

## Minutes from 4th amoA project meeting

Location: September 2nd 2025, Aarhus University Frederiksborgvej 399, 4000 Roskilde

Date: September 2nd, 2025

---

### Participants

Louise Feld (LF), Anne Winding (AW), Carsten Suhr Jacobsen (CSJ), Lea Ellegaard-Jensen (LEJ), Lucas Horstmann (LH), Erkin Gozdereliler (EG), Pedro Carvalho (PC), Linyan Zhu (LZ), Søren O. Petersen (SOP), Christian Nyrop Albers (CAL), Ulla Bollmann (UB), Anders Priemé (AP), Nanna Baggesen (NB), Thorkild Frandsen (TF), Mathias Ejby Djurhuus (MED), Åsa Frostegård (ÅF).

Excuse: Cecilie Skov Nielsen (CSN)

---

### Minutes

#### Work Package Updates

##### **WP1: Sorption and Degradation of NIs (UB, GEUS)**

UB reported on the progress of WP1. Analytical methods have been successfully developed for both GC and LC, although the detection limits for LC-MS remain high. The liquid–liquid extraction method using isoctane is nearly complete (EG). Degradation studies have shown that manure accelerates the process, although residues remain present in all cases. Degradation slows down at greater soil depths. ÅF suggested that sterilization of soil by triple autoclaving may affect its chemical composition, and alternative sterilization approaches should be considered. LC-HRMS studies revealed several unknown transformation products, with nitrapyrin showing many unknown peaks of particular interest. Comparison with DMPP indicated that nitrapyrin degradation products may be more relevant for further study. Sorption studies have been postponed to allow inclusion of new transformation products and will be performed within the coming weeks.

##### **WP2: Leaching of NIs (CAL, GEUS)**

CAL presented results from WP2. Leaching studies using bromide and D2O as tracers were successful, with recoveries of 80–100%. Nitrate concentrations in the leachate were similar across columns, although CAL noted that one column likely became anoxic over time at the bottom, which led to denitrification. In experiments with Instinct, less nitrate was detected, but the difference was not statistically significant. 3-MPA was found to leach in the column that turned anoxic, where it could not be degraded, although this was not observed under aerobic conditions. DMPP largely remained in the upper 5 cm of the column, with 80–90% degraded and only 1% leached. For nitrapyrin, the degradation product 6-CPA was detected at levels above the project threshold of 0.1 µg/L, although results varied considerably. Microencapsulation of nitrapyrin appeared to be effective, though the company has not shared details of the process. Further nitrapyrin analyses are scheduled for October (EG), with suction cup sampling continuing until January 2026. Around 60 nitrapyrin samples from suction cups are planned for analysis in January and will be processed by EG. After the fourth sampling point, nitrate levels increased in all setups, but no clear evidence of significant leaching was observed.

### **WP3: Field Site Experiments (CSN, SEGES)**

SEGES reported that cumulative methane flux measurements showed no strong effects of nitrification inhibitors. Data from 2024 and 2025 were compared, with 2025 appearing more representative of normal conditions than 2024. In 2025, some effects of inhibitors were observed, but the results were not statistically significant and may have been influenced by outliers. The discussion emphasized the need to understand why observable effects sometimes occur and sometimes do not across different years.

### **WP4: Microbial Gene Expression (AP, KU)**

AP presented updates on gene expression studies. Transcription levels in archaea increased with the application of inhibitors(T1), while bacterial copy numbers remained low despite the agricultural setting. Soil water content was highlighted as an important factor influencing interpretation of results. Amplification of nirK and nosZII will be done if evidence for denitrification occurs in total RNA dataset. CSJ suggested that it would be useful to also assess DNA levels and DNA/RNA ratios, particularly for amoA. The team discussed technical challenges with digital PCR, including overloading issues, and agreed that normalization of mRNA to rRNA would improve data reliability.

### **WP5: Non-Target Effects on Microbial Soil Ecosystems (LH, AU)**

LH presented rRNA data from Field 3 at time points T0, T1, and T2, while mRNA data are still being processed. The importance of manure composition was emphasized, as it may influence microbial responses. Fungal responses were observed, which CSJ noted as evidence of broader system-level impacts. At T0, RNA quality was low, which may have been related to cold weather conditions at the time of sampling. LEJ suggested checking historical weather data from DMI to clarify this effect.

---

### **Discussion on Data Priorities**

The group discussed priorities for data collection and analysis across work packages. WP1 is on schedule, and GC-Orbitrap analyses are expected to be completed before Christmas. For 2025 chemical analyses, time point T1 is considered essential, with T4 and T6 suggested as additional priority points. CAL proposed waiting for microbial data before finalizing time point selection, while PC suggested that student helpers at AU could assist with soil extractions to accelerate progress. SEGES was asked to provide a precise description of manure application procedures as soon as possible, as CSJ stressed the need for certainty and consistency in sampling. In WP2, around 60 nitrapyrin samples collected with suction cups will be analyzed by EG in January. WP4 will prioritize amoA (both archaeal and bacterial) and denitrification genes, with methane oxidizers considered optional. WP5 reported delays in 2024 due to RNA extraction difficulties; processing of 2024 data will take approximately one month, while RNA extracts from 2025 appear of higher quality.

---

### **Reflections by TF**

TF reflected on the discussions, noting that while it is important to assess products such as Instinct and Vizura, the second deliverable, focusing on implications for future NI products, is of greater strategic significance. So far, no alarming results have emerged regarding DMPP or nitrapyrin, which is encouraging. TF emphasized that the uptake of inhibitors by farmers will depend on the strength and clarity of project results. The group discussed the project's 0.1 µg/L leaching threshold, with CAL

arguing that column experiments represent a worst-case scenario that may be harsher than real field conditions.

---

### Preliminary Evaluation Framework

PC presented guidelines from the EU for evaluation of nitrification inhibitors, while ÅF noted that Sweden has already published a national risk assessment framework.

---

### Group Discussions

In group discussions, participants shared perspectives on regulation and assessment of NIs. Group 1 (ÅF, EG, UB, NB) emphasized the need for EU-level regulations and threshold limits similar to those applied to pesticides. They highlighted the importance of establishing indicator microorganisms for soil health, while noting that nitrifiers may not be suitable targets since they are directly affected by inhibitors. The group also argued that evaluations must include leaching as well as degradation and sorption, since pesticides in Denmark are no longer evaluated on leaching alone.

Group 2 (AP, CSJ, LZ, LH) supported regulating NIs as pesticides, though they acknowledged challenges in using nitrifiers as target organisms. They suggested methane oxidizers might serve as an alternative. They recommended laboratory-based risk assessments under controlled conditions rather than relying solely on field trials. The group stressed the importance of studying repeated applications, which better reflect real agricultural practices, and suggested accelerated leaching experiments simulating multiple years of application. They also proposed organizing a joint session with the German EPA representative, who will be in Copenhagen in November, to exchange experiences.

Group 3 (PC, LEJ, CAL, LF, MED) underlined the importance of dose-response studies, given that NIs are often applied at higher rates than pesticides. They also noted that degradation byproducts could pose risks and should be included in future evaluations.

Across all groups, common conclusions emerged: nitrification inhibitors should be regulated, either as pesticides or biopesticides; clear standards for analysis must be established; nitrifier-based regulation poses methodological challenges; and the project's reliance on a limited range of soil types is a weakness, reinforcing the value of controlled laboratory experiments.

---

### Conclusions and Next Steps

The group agreed to postpone the project timeline, noting that it has already been delayed by four months due to issues with Danish Crown. The new deadline for completion is set for the end of June 2026. Ulla will provide confirmation of the German EPA visit dates. A two-day workshop on impact evaluation has been scheduled for February 5–6, 2026 (week 6).