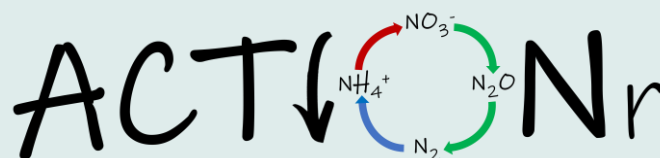


ACTIONr NEWSLETTER

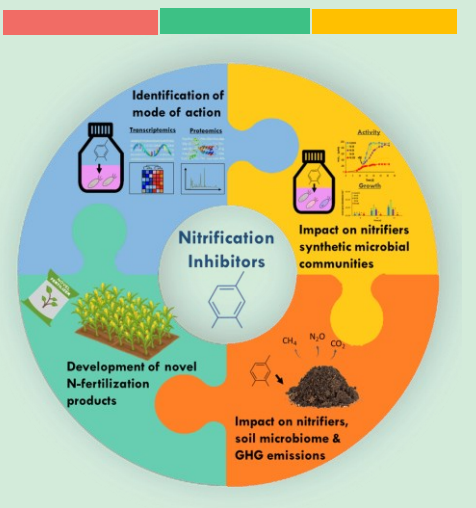


Issue 6/ October 2025

ACTIONr

Research Action Network
for Reducing Reactive
Nitrogen Losses from
Agricultural Ecosystems

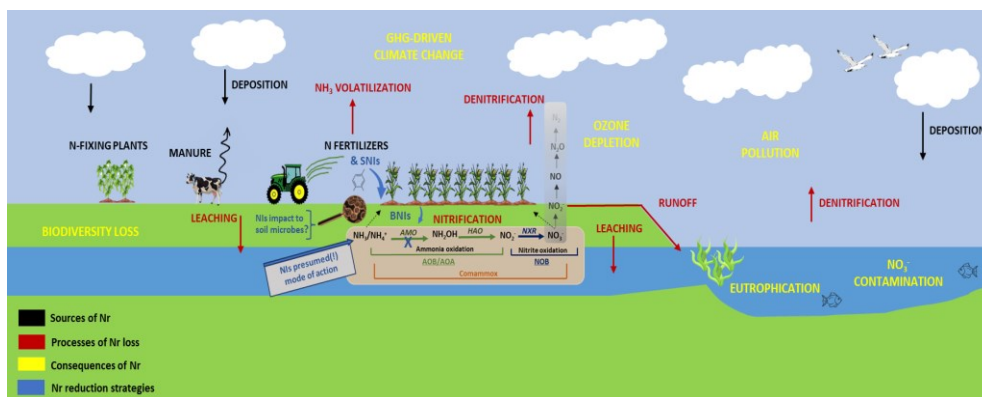
It is a Horizon 2021-2027
Research and Innovation
program funded by the
European Commission under
Grant Agreement
No 101079299.



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

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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“Insights from ACTIONr and Side Projects on Nitrification Inhibitors Mechanisms, Performance and Practical Implications”

The use of nitrification inhibitors (NIs) has long been regarded as a key approach to reducing nitrogen losses from agricultural soils. Among them, synthetic nitrification inhibitors (SNIs) such as DCD, DMPP, and nitrapyrin, have been widely applied in fertilizers for decades, whereas biological nitrification inhibitors (BNIs), naturally produced by plant roots, represent a newer, more environmentally aligned concept.

Despite their different origins, both groups of inhibitors are still associated with key open questions regarding their precise cellular targets, persistence in soils, and ecological side effects. These remain central challenges in understanding how nitrification can be managed sustainably.

Through its research activities and collaborations, ACTIONr has helped shed new light on these issues, offering mechanistic, ecological, and practical insights into how synthetic and biological inhibitors function and interact with the soil nitrogen cycle.

How they work

Through transcriptomic analysis, we have shown that the synthetic inhibitor DCD disturbed the energy metabolism and activity of ammonia-oxidizing microorganisms. The two main groups of ammonia-oxidizers reacted in different ways: ammonia-oxidizing bacteria (AOB) compensated for the energy loss by reorganizing their metabolism and forming aggregates, while ammonia-oxidizing archaea (AOA) adopted an energy-saving, free-living strategy (Perruchon et al., 2025). These contrasting responses may explain why synthetic inhibitors like DCD tend to suppress AOB more strongly, while AOA can recover faster, allowing partial restoration of nitrification activity over time.

In contrast, the second commonly used SNI, DMPP, did not trigger such extensive physiological adjustments. In *Nitrososphaera viennensis* (AOA), exposure caused only limited transcriptional changes, mainly in genes related to energy regulation. These results suggest that in AOA, DMPP acts indirectly, most likely by altering cellular redox state or regulatory networks, rather than by directly inhibiting key enzymes like ammonia monooxygenase. Its more regulatory interaction with microbial metabolism, together with its chemical stability, likely contributes to its reliable performance under field conditions (Dalkidis et al., 2025a).

BNIs, by contrast, revealed an even broader range of effects. Each plant-derived compound—zeanone, MHPP, sakuranetin, or 1,9-decanediol—triggered distinct cellular adjustments, influencing enzymes related to nutrient uptake and stress tolerance. Rather than acting on a single target, BNIs modulate multiple physiological pathways at once, reflecting their natural complexity. They therefore emerge as broad-spectrum regulators of microbial metabolism that continue to shape the balance of nitrifying communities in the soil (Dalkidis et al., 2025b).

How long they last

The persistence of NIs in soil proved to be a critical factor shaping their overall performance. DCD tended to degrade faster in soils with no previous exposure, while in soils with a long history of use, microbial adaptation led to a gradual loss of its inhibitory effectiveness, evidence that microorganisms can adapt to degrade DCD more efficiently over time, reducing its long-term performance. In contrast, DMPP showed slower dissipation and more consistent inhibitory activity across different soil types, underscoring the importance of compound-specific interactions with soil

Glossary

NIs - Nitrification Inhibitors:

Compounds that slow down the microbial conversion of ammonium (NH_4^+) to nitrate (NO_3^-).

SNIs – Synthetic Nitrification Inhibitors:

Man-made inhibitors (e.g. DCD, DMPP, nitrapyrin) used in fertilizers.

BNIs – Biological Nitrification Inhibitors:

Natural compounds produced by plant roots that suppress nitrification.

AOB / AOA – Ammonia-Oxidizing Bacteria Archaea:

Key microbial groups that drive soil nitrification.

AMO – Ammonia Monooxygenase:

Enzyme catalyzing the first step of nitrification.

Microbial catabolism:

Microbial breakdown of organic compounds

Biofilm:

Structured microbial community attached to surfaces, often more resistant to environmental stress.

Persistence defines performance — how long an inhibitor lasts in soil determines how well it works.

chemistry and microbial communities (Amanatidou et al., 2025).

For BNIs, rapid dissipation was identified as a key limitation to their performance. Compounds such as MBOA, MHPP, zeonone, sakuranetin, and 1,9-decanediol were highly effective immediately after application (Moutzourelli et al., 2025), but their activity declined rapidly as they were broken down by soil microbes. Experimental data showed that soil pH, silt or clay content, and microbial activity strongly influence how long these compounds remain active, with acidic soils slowing their degradation. The dominant role of microbial catabolism was confirmed by laboratory tests in sterilized soils, where the compounds persisted substantially longer (Papadopoulou et al., 2025).

These findings show that BNI persistence is governed by a combination of biotic and abiotic factors, including microbial metabolism, pH, and soil texture. Their limited stability underscores the need for improved formulation strategies, such as combining multiple BNIs with complementary properties or using encapsulation and slow-release carriers to extend their activity in the field. Prolonging their effective lifespan is essential to enhance their agronomic impact while reducing application frequency and costs.

Performance, interactions, and wider effects

In practice, the performance of NIs depends not only on their persistence but also on how they interact within the soil ecosystem. Our experiments showed that combining SNIs with complementary properties, each targeting different groups of ammonia oxidizers (AOB versus AOA), resulted in stronger and longer-lasting inhibition compared to single-compound applications (Moutzourelli et al., 2025). These findings highlight the potential of tailored inhibitor mixtures to achieve broader and more balanced control of nitrification under field conditions.

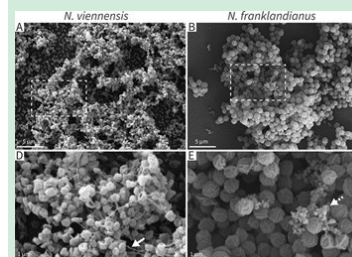
Beyond SNIs, metabolomic profiling of wheat root exudates identified natural combinations of metabolites, such as organic acids, amino acids, and flavonoids, that collectively suppress nitrification (Ghatak et al., 2025). These insights open new possibilities for breeding crop varieties with enhanced biological nitrification inhibition, offering a sustainable pathway toward improved nitrogen-use efficiency and reduced fertilizer losses.

To study these interactions under realistic yet controlled conditions, we developed synthetic microbial communities of nitrifiers that served as reliable and ecologically relevant model systems. These “miniature ecosystems” enabled us to observe how different inhibitors influence microbial activity, cooperation, and resilience within nitrifying networks. Complementary work on AOA biofilms further showed that biofilm formation can substantially alter the physiological responses of archaeal nitrifiers (Dreer et al., 2025), potentially affecting how NIs perform in natural environments.

We also found that both SNIs and BNIs can influence non-target microbial groups, extending their effects beyond nitrifiers. Compounds such as DCD and DMPP affected nitrite oxidizers, denitrifiers, and other functional microbial groups, including those involved in carbon, phosphorus, and sulfur cycling (Amanatidou et al., 2025), while certain BNIs temporarily reduced methane oxidation. Interestingly, the impact on methanotrophs often lasted longer than on nitrifiers, suggesting that NIs can indirectly influence greenhouse gas dynamics in soil. Although these effects were generally short-lived, they highlight the importance of evaluating inhibitors within the broader soil microbial network, not just their immediate targets.

Key ACTIONr and Related Projects Insights:

- SNIs (like DCD and DMPP) differ in how they target nitrifying microbes.
- BNIs act more broadly, reshaping microbial metabolism rather than blocking a single enzyme.
- Microbial adaptation can alter NI performance over time.
- Soil pH, texture, and microbial activity are key drivers of NI persistence.
- Combining inhibitors or using BNI-producing crops may offer a path to sustainable nitrogen management.



Scanning electron microscopy (SEM) images of soil ammonia-oxidizing archaea (AOA), *N. viennensis* and *N. franklandianus*, forming biofilms with visible extracellular polymeric structures (EPS) (by Dreer et al., 2025). Such biofilms may influence how these microbes respond to nitrification inhibitors in soil environments.

The efficiency of nitrification inhibitors depends on their ecological context—their true impact emerges from interactions among soil chemistry, microbes, and management history.

What does this mean for agriculture

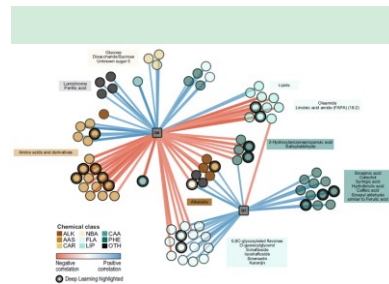
Overall, our results show that the effectiveness and sustainability of NIs depend strongly on soil type, management history, and microbial diversity. Synthetic inhibitors, such as DMPP, remain reliable across a wide range of conditions, but their long-term use should be applied strategically and alternated to prevent microbial adaptation. BNIs represent a promising and environmentally friendly alternative, yet their rapid dissipation calls for improved formulations, through encapsulation, carrier technologies, or combined use with synthetic products, to extend their activity in the field. Another promising approach is to harness plants themselves as continuous sources of BNIs, by cultivating BNI-producing species or incorporating them in suitable crop rotations and intercropping systems. Such strategies could provide a steady, in situ supply of biological inhibitors, reducing the need for external inputs while supporting long-term soil health. Monitoring their broader ecological effects will be crucial to ensure that these tools support both crop productivity and environmental protection. Informed management can help integrate NIs into sustainable fertilization strategies that minimize nitrogen losses while maintaining yield efficiency.

Looking ahead

Through ACTIONr and its associated research, nitrification inhibition has evolved from a largely empirical agronomic practice into a mechanistically informed and ecologically aware approach. This progress lays the groundwork for the next generation of NIs—effective, selective, and environmentally compatible—that can help balance agricultural productivity with climate and ecosystem goals.

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7. [Ghatak et al., 2025 *Plant Biotechnology Journal*](#)
8. [Dreer et al., 2025 *The ISME Journal*, 19 \(1\): wrafi82](#)



Network of wheat root metabolites showing positive (blue) and negative (red) correlations with nitrification inhibition (by Ghatak et al., 2025). Diverse compounds — amino acids, flavonoids, lipids, and organic acids — act together to slow down ammonia oxidation.



The ACTIONr Consortium
University of Thessaly
University of Vienna
University of Lyon

A Research Action Network for
Reducing Reactive Nitrogen
Losses from Agricultural
Ecosystems

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Eleftheria Bachtsevani

holds a first degree in Biochemistry and Biotechnology and an MSc in Biotechnology–Quality Assessment in Nutrition and the Environment from the Department of Biochemistry and Biotechnology, University of Thessaly, Greece. She completed her Ph.D. at École Centrale de Lyon, France, where her thesis focused on the toxicity of pesticides on soil nitrifiers, aiming at the development of a first Tier I ecotoxicity tool for pesticide risk assessment. Since 2024, she has been a postdoctoral researcher in the ACTIONr project at Université Claude Bernard Lyon 1.

“My main research interest lies in assessing the impact of agrochemicals on microbial functioning in the nitrogen cycle, with particular attention to the physiology of microbial groups involved in nitrification.”

INTERVIEWING RESEARCHERS OF ACTIONr

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

I joined the ACTIONr project a year ago as a postdoctoral researcher at Université Claude Bernard Lyon 1. My research focuses on assessing the impact of nitrification inhibitors (NIs) on soil nitrifier activity, community composition, and their contribution to greenhouse gas emissions. I am also particularly interested in the off-target effects of NIs on methanotrophs, microorganisms that play a crucial role in reducing methane emissions. In addition, I collaborate closely with the ACTIONr PhD student to evaluate the effects of biological nitrification inhibitors (BNIs) across experimental systems of increasing complexity — starting with controlled in vitro assays, progressing through soil slurry experiments, and finally reaching soil microcosms. This joint approach allows us to connect fundamental microbial processes with more realistic soil environments, helping us better understand how nitrification management strategies influence both targeted and non-targeted microbial communities in agriculture.

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

One of the challenges for me has been starting to work with methanotrophs, since my background is mainly with nitrifiers. While both groups are central to the nitrogen and carbon cycles, they require different experimental approaches, and this transition has pushed me to develop new skills and techniques. More broadly, a constant challenge is the complexity of soil microbial communities. Soils host a vast diversity of microorganisms that interact in ways that are often difficult to disentangle, making it challenging to pinpoint the specific effects of nitrification inhibitors.

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

My motivation to integrate microbial ecology into environmental research comes from recognizing how central microorganisms are to ecosystem functioning. Microbes drive key processes in the nitrogen and carbon cycles, influencing soil fertility, greenhouse gas emissions, and ultimately climate change. Among them, nitrifiers captured my interest early on because of their pivotal role in regulating nitrogen availability for plants while also contributing to nitrous oxide emissions. During my studies, I realized that while agrochemicals can improve crop productivity, they may also have unintended consequences for these invisible but vital microbial groups. This inspired me to focus on bridging microbiology with environmental sciences, with a particular emphasis on nitrifiers, to better understand and predict how human activities shape soil ecosystems and to support the development of more sustainable agricultural practices.

What are some key highlights from your recent research?

Recently, I have been focusing on the off-target effects of biological nitrification inhibitors (BNIs) on methanotrophs, the microorganisms responsible for consuming methane in soils. To investigate this, I am combining gas analysis — to directly track *methane consumption* — with *molecular tools that reveal changes in microbial activity and community composition*. Although the work is still ongoing, our preliminary findings suggest that BNIs can have unexpected effects on methanotroph activity. This is an exciting direction because methanotrophs play a crucial role in mitigating greenhouse gas emissions, yet their response to BNIs has rarely been studied. Gaining this understanding could provide valuable insights into how nitrification management practices influence multiple greenhouse gases simultaneously, supporting both agricultural productivity and climate sustainability.

LATEST NEWS OF ACTIONr NETWORK

ACTIONr brings science to the field at Thessaloniki International Fair 2025



ACTIONr took part in the Thessaloniki International Fair (6–14 September 2025), proudly representing the University of Thessaly. Visitors explored our research on sustainable nitrogen management through interactive displays and hands-on demonstrations.



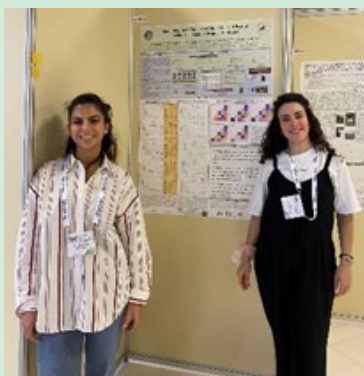
ACTIONr participated in the 28th Thessaloniki International Fair (6–14 September 2025), proudly representing the University of Thessaly and its commitment to sustainable agriculture. Visitors to our stand learned about the project's goals and the importance of improving nitrogen use efficiency, reducing losses, protecting water quality, and enhancing soil health for productive, climate-smart farming. The team engaged the public through hands-on demonstrations, including microscope observations and colorful reagent tests, and explained common tools used to study soil nitrogen cycling. Discussions with farmers, students, and other stakeholders also highlighted practical ways to apply the project's findings in the field. A warm thank-you to everyone who visited our stand! We look forward to building on your feedback and sharing the next results and events from ACTIONr.

ACTIONr at the MikroBioKosmos & CEESME Joint Conference (Thessaloniki, 22–24 September 2025)

The ACTIONr consortium had a strong presence at the Joint Conference of the MikroBioKosmos Society and the Central and East Europe Symposium of Microbial Ecology (MBK-CEESME 2025), held in Thessaloniki, Greece. Researchers from the University of Thessaly shared new insights into nitrification inhibitors (SNIs and BNIs) and their roles in the soil nitrogen cycle, combining environmental, physiological, and multi-omics perspectives. The programme featured an invited keynote lecture by Prof. Christa Schleper (University of Vienna) on “What Asgard Archaea can tell us about early eukaryotic evolution.” The ACTIONr team contributed four posters and two oral presentations, highlighting key advances:

- (i) Persistence and dissipation of plant-derived BNIs across agricultural soils, influenced by soil pH and microbiota.
- (ii) Culture–soil disparities in BNI efficacy, emphasising the complexity of real-soil environments.
- (iii) Plant triterpenoids as novel BNIs with selective activity against archaeal nitrifiers.
- (iv) Time-resolved transcriptomic and proteomic analyses revealing rapid microbial responses to synthetic and biological inhibitors.

The conference showcased ACTIONr's collaborative research and its leadership in nitrogen-cycle microbiology, bridging fundamental science with agricultural applications.



**.Conference highlight –
Thessaloniki, 22–24 Sept 2025:**
ACTIONr presented four posters and two talks at the MikroBioKosmos & CEESME Joint Conference, featuring new findings on nitrification inhibitors, microbial ecology, and nitrogen cycling. Key topics included BNI persistence, plant-derived inhibitors, and multi-omics insights into nitrifier physiology.



Researchers' Night, Larissa (26 Sept 2025):

ACTIONr's "Nitrogen Races" turned visitors into nitrogen molecules exploring the cycle—fun, hands-on, and educational! Big thanks to everyone who took part!

ACTIONr Webinar Series : "Unlocking Knowledge of Ecophysiology and Ecogenomics of the N Cycle"

When: Every two months

How: Online via Zoom

Who: Researchers, professionals & stakeholders interested in nitrogen cycle microbiology

Why: To share advances and foster collaboration in ecophysiology, ecogenomics, and microbial ecology

Highlights:

9 webinars, connecting 100+ participants across Europe & beyond.

Speakers from the University of Vienna, INRAE, University of Washington, and others.

Topics included:

- Ammonia-oxidizer genomics & physiology
- Nitrifiers in oceanic & soil systems
- Microbiome engineering & soil health
- BNIs and greenhouse gas emissions

ACTIONr "Nitrogen Races" at Researchers' Night in Larissa

On the evening of 26 September 2025, ACTIONr brought science



and fun together at Researchers' Night in Larissa, held in the courtyard of Mylos of Pappa. Through the interactive floor game "Nitrogen Races," young

participants became nitrogen molecules and travelled through the nitrogen cycle in a creative and educational way. They discovered the roles of legumes and microorganisms, explored human impacts on the cycle, and learned about science-based solutions for a more sustainable future. For visitors who missed the floor game, a tabletop version kept curiosity high and engagement strong. A big thank-you to everyone who joined and helped make science fun!



ACTIONr Webinar Series Comes to an End – A Great Journey of Scientific Knowledge and Engagement!

ACTIONr Webinar series

"Unlocking Knowledge of Ecophysiology and Ecogenomics of the N cycle"

Dates 
March 2024 to July 2025

Thank you all !!!

Invited presenters













ACTIONr

Researcher Action Network

Unlocking Knowledge of Ecophysiology and Ecogenomics of the N cycle

It is a Horizon 2021-2027 Research and Innovation program funded by the European Commission under Grant Agreement No 101079259.




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

Between March 2024 and July 2025, the ACTIONr project hosted a bi-monthly webinar series entitled "Unlocking Knowledge of Ecophysiology and Ecogenomics of the N Cycle". This initiative aimed to foster dialogue and share the latest scientific advances in nitrogen-cycle research, bringing together experts and participants from across Europe and beyond. Each session featured a distinguished researcher presenting cutting-edge

findings on diverse yet interconnected aspects of nutrients transformations in natural and managed ecosystems. Topics ranged from the genomic and physiological traits of ammonia-oxidizing

ACTIONr Webinar series

“Unlocking Knowledge of Ecophysiology and Ecogenomics of the N cycle”

Webinar title:

“Unexpected genomic and physiological diversity in ammonia-oxidizing microorganisms”

Date: Monday, March 10, 2025

🕒 Time: 17:00-18:00 CET

Presentations are available on the scientific platform of ACTIONr @

<https://www.actionr.eu/educational-scientific-platform/>

microorganisms (“Decoding the Ammonia-Oxidizers: From Genomes to Physiology and Back”, “Unexpected genomic and physiological diversity in ammonia-oxidizing microorganisms”), to their roles in broader biogeochemical contexts such as the oceanic carbon cycle and oxygen-depleted environments. Other sessions explored innovative methodological and ecological perspectives, including “Retooling Microbiome Engineering for a Sustainable Future”, “Hydrogels as a Technology to Better Understand and Address Soil Dysbiosis”, and “Ecophysiological Implications of the Millipede Microbiome”. The series also addressed climate-relevant processes, such as microbial responses to warming and the influence of tree species with Biological Nitrification Inhibition capacity on greenhouse gas emissions. Each webinar concluded with vibrant discussions among participants, fostering knowledge exchange, interdisciplinary connections, and the identification of emerging challenges and opportunities in the study of nitrogen cycling. Collectively, this series significantly contributed to building a dynamic, informed, and collaborative community around the ecophysiology and ecogenomics of nitrogen-transforming organisms.

Research Beyond Borders – UTH PhD Students Shine in ACTIONr’s Final Secondments

In the final phase of ACTIONr, two PhD students from the University of Thessaly successfully completed their 2025 research secondments within the consortium.

Dimitris Dalkidis joined Prof. Christa Schleper’s group at the University of Vienna (February–August 2025), advancing his research on ammonia-oxidizing microorganisms (AOM). Building on his previous visit, this time he focused on an ammonia-oxidizing bacterium (AOB), gaining advanced skills in bioinformatics and microbial physiology while broadening his understanding of AOM diversity. Beyond the lab, Dimitris collaborated with early-career researchers, expanded his professional network, and engaged with Vienna’s vibrant scientific community and culture. The experience will directly contribute to ACTIONr’s work linking transcriptomic data to corresponding protein expression and refining our understanding of nitrification pathways.

Meanwhile, Valia Moutzourelli carried out her secondment at the Laboratory of Microbial Ecology (Université Claude Bernard Lyon 1) under the supervision of Dr. Christina Hazard and Dr. Graeme Nicol (April–October 2025). Working in their newly established facilities, she received advanced training in Stable Isotope Probing (SIP) and performed high-precision N₂O flux measurements using a Picarro analyzer. These exchanges significantly enhanced both students’



Two UTH PhDs finished their secondments in 2025 —D. Dalkidis (Vienna, Austria) and V. Moutzourelli (Lyon, France)— gaining advanced lab and bioinformatic expertise for their thesis wrap-up.



Training across borders

Ongoing secondments (UNIVIE→UTH, CNRS/ECL→UTH) strengthen expertise exchange and build lasting capacity in proteomics and SIP-based GHG analysis.

Wrapping up three years of ACTIONr!

The consortium gathers in Larissa (29–31 October 2025) for the final meeting and SIP Workshop — three days of science, collaboration, and hands-on exploration of the nitrogen cycle.



ACTIONr partners during a previous consortium meeting - looking ahead to the final gathering in Larissa.

technical expertise, strengthened collaboration within the ACTIONr network, and energized the final stages of their doctoral research—further supporting the project’s mission to advance excellence in nitrogen-cycle microbiology

Expanding Collaboration: ACTIONr Launches Bidirectional Secondments

As ACTIONr enters its final phase, new bidirectional secondments are strengthening collaboration and technology transfer across the consortium. From the University of Vienna (UNIVIE), Dr. Logan Hodgkiss joined the University of Thessaly (UTH) for a two-month exchange focused on establishing proteomic analysis workflows and training in synthetic microbial community systems. In parallel, a second exchange from CNRS, Lyon, brought Dr. Graeme Nicol and Dr. Christina Hazard to UTH for one month, facilitating technology transfer in Stable Isotope Probing (SIP) and greenhouse gas (GHG) emission measurements. Their visit also included hands-on sessions on analytical monitoring of nitrification inhibitor fate. These exchanges reflect ACTIONr’s strong spirit of collaboration — connecting expertise, building new skills, and ensuring that advanced methods are shared and applied across partner teams. Together, they help strengthen the project’s scientific ties and long-term contribution to sustainable nitrogen management.

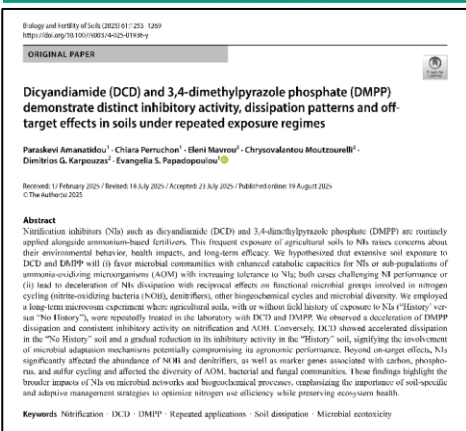
FORTHCOMING EVENTS

ACTIONr Closing Meeting and SIP Workshop – Wrapping up with Collaboration and Knowledge Sharing

The final ACTIONr Closing Meeting will take place on 29–31 October 2025 at the University of Thessaly, Larissa, bringing together all consortium partners to review project achievements and chart next steps for future collaborations. Over three days, sessions will cover the project’s scientific results, training actions, dissemination and sustainability plans, and upcoming research perspectives. Presentations from UTH, UNIVIE, and CNRS will highlight advances in nitrification inhibitor research—from their mechanisms of action to interactions within soil microbiomes and implications for greenhouse gas emissions. As part of the meeting, a dedicated technical workshop on Stable Isotope Probing (SIP) will be held on 31 October, led by Dr. Graeme Nicol and Dr. Christina Hazard (CNRS, France). The workshop will combine theoretical and hands-on training, guiding participants through the design, application, and interpretation of SIP experiments in nitrogen-cycle research. Practical exercises will focus on data integration and future opportunities for joint SIP-based activities across the ACTIONr network.

NOVEL RESEARCH IN THE THEMATIC AREA OF ACTIONr

Distinct Pathways of Nitrification Inhibition Revealed Under Repeated Soil Exposure



A major scientific output within ACTIONr comes from the FRIDA team at the University of Thessaly. In their recent paper published in *Biology and Fertility of Soils* (Amanatidou et al., 2025), Dr. Chiara Perruchon and PhD student Paraskevi Amanatidou, together with ACTIONr members Valia Moutzourelli, Dimitrios G. Karpouzias, and Evangelia S. Papadopoulou, investigated how repeated soil exposure alters the behavior and effectiveness of two widely used nitrification inhibitors (NIs)—dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate

(DMPP). Their long-term microcosm experiments revealed that DCD loses its inhibitory efficiency over time, likely due to microbial adaptation, while DMPP remains more stable yet induces broader changes in soil microbial networks. Both compounds also affected microorganisms linked to carbon, phosphorus, and sulfur cycling, underscoring their wider ecological footprint. These findings provide new mechanistic evidence that DCD and DMPP act through distinct dissipation and inhibition pathways, highlighting that repeated NI applications can trigger soil-specific microbial responses. The study highlights the importance of adaptive, soil-tailored nitrogen management strategies—a key objective of the ACTIONr project.

EDITORIAL CLOSING NOTE – THREE YEARS OF ACTIONr

As ACTIONr comes to its close in October 2025, we take pride in what has been achieved and gratitude for all who made it possible. Over the past three years, the project has built new research capacity at the University of Thessaly and strengthened lasting partnerships with the University of Vienna and the University of Lyon. Together, we have explored the hidden dynamics of the nitrogen cycle—from microbial physiology to soil ecology and agricultural applications. Through joint experiments, secondments, training activities, and open scientific exchange, ACTIONr has helped advance both knowledge and collaboration in sustainable nitrogen management. Beyond the results, what truly defines ACTIONr is its people—the researchers, students, and partners who worked together across disciplines and borders, sharing expertise, curiosity, and commitment. The project may be ending, but the collaboration, skills, and ideas it inspired will continue to grow. On behalf of the ACTIONr Team, thank you for being part of this journey!

“Dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP) demonstrate distinct inhibitory activity, dissipation patterns and off-target effects in soils under repeated exposure regimes”

Amanatidou et al. (2025) in *Biology & Fertility of Soils* report that DCD and DMPP behave very differently under repeated soil exposure. DCD’s activity declines, suggesting microbial adaptation, while DMPP remains effective but reshapes wider microbial communities, influencing N, C, P, and S cycles. The study, led by Dr. Chiara Perruchon and Paraskevi Amanatidou with V. Moutzourelli, D.G. Karpouzias, and E.S. Papadopoulou, highlights the need for soil-specific sustainable nitrogen management.

<https://doi.org/10.1007/s00374-025-01936-y>



Part of the coordinating team from the University of Thessaly! Three years of hard work, collaboration, and shared passion that made ACTIONr a success.

THE ACTIONr CONSORTIUM



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Reducing Reactive Nitrogen
Losses from Agricultural
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