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EDITORIAL

Denitrification in Agricultural Soils – Integrated control and Modelling at various scales (DASIM)

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Abstract
The special issue summarises and highlights key findings of the research unit DASIM funded by the German Research Foundation (DFG) on the process of denitrification. Progress was made in several areas including the development of new and advanced methods to quantify N_2 fluxes such as a new ^{15}N gas flux method, enhanced Raman spectroscopy and a new incubation system to study plant-soil interactions in He-O_2 atmosphere. Understanding of denitrification in disturbed and structured soil was gained by combining X-ray CT scanning and microbial ecology methods. High resolution models developed as part of DASIM were able to successfully simulate experimental data and provide valuable insights for the improvement of existing ecosystem models. Improved ^{15}N tracing tools for the analysis of ^{15}N tracing data in soil-plant systems have been developed that are extensively used by associated partners. DASIM brought together an interdisciplinary network of researchers interested in analytical but also modelling aspects. This includes close collaboration with the FAO/IAEA centre of Nuclear Techniques in Food and Agriculture of the United Nations which resulted in an open access book that describes the methods used in DASIM. The impact of the DASIM research unit on the scientific community is manifold and will most likely have a lasting impact on the understanding of nitrogen cycling in terrestrial ecosystems.

Introduction

The atmosphere consists of around 78 vol.-% dinitrogen (N_2), a gas that is produced by microbial denitrifiers under anoxic conditions. Denitrification is an essential process of the nitrogen (N) cycle returning organically bound and mineral N in gaseous form to the atmosphere. Dinitrogen was discovered by Rutherford (1772) (Fig. 1), who referred to it as "noxious air" or "phlogisticated air", indicating the view at the time that N_2 is produced by combustion in the sense of the phlogiston theory (Dobbin 1935). This is also reflected in the name "azote" (engl. "without life"), which Lavosier (1790) gave to the new element. In his doctoral thesis, Rutherford already referred to Joseph Priestley who discovered oxygen (O_2, "dephlogistigated air") and nitrous oxide (N_2O), another gas in the denitrification process (Priestley 1772; Smith 1972). In the middle of the nineteenth century, Reiset (1856, 1868) discovered that denitrification is essentially a process in which nitrate (NO_3^-) is reduced to gaseous N forms. However, it took another 25 years before Gayon and Dupetit (1882, 1886) showed that denitrification is a reduction of NO_3^- to N_2, carried out by bacteria and proceeding in two steps: (i) NO_3^- to nitrite (NO_2^-) and (ii) NO_2^- to N_2. The proof that N_2O is a free intermediate in the denitrification sequence was carried out by ^{15}N trapping experiments (Hollocher 1982). While N_2 is environmentally benign, N_2O is a climate-relevant trace gas contributing to ozone destruction (Crutzen 1970) and global warming (Tyndall 1861). It is now clear that denitrification is a facultative anaerobic process in which the reduction of NO_3^- to N_2 proceeds via a series of enzymatic steps translated by several functional genes. These are responsible for the various reduction steps leading to intermediate compounds ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$).

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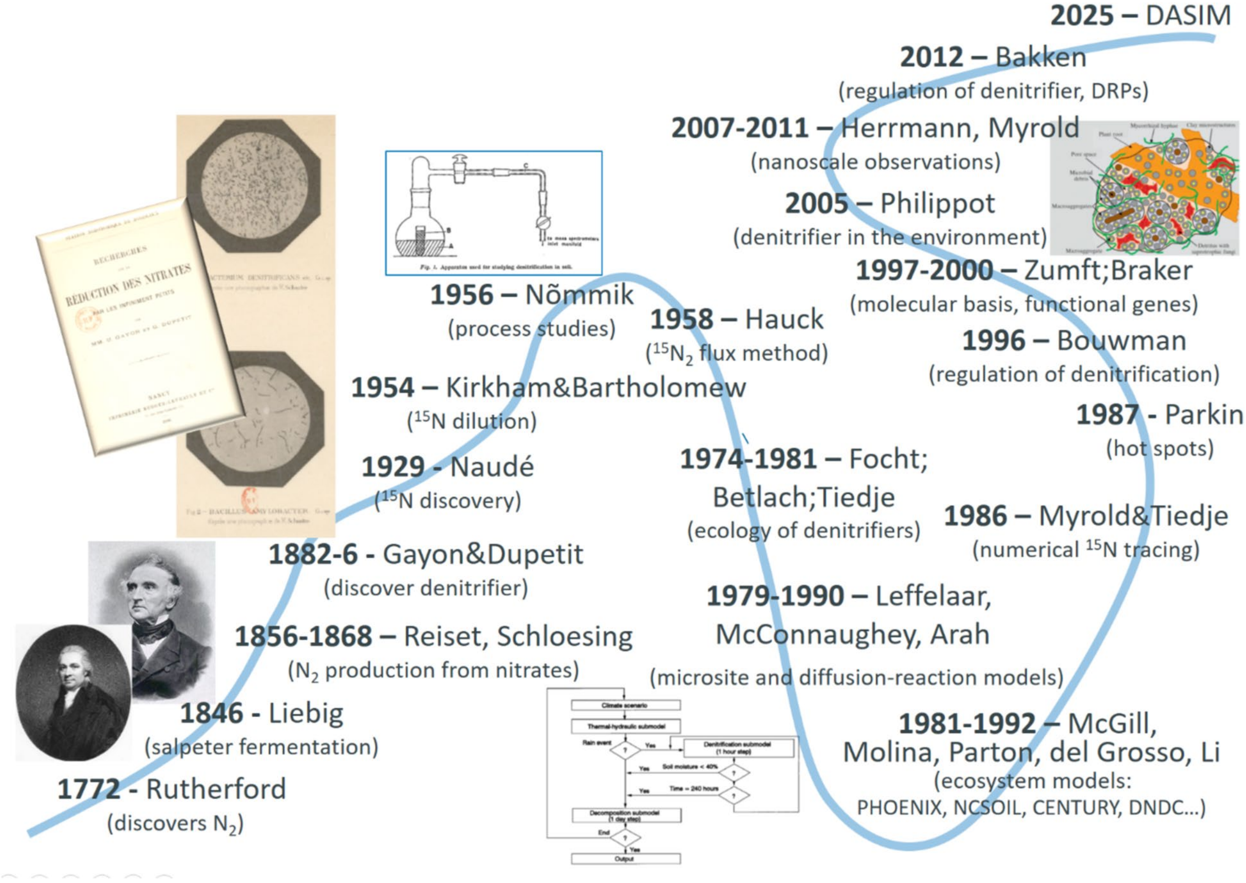

Fig. 1 Denitrification history with some scientific highlights since the discovery of N_2 in 1772

Arnold (1954) showed that N_2O and N_2 are most likely produced in anoxic microsites in otherwise oxic soil. Process studies by Nõmmik (1956) highlighted the importance of NO_2^- to explain gaseous N emission pathways. How much and in what proportion (N_2O/N_2 ratio) the gases are emitted depends on a large range of interrelated abiotic and biotic factors, especially NO_3^- and O_2 concentration, pH value and availability of organic C substrates which affect microbial activity (Groffman et al. 1988). Studies have also shown that the composition of the microbial community determines the N_2O/N_2 ratio when, for example, denitrification is carried out by fungi that lack N_2O reductase (Aldossari and Ishii 2021; Shoun et al. 1992). Fungi have the ability for co-denitrification, a hybrid reaction between NO_2^- and another reduced N compound, a process that can dominate N_2O production from soils (Kirkby et al. 2025, this issue; Laughlin and Stevens 2002). Thus, the decomposition of NO_2^- interacting with soil organic matter (SOM) is an important reaction for gaseous N production. Furthermore, self-decomposition of NO_2^- to gaseous N compounds can occur at pH values below pH 5.5 possibly at microsites in soils that are otherwise dominated by higher pH values (van Cleemput and Samater 1996). In the last decades research into the microbial taxa and their ability to denitrify has shown that denitrification is an ubiquitous feature in nature and present in many phylogenetically unrelated microbial taxa belonging to bacteria (Alpha-, Beta-, Gamma-, and Epsilon-Proteobacteria, Firmicutes), archaea, and fungi (Zumft 1997). Concepts such as the denitrification regulatory phenotypes (DRPs) have been proposed to characterize denitrifiers and their functional response to the various controlling factors (Bergaust et al. 2011). With the advent of high-throughput sequencing techniques, an unprecedented in-depth analysis of denitrifiers became manageable (Palmer et al. 2012). Other NO_3^- reduction processes competing with denitrification in soil–plant systems are assimilatory NO_3^- reduction by plants and microbes, dissimilatory NO_3^- reduction to ammonium (NH_4^+) (DNRA), the above-mentioned co-denitrification, and chemical transformations (chemo-denitrification) at low pH values (Tiedje 1988).

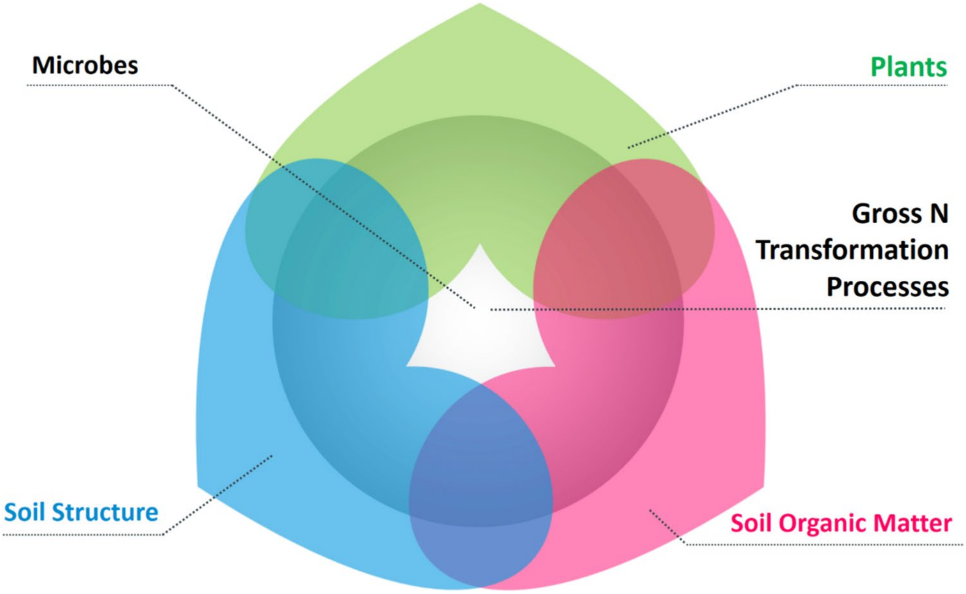

Fig. 2 DASIM concept to understand denitrification in soil–plant systems

The DASIM concept

The overall objective of the DASIM research unit was the investigation, assessment, and modelling of denitrification in its complexity in agricultural soils at various scales and in response to various atmospheric boundary conditions at, to date, unmatched resolution. We intended the development and improvement of methods to determine denitrification activities and dynamics of N transformation processes including N_2 emissions at unprecedented process resolution by taking advantage of interdisciplinary cooperation in experimental laboratory and field work as well as modelling. The innovative combination of the latest physical, biological, and analytical tools was enabled to disentangle the complex interactions of physical and biochemical processes in agricultural soils. Key was to elucidate the impact of difficult to quantify control factors such as soil pore structure, microbial community structure, plant effects, quality and spatial distribution of soil organic matter and others as well as their interactions (Fig. 2).

Soil organic matter has a direct and an indirect influence on denitrification, in particular its quality and distribution within the soil matrix (Miller et al. 2009). However, their individual contribution to fuel denitrification is not very well understood. Plants can actively exudate carbon (C) compounds acting as easily degradable substrate for denitrification. Root biomass degradation also represents a substantial source of organic substrates for denitrifiers. Furthermore, plants strongly compete with soil microorganisms for O_2 and NO_3^- and affect soil moisture, thus influencing major regulators for denitrification such as oxic-anoxic soil conditions in microsites (Müller and Clough 2014). Thus, structurally and functionally distinct denitrifier populations may develop in preferentially colonized sites. Soil structure plays an important role because it defines the network of air-filled pores which has implications for the distribution of water and microbes, diffusive exchange of C and N compounds and O_2. The interactions of these factors determine the composition and activity of the microbial community, which carries out various enzymatic reactions characterized by interrelated gross N transformation processes such as mineralization, immobilization, nitrification and denitrification, comprising the N cycle (Fig. 2).

Bringing together the complex network of interacting factors and conditions is typically the task of denitrification models considering habitat properties and microbial interactions therein, as well as the various transformation processes controlled by O_2 and the various C and N species. A key challenge is to simulate the temporal dynamics of the suite of regulators and its spatial distribution at various scales (e.g., from microsite to plot scale) (Leffelaar 1987). Moreover, there is generally a lack of validation of the modelled denitrification rates by measurements of the gaseous products, including N_2, which makes further development and refinement of models difficult. The aim of DASIM was therefore to bring together all the above aspects, which is a prerequisite for modelling denitrification at different scales to improve predictions.

Experiments had been designed with the aim to provide data for the evaluation of existing denitrification models at different scales and the development of high-resolution simulations. We focused on two arable soils and one grassland soil that differed in soil organic matter content and texture for individual, associated, and central experiments. Initially, simplified systems (i.e., using glass beads and unplanted, homogenized soils) were used under controlled conditions, followed by mesocosm studies with intact soil cores and finally, plot scale experiments (with undisturbed soils) under natural environmental conditions.

Key questions addressed in DASIM were:

- How are the activity of denitrifiers and their community structure controlled at the micro scale?
- How is denitrification affected and controlled by other simultaneously occurring N cycling processes such as mineralization, immobilization or nitrification?
- How can small scale features of spatial heterogeneity and biological activity ("hot spots" and "hot moments") be adequately represented at larger scales up to the field scale?
- How can the effect of rhizosphere processes (plant root exudation and turnover) and functional soil organic matter fractions on denitrification be elucidated?
- Do fundamental relationships exist on spatio-temporal controls of denitrification at various scales (from micro- to meso- and plot-scale)?
- To what extent is it possible to predict denitrification of a given soil in response to atmospheric boundary conditions (e.g. O_2 concentrations) based on measurable structural and biochemical properties?

Methodological developments

The key methods used in DASIM were published together with the International Atomic Energy Agency (IAEA) as an open-access book (Zaman et al. 2021c). Several methodological developments have been carried out to obtain advanced data that could be used in various modelling studies. The ^{15}N gas flux (^{15}NGF) method (Hauck et al. 1958) was developed further (Micucci et al. 2023) to enhance its sensitivity for N_2 flux measurements, especially under field conditions, using an N_2-reduced atmosphere with 0.5 to 2% N_2 in helium–oxygen (He-O_2), which improved the limit of detection of N_2 fluxes by two orders of magnitude (Well et al. 2019; Zaman et al. 2021b). This method was used to study the effect of soil structure (Lucas et al. 2025, this issue; Rohe et al. 2021b), manure placement (Grosz et al. 2025, this issue), earthworms and cropping systems (Kemmann et al. 2021), water-logging (Kemmann et al. 2023) and pH / litter interactions on N_2 and N_2O dynamics (Pfülb et al. 2025, this issue). In DASIM it was also used to study N_2 and N_2O fluxes in small lysimeters in winter wheat during 10 weeks following organic fertilization in comparison to mineral fertilization (Buchen-Tschiskale et al. 2023), during an entire cropping season in winter wheat and sugar beet (Eckei et al. 2025, this issue) and in connection with a full N balance on an alpine grassland site (Dannenmann et al. 2025, this issue) and an arable site (Yankelzon et al. 2025a, this issue).

To measure the N_2 emissions and $N_2O/(N_2+N_2O)$ ratios (R_{N2O}) under controlled conditions a soil–plant incubation system was developed that allows to grow plants over weeks to months in large gas-tight chambers in a He-O_2 atmosphere under a tunable light source, while being irrigated with N_2-free water (Yankelzon et al. 2025b, this issue).

Another approach that enables the sensitive and selective detection and isotopic differentiation of N_2, N_2O and ammonia (NH_3) is Enhanced Raman gas spectroscopy (Blohm et al. 2024). The technique is suitable for experiments with natural or He atmospheres with or without isotopic labels. As part of DASIM, this method was further developed so that advanced Raman spectroscopy now allows the quantification of N_2 concentrations at the parts per billion (ppb) level (Merian et al. 2024; Zaman et al. 2021d), i.e. similar to the detection limit achievable for soil-emitted N_2 with the ^{15}NGF under N_2-depleted atmosphere (Zaman et al. 2021b). Furthermore, automatic laboratory and field-based greenhouse gas (GHG) measuring systems were enhanced (Zaman et al. 2021a). To allow homogenous ^{15}N tracer applications, improved labelling methods were developed for laboratory (Zhu et al. 2024) and field applications (Tenspolde et al. 2025, this issue). To provide globally comparable isotopic data, the DASIM consortium also led an initiative for international $^{15}N_2O$ and $^{15}N_2$ isotope and isotopomer standards that are suitable for both, Isotopic Ratio Mass Spectrometry (IRMS) and Cavity Ring-Down Spectroscopy (CRDS) systems (Well et al. 2025a).

For the analysis of ^{15}N tracing data obtained from plant-soil systems advanced numerical tools were developed that enable the concomitant analysis of soil N transformations and plant N uptake (He et al. 2020). Moreover, the ^{15}N tracing tool *Ntrace* was further refined and re-developed with advanced parameter optimization routines to improve the accuracy and speed of ^{15}N tracing analyses (Jansen-Willems et al. 2022).

To include soil structural information that allows microsite modelling of a three dimensional system, X-ray CT scanning approaches were applied (Schlüter et al. 2025, this issue) providing data for spatially-resolved, process-based modelling of denitrification in intact soil cores (Schulze et al. 2025; Zawallich and Ippisch 2025, this issue; Zawallich 2024).

Based on the data, new model strategies were developed to enable a more realistic prediction of denitrification activity in space and time. They are not only dependent on the anaerobic volume fraction but also on factors that determine hotspots for denitrification by including, for instance, microstructural information on air distances and particulate organic matter (POM) (Lucas et al. 2024; Schlüter et al. 2025, this issue).

DASIM results

In the following we present some key results of DASIM in relation to the various components outlined in the DASIM concept (Fig. 2).

Soil structure and the role of organic substrates, nitrate and oxygen

Experimental studies started with simplified systems made of porous glass beads, representing microbial hotspots that were supplied with labile C and NO_3^- so that denitrification activity scaled in a predictable way with O_2 limitation (Schlüter et al. 2018). The hotspot size and external O_2 concentration at its boundary determined O_2 concentration gradients and thus the diffusive O_2 flux into the hotspots once anoxia was fully established. When embedded into a sandy matrix the distance of hotspots determined growth and the rate at which the hotspots developed anoxia (Schlüter et al. 2019). This in turn governed the accumulation of denitrification intermediates which was confirmed by diffusion–reaction models that were based on independent measurements (Schlüter et al. 2025, this issue; Zawallich and Ippisch 2025, this issue). Models revealed that the notion of anoxic centres fuelling denitrification might not always apply. The highest modelled denitrification rates are located at the interface of anoxic hotspot centres and the hypoxic seam around it, as this is where the number of active cells are highest and all reactants are present (Schlüter et al. 2025, this issue; Zawallich and Ippisch 2025, this issue).

In real soils, the regulation of denitrification by soil structure has to be taken into account. Carbon substrate, NO_3^-, denitrifiers, and O_2 concentrations are often spatially separated and the diffusion constraints imposed by soil structure and water content determine their local availability (Lucas et al. 2025, this issue; Schlüter et al. 2025, this issue). Oxygen availability depends on the distribution of air-filled pores with connection to the atmosphere and the volume fraction of the wet soil matrix that may develop anoxia. These patches of oxic and anoxic volumes emerge at a scale of µm to mm (Kravchenko et al. 2017; Lucas et al. 2025, this issue; Schlüter et al. 2025, this issue). Also high microbial activity fuelled by labile organic C in form of fresh POM may impose steep O_2 gradients at µm to mm scales and decouple denitrification activity from the bulk O_2 availability in the soil (Lucas et al. 2024; Ortega-Ramírez et al. 2023). The occurrence of substantial denitrification rates, despite average O_2 concentrations of 50 to 80% of atmospheric concentrations, has been confirmed both by experiments in repacked (Rohe et al. 2021b) and in undisturbed soils (Lucas et al. 2025, this issue) and by spatially explicit simulations (Schulze et al. 2025, this issue; Zawallich and Ippisch 2025, this issue). In batch incubations microbial activity and denitrification activity scaled linearly with the water-extractable organic matter (WEOM) from plant residues (Schlüter et al. 2022; Surey et al. 2021) which agrees with the observations by Parkin (1987) that > 90% of N_2O emissions originated from a single leaf fragment in an otherwise well-aerated soil. For the first time, O_2-microsensors were used in combination with the X-ray CT information to create 3D images of microscale O_2-distribution (Lucas et al. 2024). Pore structure alone was not sufficient to predict the O_2-distribution in intact soil cores. Heterotrophic respiration, and thus O_2 depletion, was strongly associated with the volumetric POM content in direct vicinity to air-filled pores, whereas the contribution of poorly aerated POM further inside the wet soil matrix was low. However, total denitrification rates were associated with the amount of POM located inside the wet soil matrix, but independent of the well-aerated POM (Lucas et al. 2024). This highlights that the erratic nature of denitrification rates can be understood much better if microscale information about the location of microbial hotspots is considered. These locations do not necessarily depend on the distance to air-filled pores (van Dijk et al. 2025, this issue). Genes that encode for denitrification steps were more abundant at shorter pore distances in line with general *16S* bacterial gene abundance, but its relative abundance showed no clear pattern. Thus, denitrification can potentially be induced everywhere but is most likely triggered by vicinity and distribution of POM (Schlüter et al. 2025, this issue; van Dijk et al. 2025, this issue). Moreover, results by Wang et al. (2025a, this issue) show that conditions promoting rapid denitrification in arable soils have an effect on other gross N transformation processes. Under water-saturated (O_2-limiting) conditions an increase in NH_4^+ immobilization and a concomitant decrease in autotrophic nitrification occurred which was also reflected in gene expression.

Based on the new understanding, a model concept has been proposed that incorporates microscale information into effective denitrification models operating at the soil profile scale (Schlüter et al. 2025, this issue; Schulze et al. 2025, this issue).

Organic substrates

The relevance of specific soil organic matter fractions was confirmed by incubation experiments using soils from a long-term fertilization experiment that resulted in different amounts of plant residues, their WEOM fractions, and mineral-associated organic matter (MAOM) (Surey et al. 2021, 2020a, b). The denitrification potential of a Chernozem soil decreased in the order: farm yard manure > pure mineral fertilization > no fertilization, coinciding with the finding that especially soil receiving farm yard manure contained larger portions of labile POM and WEOM. Denitrification-related N_2O emissions were closely related to water-extractable organic C (WEOC), which was well linked to the abundance of free POM. Thus, free POM serves as major substrate for denitrifiers and WEOC could serve as an easy-to-use indicator of a soil's denitrification potential. Using the three DASIM soils confirmed that the source function of POM relative to MAOM for delivering WEOC is much higher (Surey et al. 2021). WEOC derived from POM accounted for 53 to 85% of total denitrification while WEOC released from MAOM accounted for only 15 to 47%. Thus, WEOC, originating from fresh plant residues, appears to be the most critical organic matter fraction promoting denitrification. However, the quality of fresh POM varies in agricultural soils, depending on plant species and changes with decomposition time, which also affects the molecular composition of WEOC and its potential for driving denitrification (Lucas et al. 2024). Regression analyses suggest that WEOC can be reasonably predicted based on common soil variables such as clay and organic C and to some extend pH, because under acidic conditions more recalcitrant aromatic compounds contribute to WEOC. This in turn reduces the overall denitrification potential (Surey et al. 2020b), which is in line with observations by Čuhel et al. (2010). This is also consistent with the results of Wang et al. (2025b, this issue), who used a garlic-rice and wheat-rice rotation to show that the addition of straw, which leads to a higher C/N ratio, can reduce the denitrification potential. Furthermore, the role of organic C and N compounds and their quality on regulating the N cycle was confirmed by Zhu et al. (2025, this issue) in a study looking at effects of land-use conversion.

Microbial ecology

A variety of experiments with soil samples and pure cultures addressed whether the DASIM soils show contrasting denitrifier and DNRA community structures in response to organic C quality, pH, temperature and NO_3^- or NO_2^- concentrations. These investigations were carried out under controlled conditions with an automated incubation and gas measuring system (Zaman et al. 2021a).

Pure culture experiments revealed that denitrifying bacteria and DNRA communities have different affinities to their substrate (N oxides), and differ also in their maximum reaction velocity. DASIM soils showed different denitrification kinetic potentials which coincided with different community compositions (van Dijk et al. 2025, this issue) with respect to their response to pH, temperature, quality and content of organic matter and O_2 level (Surey et al. 2020a). Soil slurry experiments showed that denitrification rates are highest when the soil pH is neutral to slightly alkaline and ambient temperature is in the range of 25 to 45 °C. Denitrifier communities were impacted by temperature and pH as indicated by denitrification gene diversity, and results defined pH and temperature preferences of certain uncultured denitrifier genotypes (van Dijk et al., unpublished data). Incubation experiments with *Azospirillum brasilense* Sp7 indicated that increasing O_2 concentrations inhibited expression of denitrification-relevant genes like *napA*, *nirS* and *nosZI* with a rather high O_2 tolerance (Henjes et al., unpublished data). Experiments addressing the influence of organic matter showed that WEOC shapes the short-term microbial community composition and the $N_2O/(N_2O + N_2)$ ratio (Surey et al. 2020a). This is in line with a study by Wu et al. (2025, this issue) showing that differences in the microbial composition and N cycling of two different paddy fields can be explained by different physicochemical properties, differences in C availability and denitrification gene expressions (Yuan et al. 2025, this issue). Also, rapidly changing conditions for instance under freezing–thawing events of organic soil can lead to an increased nitrifier and *nirK*-type denitrifier activity. This leads to incomplete denitrification and thus to an enhanced N_2O/N_2 ratio (Kazmi et al. 2025, this issue). Thus, the physiology of uncultured denitrifiers provides evidence for a functional relationship between community structure and reaction kinetics including the competition of denitrifiers with DNRA which was also considered in subsequent modelling studies (Zawallich and Ippisch 2025, this issue; Zawallich 2024).

The influence of plants on denitrification

Plant effects on denitrification range from physical effects such as shading, root effects on soil porosity and aeration to water uptake, root respiration, competition for N, rhizodeposition of labile C compounds, and litter chemistry (Malique et al. 2019; Yankelzon et al. 2025a, this issue). In an incubation with either plant-free soil or soil sown with wheat (*Triticum aestivum* L.), N_2 and N_2O emissions increased only after the onset of plant growth (Yankelzon et al. 2025a, this issue). In general, plant roots and their interactions with the soil microbiome affect soil structure (Lucas et al. 2025, this issue). Root exudation is considered to control rhizosphere denitrification (Senbayram et al. 2020) which was confirmed by using DASIM soils showing that root exudation explained more than half of the variance of rhizosphere denitrification enzyme activity (Malique et al. 2019; Maurer et al. 2021) exerting feedback effects on plant–microbe competition for NO_3^- (Rummel et al. 2021b). Stimulation of denitrification was most pronounced in low C soil and under high soil moisture. While many rhizodeposition compounds stimulated denitrification (e.g. organic acids), the sugar lyxose seemed to inhibit denitrification (Maurer et al. 2021). However, in a climate chamber study, no stimulating effect of root-derived C, but a strong influence of soil moisture on daily $N_2O + N_2$ fluxes, cumulative

Table 1 Emission factors for N_2 emissions (EF_{N2}) and $N_2O/(N_2+N_2O)$ ratios (R_{N2O}) as derived from 18 state of the art publications from 1978 to 2023, for more information contact Dannenmann et al. (2025, this issue) (EFs are defined as N emissions as a percentage of the soil N inputs)

EF_{N2} [% of fertilizer N]						
	Mean	SE	Median	Minimum	Maximum	No. of observations
All data	10.0	1.5	5.1	0.0	69.5	63
Grassland	13.3	2.1	15.4	1.6	28.7	19
Cropland	8.6	1.9	3.7	0.0	69.5	44
Mineral fertilizer	9.8	2.6	3.7	0.0	69.5	32
Organic fertilizer	10.3	1.6	5.9	1.6	34.2	31
R_{N2O}						
	Mean	SE	Median	Minimum	Maximum	No. of observations
All data	0.15	0.02	0.06	0.00	1.00	66
Grassland	0.12	0.03	0.04	0.01	0.42	19
Cropland	0.16	0.03	0.08	0.00	1.00	47
Mineral fertilizer	0.20	0.04	0.13	0.01	1.00	32
Organic fertilizer	0.10	0.02	0.05	0.00	0.42	34

N emissions, and N_2O production pathways was observed (Rummel et al. 2021b). Rapid plant N uptake reduced the availability of NO_3^- and therefore restricted denitrification, providing evidence that root-derived C may stimulate denitrification when plants are small, while N and water uptake become the main controlling factors with increasing plant and root growth. This was confirmed by a field study with winter wheat and sugar beet (*Beta vulgaris* subsp. *vulgaris*) where plant growth, soil NO_3^- and NH_4^+, soil WEOC, and N_2O+N_2 fluxes were monitored. In this field study, WEOC content increased linearly with plant growth confirming the importance of rhizodeposits for denitrification. However, N_2O+N_2 fluxes were highest when plant N uptake und competition for mineral N (NH_4^+, NO_3^-) was low (i.e. during early plant development, ripening, and after harvest) (Eckei et al. 2025, this issue).

During the post-harvest period, crop residue incorporation can increase the availability of labile organic C leading to plant litter-associated anoxic hotspots and therefore denitrification (Kravchenko et al. 2017). The chemical quality of litter played a crucial role in regulating litter decomposition and, thus, N_2O fluxes (Lashermes et al. 2022; Rummel et al. 2020, 2021a). Litter quality measures, such as C/N ratio and WEOC content, were important indicators of the magnitude of N_2O and N_2 production after litter addition (Eckei et al. 2025, this issue). Furthermore, land management such as tillage practices influence N_2O and GHG emissions. Sun et al. (2025, this issue) show that practices such as periodic tillage, which may enhance microbial activity, increases emissions per area but, due to the higher yield, may actually reduce the yield-scaled GHG emissions (i.e. CO_2, CH_4 and N_2O). These considerations play an important role if the aim is to increase yield to maintain food security by intensifying soil cultivation. This requires a careful balance between C sequestration, tillage practices and N fertilization, taking into account the environmental costs (Sun et al. 2025, this issue). Also, land-use conversion from forest to plantation affects N transformations but careful management does not necessarily impose a negative environmental effect (Zhu et al. 2025, this issue).

Towards realistic N_2 emission factors in undisturbed soil systems

To extend the knowledge about the dynamics of denitrifying activities and their control under conditions similar or identical to the field, undisturbed, intact soils (with and without plants) were studied under controlled conditions, but also under changing environmental conditions and at the plot scale. “Hot spots” and “hot moments” were evaluated by taking into account soil moisture dynamics, spatial distribution of C sources and microbes, and the various effects of plants. *In-situ* N_2 flux measurements were obtained using the improved ^{15}NGF method (Well et al. 2019). Furthermore, the literature was screened for studies based on direct *in-situ* measurements of N_2 and N_2O to obtain more comprehensive data sets, i.e. from studies using the ^{15}NGF method or the He-soil core method for intact plant-soil systems with light availability that allows for active plants to grow under realistic conditions. Also, laboratory studies using intact plant-soil systems in dark chambers were accepted if the obtained denitrification product stoichiometry was used to derive field N_2 fluxes in combination with high resolution *in-situ* N_2O flux measurements or total fertilizer N losses obtained from field ^{15}N fertilizer balances (in the latter case including NH_3). Data were included until after fertilizer-induced peaks of N_2 and N_2O had subsided. Based on these criteria, 18 papers, starting in 1978 and including recent DASIM studies, with 63 observations of emission factors for N_2 (EF_{N2}) and $N_2O/(N_2+N_2O)$ ratios (R_{N2O}) were accepted. The mean EF_{N2} amounted to 10.0 ± 1.5% of applied N (min: 0%, max: 70%), and the mean R_{N2O} was 0.15 ± 0.02 (Table 1). Table 1 also shows EF_{N2} and R_{N2O} separately for grassland and cropland and for organic and mineral fertilizers.

The EF_{N2} obtained was about three times higher and the R_{N2O} was less than half the values provided by Pan et al. (2022) who included questionable data based on the acetylene inhibition technique. A R_{N2O} of 0.15 agrees well with the estimate provided by a recent review of terrestrial denitrification N fluxes, which concludes that N_2O accounts for about 8% (6 to 11%) of the terrestrial denitrification flux on a global scale (Scheer et al. 2020). A total N input of 161 (139 to 192) Tg N yr^{-1} to global croplands (Zhang et al. 2021) would result in total N_2 losses of 16.1 (13.9 to 19.2) Tg N yr^{-1}. Current estimates of reported denitrification losses from agricultural soils range from 17 to 87 Tg N yr^{-1} in different studies (Hofstra and Bouwman 2005; Seitzinger et al. 2006), indicating that an EF_{N2} of 10% from cropland soils is still conservative and may even be underestimated. This shows that still more field studies of N_2 emissions from agricultural soils are needed to provide robust estimates of both universal EF_{N2} and specific EF_{N2} for different fertilizer, soil and crop types and climatic conditions. In most of these studies, denitrification in the top soil was quantified. However, if denitrification in the subsoil is taken into account (van Cleemput 1998), it must be assumed that the total fertilizer loss and the EF of the entire root zone are higher.

In addition, data derived from full fertilizer N balances in lysimeter studies with ^{15}N-labelled fertilizer corroborated parallel direct N_2 measurements using the ^{15}NGF method (Dannenmann et al. 2025, this issue; Yankelzon et al. 2025a, this issue). Other studies resulted in mostly low $N_2O/(N_2+N_2O)$ ratios (Eckei et al. 2025, this issue) (Buchen-Tschiskale et al. 2023) which is in line with the estimates at the global scale (Scheer et al. 2020).

This suggests that the ^{15}NGF method delivers reliable data for in situ studies in agricultural systems (Friedl et al. 2020; Lewicka-Szczebak et al. 2020; Well et al. 2019). This is also confirmed by Kirkby et al. (2025, this issue) who presented data of high-resolution N_2O and N_2 emissions from a sugar cane site in Australia. They show that hybrid reactions (co-denitrification) are only important to consider for N_2O but not for N_2 production. Co-denitrification is of higher relevance in soils with a high organic matter content. With this method it is also possible to evaluate physicochemical effects on the N_2O/N_2 product ratio as presented by Yuan et al. (2025, this issue). They showed that Fe-loaded biochar can promote complete denitrification in arable soil, which can therefore be considered as an N_2O mitigation method (Yuan et al. 2025, this issue).

Tools to study denitrification at various scales

Another approach to fill the lack of data on denitrification product ratios is the development and application of N_2O isotopocule mapping approaches to estimate N_2O fluxes and product ratios from nitrification, fungal and bacterial denitrification and N_2O reduction to N_2 (Well et al. 2025b, this issue; Yu et al. 2020). While natural abundance stable isotope values of N_2O (average $\delta^{15}N$, $\delta^{15}N^{bulk}$; $\delta^{18}O$; ^{15}N site preference) is increasingly used to study N_2O processes, reliable predictions require consideration of uncertainties (Lewicka-Szczebak and Well 2020; Micucci et al. 2025; Wu et al. 2019). Mapping approaches allow the quantification of up to four different processes at the same time (Lewicka-Szczebak et al. 2020; Lewicka-Szczebak and Well 2020). This approach was further evaluated to show that N_2O reduction determined by the isotopocule mapping and the ^{15}NGF method agreed well when denitrification was intense and N_2O fluxes were dominated by bacterial denitrification (Lewicka-Szczebak and Well 2020).

To quantify fungal N_2O fluxes, isotopocule mapping with ^{15}N tracing and inhibitor approaches were used (Rohe et al. 2021a). It was also evaluated how $\delta^{18}O$ of N_2O can be used to distinguish N_2O from nitrification and fungal denitrification in connection with gene expression of fungal P450nor (Rohe et al. 2020).

Independent analyses of N_2O reduction by soil incubation under He-O_2 atmosphere and direct determination of N_2 production to correct isotopocule data for the isotope effect during N_2O reduction improved estimates of fungal N_2O production (Senbayram et al. 2020). Isotopocule mapping requires robust estimates of precursors to identify the active pathways and processes. In particular, NO_2^- is an intermediate of nearly all N transformations including those of N_2O turnover (Jansen-Willems et al. 2022; Lewicka-Szczebak et al. 2021). Laboratory soil incubations with parallel natural abundance and ^{15}N treatments, accompanied by isotopic analyses of soil N compounds (NO_3^-, NO_2^-, NH_4^+) and the double ^{15}N tracing method, were used to quantify N transformation processes based on natural abundance NO_2^- dynamics (Lewicka-Szczebak et al. 2021). The good agreement between both approaches demonstrated that natural abundance NO_2^- isotope studies are a promising tool to improve our understanding of soil N cycling including N_2O and N_2 dynamics based on isotopocule mapping (Well et al. 2025b, this issue).

Evaluation and further development of denitrification models

Data obtained in DASIM were used for improvement and evaluation of denitrification models. During evaluation the models DNDC, DeNi (based on the NGAS and

DailyDayCent), and COUPMODEL (http://www.coupmodel.com/) were used to test the denitrification sub-modules at different levels of complexity (Grosz et al. 2023; Schlüter et al. 2025, this issue). Three international DASIM workshops were organized to address deficiencies of the models used. Key demands and requirements identified were to improve the simulation of O_2 dynamics and to create a standardized method for model calibration and the setup of a knowledge network for modelling soil O_2 and denitrification dynamics for improved simulations of N dynamics (Yu et al. 2020). The models showed deficiencies in the calculation of anaerobic soil volume fraction (ansvf), calibration of denitrifier microbial dynamics, and decomposition of labile organic C. This is related to different approaches resulting in different values of the same biophysical variable, e.g., ansvf, but can still yield similar results for other key target quantities like N_2O emissions. This phenomenon, known as equifinality, underscores the complexity of denitrification modeling, arising from the abundance of process parameters compared to available measurements (Grosz et al. 2023). N_2 emissions may vary significantly which is often neglected in studies due to the lack of validation data. Thus, a full N balance approach and advanced measurements are recommended (Yankelzon et al. 2025a, this issue). Validation of modeled processes related to denitrification by direct measurements remains a challenge, hindering the refinement and development of models.

Thus, in DASIM the role of denitrification substrate quality and availability, especially POM and MAOM, has been stressed, especially the accessibility of various C substrates by the aerobic and anaerobic microbial communities. Moreover, to account for spatial variability a new functional relationship has been developed, considering the distance of locations with high denitrification activity from the nearest air-filled pore connected to the atmosphere. This approach benefits from measurable information about pore structures using X-ray CT scanning, potentially enabling the creation of soil-specific databases usable by ecosystem models (Grosz et al. 2023; Schlüter et al. 2025, this issue; Schulze et al. 2025, this issue).

Conclusions

The DASIM research unit shows that even after more than 200 years of studying denitrification, progress can still be made to better understand this process in the context of N cycling in terrestrial ecosystems. This concerns analytical progress such as advanced techniques, but also the tight connection of state-of-the-art observations with high-resolution modelling. A fundamental understanding at the microsite level is crucial for the prediction of denitrification at larger scales. Thus, a profound process understanding at the small scale (µm to mm) is essential for the required upscaling to the plot and field scale (m) (Butterbach-Bahl et al. 2013). With the numerical tools available today, it is possible to simulate highly complex interactions at near-natural conditions, providing new insights on the processes occurring in soils. State-of-the-art methods based on stable isotopes are key to our progress, and new techniques for direct quantification of N_2 concentrations, such as advanced Raman spectroscopy, have also emerged from this research.

The interdisciplinary nature of this research unit was absolutely essential for the development and combination of methods that have not yet been used to this extent (e.g., combination of X-ray CT scanning with high resolution modelling and microbial ecology). The information gained from DASIM was shared with a larger number of associated partners, who benefited from the progress made. The very active publication activity of this group also contributed to its world-wide recognition.

However, the most important lesson learned from this research is to not limit yourselves to your own area of expertise, but to enable genuine and effective collaboration with people you enjoy working with! It is this synergy effect of interdisciplinary research that has been crucial for the progress in DASIM.

Although we have addressed a number of key aspects to bring our understanding on denitrification to a higher level, a number of issues have not yet been resolved/addressed, such as the role of subsoils, interactions with higher animals, but also chemical transformations and investigations of soils that are important on a global scale, such as organic soils.

At the end, the quote of Bakken and Dörsch (2007) still holds true.

> *"…constructing new and more refined models to start bridging the gap between gas-flux data and the biology of denitrifying bacteria… [and physico-chemical controls] is [still!] a … task … Herculean in nature"*

which should encourage subsequent generations of scientists to continue studying this important topic.

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