market Research Future, 2024](#)

Mode of Action (of NIs):

The specific biochemical process or molecular target through which nitrification inhibitors (NIs) affect ammonia-oxidizing microorganisms (AOMs) to suppress the conversion of ammonium to nitrate. Despite their long-standing use since the 1950s, the precise mode of action of current synthetic NIs (SNIs), and even more recent biological NIs (BNIs), remains poorly understood. Clarifying this mechanism would help predict their effectiveness, tailor NI combinations targeting different steps of the ammonia oxidation pathway and ultimately improve nitrogen fertilizer efficiency and environmental safety.



Chiara Perruchon is the Principal Investigator of FRIDA and a postdoctoral fellow at the Laboratory of Environmental Microbiology and Virology, Department of Environmental Sciences, University of Thessaly, Greece.



Esteban Emanuel Nieto

has a degree in Biology with a specialization in zoology (B.Sc. + M.Sc. equivalent) from the National University of La Plata, Argentina. He completed his Ph.D. at the Centre for Research and Development in Industrial Fermentations in Argentina. During his PhD thesis, Esteban studied, through a multi-omic approach, the interactions occurring among the members of a consortium during the degradation of polycyclic aromatic hydrocarbons and the interactions between the consortium and the native soil microbial community during bioremediation processes. Currently, his work is focused on the impact of top-down regulation by protists in bacterial population dynamics and functioning.

“I think that including an ecological framework in the study of biotechnological processes is key to improving the application of sustainable solutions. Investigating how predation affects AOM could be crucial for regulating nitrification processes.”

INTERVIEWING RESEARCHERS OF ACTIONr

Esteban, please tell us what your role within the ACTIONr project is, and how it connects to the study of nitrification in soil?

My role at ACTIONr is to study the role of trophic interactions in regulating the density of nitrifying communities, with a particular focus on protist activity. Protists include some of the most important groups controlling bacterial abundance in the soil, playing a regulatory role in the diversity and functionality of this ecosystem. They also play a key role in nutrient cycling, particularly nitrogen, through the microbial loop. Trophic interactions are highly sensitive to environmental disturbances (e.g., the presence of pollutants). The main goal of my work is to investigate the ecological basis of these trophic interactions in relation to nitrification processes, understanding protists as natural regulators of nitrifier density, and to explore how environmental disturbances affect this interaction.

What are some of the main challenges you've encountered in your work so far?

Working with organisms that have very different physiological requirements, establishing systems where both microorganisms can coexist presents a significant challenge. This has required me to optimize cultivation conditions and adjust various parameters, which is a demanding and meticulous task.

What motivated your interest in integrating microbial ecology into environmental research?

Despite the abundance of protists in soil and their recognized role as regulators of bacterial communities, there are still very few studies that include them in the context of understanding bioprocesses. One of my main interests is to integrate concepts from microbial ecology into environmental processes, in order to develop a more holistic understanding of ecosystems and enable the effective application of sustainable technologies.

What are some key highlights from your recent research?

Since joining the lab of Plant and Environmental Biotechnology, I have studied the impact of various pesticides on the growth of axenic protist cultures. At the same time, I began working with *in vitro* systems combining bacteria and protists, which required optimizing culture conditions due to the significant differences in their physiological needs compared to the bacterial cultures we use as models. Preliminary results indicate that sensitivity to pesticides varies depending on the protist group. Furthermore, in mixed cultures of protists and ammonia-oxidizing bacteria, we observed an inhibitory effect on nitrite production, which appears to be density-dependent on the predator population.

What about your upcoming steps in this research project?

I am currently continuing to optimize the culture conditions to enable stable coexistence of both microorganisms in *in vitro* systems. Additionally, I will conduct soil experiments using a defined community of nitrifying bacteria and protists, applying different pesticides to evaluate trophic interactions in a more complex matrix such as soil, and to analyze how various groups of organisms respond differently to pesticide exposure.



ACTIONr Podcast Debut – First episode released, highlighting the role of nitrogen in crop production, the challenges of nitrogen loss, and the importance of smart nitrogen management.



High school students visit University of Thessaly – Welcomed by ACTIONr postdoctoral researcher Elena Papadopoulou, students were introduced to advanced biotechnology tools and the daily life of research.



ACTIONr at Innovent Forum 2025 – On February 14–15 at JOIST Innovation Park (Larissa), ACTIONr highlighted innovative approaches to reactive nitrogen challenges, engaging with stakeholders from academia and industry.

LATEST NEWS OF ACTIONr NETWORK

ACTIONr Podcast Series Launches: Episode 1 Highlights the Role of Nitrogen in Agriculture and the Environment

In January, the ACTIONr Podcast Series launched with its first episode: “Exploring Nitrogen’s Significance and Impact on Crops and the Environment.” This episode discusses the essential role of nitrogen in agricultural systems, the challenges associated with nitrogen losses, and the importance of improving nitrogen use efficiency as a key strategy for addressing environmental and food security concerns. Aimed at a broad, environmentally engaged audience, the episode provides a clear introduction to the complexities of managing reactive nitrogen in agriculture and highlights why this issue is central to the goals of the ACTIONr project

ACTIONr Joins 25th Anniversary of Biochemistry & Biotechnology Department



To celebrate the 25th anniversary of the Department of Biochemistry and Biotechnology at the University of Thessaly, ACTIONr welcomed in February local high school students for a lab visit. Postdoctoral researcher Elena Papadopoulou introduced them to ongoing research in nitrogen management, highlighting key tools and methods in environmental biotechnology and sparking their interest in science.

Showcasing Innovation: ACTIONr at Innovent Forum 2025



In mid-February, ACTIONr participated in the Innovent Forum 2025, a two-day event held at JOIST Innovation Park in Larissa and streamed online. The forum brought together stakeholders from academia, research, and industry. As part of the presence of the University of Thessaly, ACTIONr researchers presented innovative work on reactive nitrogen and sustainable agriculture, fostering new opportunities for cross-sector collaboration.



Advanced Training in Microbial Ecology and Data Analysis at the University of Vienna

In March 2025, PhD student Vivi Amanatidou from the University of Thessaly (UTH) joined seconded ACTIONr PhD candidate Dimitris Dalkidis in Vienna to participate in an intensive two-week training program hosted by the Archaea Biology and Ecogenomics Unit at the University of Vienna. Under the mentorship of Dr. Logan Hodgskiss and within the renowned group led by Prof. Dr. Christa Schleper, the training focused on advanced statistical methods and transcriptomic data analysis in the context of microbial ecology. The program combined theoretical lectures with practical sessions, covering key techniques such as parametric and non-parametric testing, principal component analysis (PCA), and data visualization. The participants were also actively involved in the lab's scientific discussions and meetings, gaining valuable insight into research workflows and collaborative practices. This advanced training significantly strengthened their analytical skills and will directly support ACTIONr's objectives in understanding microbial dynamics and the role of biological nitrification inhibitors in nitrogen cycling. The knowledge gained will also be transferred back to UTH, reinforcing the project's broader research capacity.



Specialized training for UTH PhD students – From March 10–21, 2025, two PhD students from the University of Thessaly attended an intensive program at the University of Vienna focused on microbial ecology, statistical analysis, and transcriptomic data interpretation.



ACTIONr's 2nd Summer School Highlights Global Advances in Biological Nitrification Inhibition

From the heart of Thessaly to the serene slopes of Pelion, the 2nd Summer School of ACTIONr, titled “*Biological Nitrification Inhibition: Integrating Microbial Functions, Plant Traits, and Technological Innovations for Sustainable Nitrogen Cycling,*” brought together leading academics, researchers, early-career scientists, and PhD students for a dynamic week of learning and collaboration. Spanning five immersive days—from 1.5 days in Larissa to 3.5 days at the Paos Monastery in Pelion, Greece—the program offered a unique blend of lectures, hands-on training, and research exchange focused on the latest developments in Biological Nitrification Inhibition (BNI).

The 2nd ACTIONr Summer School (May 12–16, 2025) brought together early-career scientists in Larissa and Pelion to explore Biological Nitrification Inhibition (BNI) through expert lectures, hands-on training, and collaborative sessions on sustainable nitrogen solutions.





The summer school opened in Larissa with an inspiring keynote lecture by Prof. Christa Schleper (University of Vienna) on “What is Wrong with the Nitrogen Cycle – and How Can We Fix It?” This was followed by a lecture from Assist. Prof. Evangelia Papadopoulou (UTH) on “Current Methodologies and Advancements in Screening BNI Compounds,” and a hands-on lab training session titled “Fast-track, High-throughput Screening for BNIs in Ammonia-Oxidizing Bacteria,” hosted at the Laboratory of Plant and Environmental Biotechnology, UTH. Following the Larissa sessions, participants traveled to Paos Monastery in Pelion, where the summer school continued in an inspiring setting surrounded by nature. Highlights included:

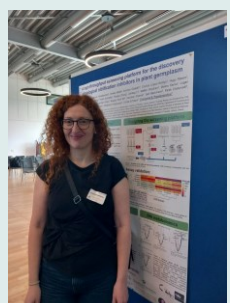
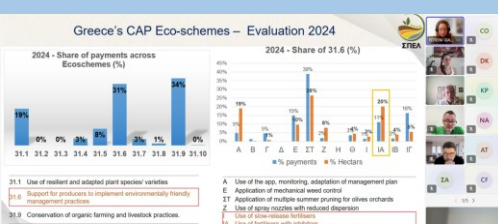
- Dr. Logan Hodgskiss (University of Vienna): “The Biofilm Lifestyle of AOA: Implications for Physiology and Nitrification Inhibition”
- Dr. Christina Hazard (University Claude Bernard Lyon 1): “Soil Virus Ecology and the Potential of ‘Soil Phage Therapy’ for Controlling Nitrification”
- Prof. Kalliope Papadopoulou (UTH): “Specialized Metabolism in Plants: Driving Growth and Adaptation to Nitrogen Availability”
- Dr. Maria Hernandez-Soriano (John Innes Centre, UK): “Wheat Genotype-Driven Recruitment of Rhizosphere Microbiome to Improve Nitrogen Use Efficiency”
- Prof. Cécile Gubry-Rangin (University of Aberdeen): “Plant Growth Stage and Conditions: Influence on BNI Efficiency”
- Assist. Prof. Dr. Sotirios Vasileiadis (UTH): Presentation-workshop on “Key and Frequently Overlooked Points in Amplicon Sequencing Analysis of Environmental Microbiomes”
- Dr. Benjamin Thiombiano (Syngenta, Switzerland): “Harnessing the Nitrogen Cycle: Chemical Ecology Driving Future Innovations in Nitrogen Management”.



This unique international training event offered multidisciplinary perspectives, bridging microbial ecology, plant biology, data analysis, and sustainable nitrogen management. It provided participants with valuable skills and insights, reinforcing ACTIONr’s commitment to advancing early-career research and collaboration in the field of BNI.



On June 20, 2025, the SPEL–ACTIONr online workshop on nitrification inhibitors explored both conventional and biological approaches to nitrogen management, including emerging ideas such as soil phage therapy, fostering dialogue between researchers and the fertilizer industry.



The ACTIONr team joined the ICoN9 conference (June 22–26, 2025) in Bremen to share new research on soil nitrification, BNIs, and plant–microbe interactions.

SPEL–ACTIONr Workshop Showcases Advances in Nitrification Inhibitors

On June 20, 2025, the University of Thessaly (UTH), in collaboration with the Hellenic Fertilizer Producers & Traders Association (SPEL), hosted a highly informative online workshop titled “Classical and Modern Trends in Nitrification Inhibitors.” The event brought together academic experts and fertilizer industry representatives to discuss the evolving landscape of nitrogen management in agriculture. The workshop opened with an overview of the fertilizer sector’s role in nitrogen management by Dr. Fotini Giannakopoulou, General Director of SPEL. Prof. Dimitrios Karpouzas and Assist. Prof. Evangelia Papadopoulou from UTH presented insights into innovative biological approaches for delaying soil nitrification, emphasizing the transition from synthetic to biological inhibitors and their environmental implications. As a guest speaker, Dr. Graeme Nicol (Université Claude Bernard Lyon 1) introduced the emerging concept of “soil phage therapy” as a promising alternative for microbial-based nitrification control. The workshop concluded with a forward-looking discussion on integrated and sustainable nitrogen strategies, reinforcing the importance of collaboration between research and industry.

ACTIONr at ICoN9 Conference (June 22–26, 2025)

In June 2025, the ACTIONr team took part in the 9th International Conference on Nitrification and Related Processes (ICoN9) in Bremen, Germany, presenting their latest research on soil nitrification, biological nitrification inhibitors (BNIs), and plant–microbe interactions in the nitrogen cycle.

The team shared exciting results from across the project, showing how tools like multi-omics, synthetic microbial communities (SynComs), and BNI screening platforms help us better understand how nitrifying microbes respond to nitrification inhibitors—and how we can manage nitrogen more sustainably in agriculture.

Key presentations included:

- Maria Kolovou (UTH) spoke at the Early Career Researchers Workshop and presented a poster on using SynComs to study how pesticides affect ammonia-oxidizing microbes—offering a more realistic approach than single-species lab tests.
- Eleftheria Bachtsevani (University of Lyon) presented new findings on a special nitrite-oxidizing bacterium that helps ammonia-oxidizers thrive in acidic soils.
- Valia Moutzourelis (UTH) talked about how the effectiveness of BNIs changes depending on the testing system—pure cultures, soil slurries, or real soil—highlighting the challenge of applying lab results to the field.



On June 27, 2025, the ACTIONr project successfully organized a dedicated workshop on BNI as a satellite event of the 9th International Conference on Nitrification and Related Processes (ICoN9), hosted at the Max Planck Institute for Marine Microbiology in Bremen, Germany.

Several posters from ACTIONr researchers added even more to the conversation:

- Paraskevi Amanatidou tested the BNI potential of fenugreek under different nitrogen levels.
- Dimitrios Dalkidis explored how *Nitrososphaera viennensis* reacts to BNIs at the molecular level.
- Elena Papadopoulou studied how BNIs break down in different soils, influenced by pH and microbial activity.
- Evangelia Papadopoulou shared a new platform for screening wheat varieties for BNI activity using root exudates.

ACTIONr's strong presence at ICoN9 showed its growing role in advancing research on nitrogen cycling—and sparked new connections with scientists working in soil microbiology, agroecology, and environmental sustainability around the world.

BNI Workshop Hosted by ACTIONr (June 27, 2025) as part of ICoN9

On June 27, a dedicated workshop on Biological Nitrification Inhibition (BNI) hosted by the ACTIONr project at the Max Planck Institute for Marine Microbiology in Bremen. The event held as a satellite event of ICoN9 and provided a focused platform for exploring the role of BNIs in sustainable nitrogen management—from molecular mechanisms to field applications. The workshop began with an introduction to ACTIONr by Prof. Dimitrios Karpouzias (UTH) and continued with keynote talks from international experts, such as:

- Dr. Cecile de Klein (AgResearch, New Zealand) on reducing nitrous oxide emissions in grazing systems through BNIs, and
- Prof. Sakiko Okumoto (Texas A&M, USA) on the BNI potential of sorghum.

Presentations from ACTIONr researchers included:

- Multi-omics insights into nitrifier responses to inhibitors (Logan Hodgskiss, Dimitris Dalkidis)
- SynCom development for agrochemical testing (Maria Kolovou)
- Soil persistence of BNIs (Elena Papadopoulou)
- BNI screening of fenugreek genotypes (Paraskevi Amanatidou)

The day concluded with a roundtable discussion on future directions in BNI research and application.

Special thanks to Dr. Boran Kartal, ICoN9 local organizer, for supporting the workshop and providing access to MPI's excellent facilities.

FORTHCOMING CONFERENCES AND EVENTS

Joint International Conference of MIKROBIOKOSMOS & CEESME 2025



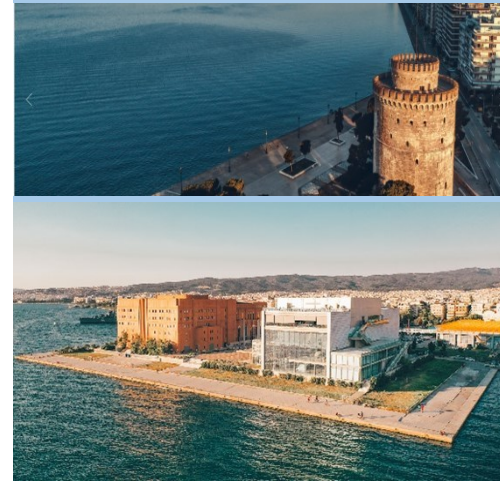
Excited to share that the ACTIONr consortium will participate actively in the upcoming Joint International Conference of Mikrobiokosmos and CEESME, to be held in Thessaloniki, Greece, from 22 to 24 September 2025. The event will gather scientists from across Europe and beyond who are working on a wide range of topics in microbial ecology and environmental microbiology. It's a great opportunity to exchange ideas, explore shared challenges, and strengthen collaborations, especially across countries in Central and Eastern Europe. As

microbial communities play an increasingly important role in tackling issues like climate change, soil health, and sustainable agriculture, the conference offers a timely space for meaningful discussion and knowledge-sharing. The ACTIONr team will contribute through oral and poster presentations and join sessions closely linked to the project's goals. With its focus on early-career support and cross-border collaboration, the event is an ideal setting to share our work, connect with peers, and build new partnerships.

NOVEL RESEARCH IN THE THEMATIC AREA OF ACTIONr

Research supported by the Grantham Foundation and the ACTIONr project has contributed to a new publication in *Plant Biotechnology Journal*, highlighting key advances in wheat root exudate profiling and BNI activity. This collaborative study sheds new light on how natural variation in wheat root chemistry can help reduce the reliance of agriculture on chemical nitrogen fertilizers. The research team, led by partners from Austria, Greece, and India, profiled the root exudate metabolome of 44 diverse wheat genotypes using advanced GC-MS and LC-MS metabolomics. The findings revealed an unexpectedly rich and diverse set of metabolites—over 6,000 features—demonstrating the untapped biochemical potential within existing wheat germplasm. To assess how these compounds influence Biological Nitrification Inhibition (BNI)—a natural process where plants suppress nitrifying microbes—the team developed a new high-throughput screening bioassay, targeting both ammonia-oxidizing bacteria and archaea. A key insight from the study: it's not single compounds, but specific metabolite combinations, that drive effective BNI. Machine learning tools were instrumental in identifying these metabolite signatures. The study was co-led by Dr. Palak Chaturvedi, Prof. Wolfram Weckwerth and Prof. Christa Schleper (University of Vienna), and Prof. Evangelia Papadopoulou (University of Thessaly), in collaboration with an international team of plant scientists and microbiologists.

The Joint conference of the Mikrobiokosmos Society & The Central and East Europe Symposium of Microbial Ecology will be held in Thessaloniki, Greece, from 22 to 24 September 2025.



Website

<https://afea.eventsair.com/mbkceesme2025/>

“Natural variation of the wheat root exudate metabolome and its influence on biological nitrification inhibition activity” – *Plant Biotechnology Journal* by Ghatak, Kanellopoulos, López-Hidalgo, Malits et al.



A joint study by the University of Vienna and UTH revealed major differences in the root exudate profiles of 44 wheat genotypes. Using GC-MS and LC-MS, researchers identified over 6,000 metabolites, with a new high-throughput bioassay showing genotype-specific BNI activity against both ammonia-oxidizing bacteria and archaea. Machine learning pointed to metabolite combinations, rather than single compounds, as the drivers of this activity, paving the way for integrating BNI traits into wheat breeding for more sustainable nitrogen use.

<https://doi.org/10.1111/pbi.70248>

THE ACTIONr CONSORTIUM



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