# Reducing soil $N_2O$ emissions by steering nitrogen cycling microbiome with grassland species composition and fertilization

**Focus and aim of the project (max. 250 words)**

This project aims to determine the impact of grassland species composition in combination with nitrogen (N) fertilization rates on soil nitrous oxide ($N_2O$) emissions, nitrogen cycle genes, and the main biological process responsible for $N_2O$ production in intensively managed temperate grassland systems [1–5]. At one of the WUR experimental fields at Bornsesteeg swards with monocultures and mixtures of grasses (*Lolium perenne* and *Festuca arundinacea*), legumes (*Trifolium pratense* and *T. repens*) with high rate of biological N-fixation[6–8], and a herb *Plantago lanceolate* with demonstrated biological nitrification inhibiting capacity[9–11] have been established. Throughout 2025, $N_2O$ emissions from these swards will be measured, as well as microclimatic factors, sward yield and N uptake, soil mineral N and dissolved organic carbon concentrations. Additionally, soil samples will be taken at 6-time points before and after fertilization at contrasting levels of $N_2O$ emissions. The total DNA from the samples will be used to quantify the total bacteria, archaea and fungi, as well as the N-cycle genes (Fig. 1) by qPCR. The abundance of N-cycle genes will be linked to $N_2O$ emissions to determine the main biological process driving $N_2O$ emissions. Additionally, we will integrate N-cycle gene abundance data and other acquired data to evaluate the $N_2O$-mitigating potential of species selection and fertilization rates. We hypothesize that species mixtures that include *P. lanceolata* will more effectively reduce $N_2O$ emissions, which is associated with decrease *amoA* bacteria and archaea genes abundances (nitrification) and increase *nifH* (N fixation), compared to monocultures at low N-application rates.

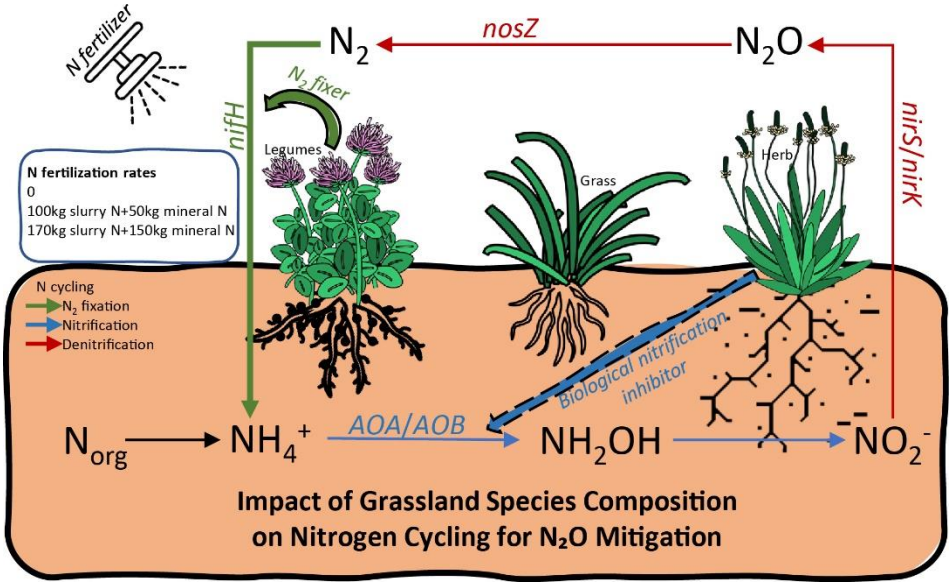


Fig. 1 Nitrogen cycle genes to be quantified in this study include those related to nitrogen fixation (*nifH*), nitrification (*amoA* for AOA, archaea; and AOB, bacteria), and denitrification (*nirK*, *nirS* and *nosZ*). The abundance of total bacteria and archaea will be determined by 16S rRNA and total fungi by the ITS region.

**Relevance given the PE&RC mission (Understanding the functioning of natural and managed ecosystems to improve the quality of life) (max. 150 words)**

This research aligns with the PE&RC mission by advancing our understanding of managed

ecosystems, specifically within temperate grasslands. By investigating how the combination of plant species combinations and N fertilization rates affect soil $N_2O$ emissions and by identifying the microbial processes responsible, this study addresses critical environmental challenges. $N_2O$ is a potent greenhouse gas, with a radiative forcing of 275 times that of $CO_2$, and agricultural practices are a major contributor[12]. Reducing these emissions is vital to mitigate climate change and meet the European Green Deal's goal of cutting greenhouse gas emissions by 55% by 2030 and achieving climate neutrality by 2050. Optimizing N use through better fertilization strategies and plant species selection can also improve soil health and decrease other N losses[13–16]. The qPCR analysis of nitrogen-cycling genes will pinpoint key microbial processes that can be targeted through management practices to significantly reduce $N_2O$ emissions[3,17].

**The manner in which the proposed project will strengthen the institutional collaboration, identifying the specific role of the proposed candidate(s) (postdoc/PhD), including the expected output of the project (max. 150 words)**

This project will bring together two leading research teams to fundamentally understand the processes and management options that impact soil $N_2O$ emissions from managed grassland. The Microbial Ecology Department of NIOO-KNAW has a strong track record in research on soil and plant microbiome specifically in N-cycling processes to $N_2O$ production and consumption in nature and agriculture systems. Concurrently, the Soil Biology Group of WUR conducts research on plant-soil interactions and sustainable management options for agriculture, and has longstanding expertise in determining $N_2O$ emissions from soils. By merging these complementary expertise areas, the project will provide a comprehensive understanding of how plant species and N fertilization practices influence the abundance of N-cycling genes and $N_2O$ emissions. The findings will be disseminated through peer-reviewed publication and social media channels for the general public, including farmers. The output of this research will establish a foundation for future collaborative research between these two groups.

**Groups involved and time to be spent by a specific postdoc / PhD candidate(s)**

This project involves two groups within PE&RC: Prof. Kuramae and her team from the Microbial Ecology Department at NIOO-KNAW with expertise in microbial processes related to $N_2O$ emission and consumption in natural and agroecological systems, and Prof. Velthof and colleagues from the Soil Biology Group (SBL) at WUR, with expertise in $N_2O$ emissions and plant-soil interactions. The project will be carried out by the PhD candidate Han Wang, working closely with Prof. Kuramae's team members Akari Mitsuta (N cycle microbiome, bioinformatics), Agata Pijl (molecular biology) and Gabriel Rocha (biological nitrification inhibitors), and with Dorien Westerik (PhD candidate), Prof. Velthof and Prof. Van Groenigen from SBL. Han Wang will spend the first month on soil sample selection based on $N_2O$ emissions together with Prof. Veltholf and the SBL team, and on DNA extraction of all samples at NIOO-molecular biology lab. The final three months will consist of performing qPCR analysis of N cycling genes, total bacteria, total archaea and total fungi followed by data analysis, interpretation, and manuscript preparation. The candidate and the team will also produce a report to disseminate the results to a wider audience.

**Appointment details: name of person(s) employed, employment period, chair group**

**Name of person(s) employed:** Han Wang (PhD candidate, Utrecht University and NIOO-KNAW).

**Employment period:** Four-month period.

**Chair group:**

1. **Soil Biology Group, Wageningen University & Research (WUR)**
   -Prof.dr.ir. Gerard Velthof (Chair of Soil Nutrient and Carbon Management)
   -Prof.dr.ir. Jan Willem van Groenigen
   -Dorien Westerik (PhD candidate)
2. **Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW)**
   -Prof.dr.ir. Eiko Kuramae (Senior Scientist and chair of Microbial Community Ecology & Environmental Genomics of Utrecht University)

**The budget requested**

We are requesting funding for the gross salary costs of one PhD candidate (last year) for four months, plus 35% overhead. Laboratory work will be conducted at WUR and NIOO-KNAW, research expenses for consumables, such as DNA extraction kits and qPCR reagents, will be covered by the chair group.

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15. Duncan, E. W., Dell, C. J., Kleinman, P. J. A. & Beegle, D. B. Nitrous Oxide and Ammonia Emissions from Injected and Broadcast-Applied Dairy Slurry. *Journal of Environmental Quality* **46**, 36–44 (2017).
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