



# Use of $^{15}\text{N}$ tracers to study nitrogen flows in agro-ecosystems: transformation, losses and plant uptake

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Nitrogen (N) fertilization is a prerequisite to sustain crop yields, but this often goes hand in hand with N losses that cause damage to the environment and an economic loss to farmers. Currently, less than 50% of the fertilizer added to croplands is converted into harvested products (Lassaletta et al. 2014) and reactive N compounds are lost to the environment causing serious environmental impacts, including eutrophication, biodiversity loss, human health problems and perturbations of the climate system (Galloway et al. 2003). How to bring global use of reactive N back within environmental limits while meeting an increasing demand for food, feed, fuel and fiber is one of the most important challenges for humanity in this century and requires substantial improvements in N use efficiency (NUE) of global cropping systems. However, to achieve this goal a better quantitative understanding of N flows, transformations and loss

pathways, and their biophysical control in current agro-ecosystems is required.

For 80 years, researchers have used the stable isotope  $^{15}\text{N}$  as a tracer to study the N dynamics in soils and the plant N use. Already the pioneers of  $^{15}\text{N}$  work in the 1940s and 1950s used  $^{15}\text{N}$  as a tracer of the N cycle, for identification of pathways as well as for quantification of process rates (Norman and Werkman 1943; Kirkham and Bartholomew 1954; Jansson 1958). Over the decades, novel methodologies have been developed and refined, a work that is still going on as highlighted by several contributions to this special issue.

This special issue seeks to explore latest trends and emerging topics in the use of  $^{15}\text{N}$  tracers to study cycling and flows of N in agroecosystems. It contains a collection of 14 contributions, which discuss latest advances and challenges in this field, advancing our mechanistic understanding of N cycling and flows at the field scale.

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## Fate of mineral and organic nitrogen fertilizer

This special issue includes several papers that use  $^{15}\text{N}$  techniques to trace the fate of N applied in mineral or organic form (crop residues, animal excreta, composts). Since N inputs as mineral or organic fertilizer are small compared to the large size of the indigenous soil N pool it is almost impossible to directly quantify the fate of N fertilizer in the plant-soil system

and assess the use efficiency of applied N (Fillery and Recous 2001).  $^{15}\text{N}$  tracer techniques allow for the accurate quantification of fertilizer N flows (e.g. plant uptake and soil retention) needed to assess the use efficiency and losses of the applied fertilizer. Consequently,  $^{15}\text{N}$  tracers provide an important tool to assess the effect of N management practices on NUE and their impact on the environment and are being used since many decades to study the fate of N in agroecosystems (Hauck and Bremner 1976).

Four of the published papers used  $^{15}\text{N}$  labeled mineral fertilizer as a tracer to quantify plant uptake and losses of fertilizer N and to test agronomical practices to improve NUE in different cropping systems. The first article in this section studies fertilizer NUE of 24 different cotton production system at five different commercial farms in Australia and investigates how fertilizer management and irrigation can be used to improve NUE in irrigated cotton production (Scheer et al. 2023). They find that NUE under current management strategies is low and demonstrate how optimized management strategies that reduce N losses and improve fertilizer and water use efficiency would make cotton production more sustainable. The study presented by Muller et al. (2022) shows how combining nitrification inhibitors with a reduced fertilizer N rate can significantly reduce nitrous oxide ( $\text{N}_2\text{O}$ ) emissions as well as overall N fertilizer losses from a vegetable cropping system without yield penalties. Li et al. (2022) demonstrate how plastic film mulching and straw return can be used to reduce the fertilizer N loss in rain-fed wheat production. However, they stress the urgent need to find substitute biodegradable plastics for the mulching to avoid environmental pollution. Finally, Webb et al. (2022) present the first on-farm investigation on the impact of poultry litter amendment on  $^{15}\text{N}$ -labeled urea recovery in irrigated cotton. Their findings demonstrate that poultry litter amendment can improve soil N reserves and total plant N uptake, while limited improvements were seen on fertilizer  $^{15}\text{N}$  plant uptake and recovery. Interestingly, across all four studies only a small amount (10–40%) of the N taken up by the crop was derived from the applied mineral fertilizer while the crop sourced most of its N (60–90%) from existing soil N reserves, clearly demonstrating that these cropping systems rely on mineralization of soil organic N and residual fertilizer as the primary N source.

Due to the increased demand for organic farm products, and restrictions on the use of synthetic N fertilizers in many industrialized countries, animal manures have become an important N fertilizer in many cropping systems (Chalk et al. 2020). However, animal manures are highly variable in their composition and consist of a high proportion of organic N compounds that must be mineralized before N becomes plant available. Consequently, N dynamics after manure applications are hard to predict, and NUE and flows of animal manure N are still poorly understood (Zistl-Schlingmann et al. 2020). Frick et al. (2022) applied  $^{15}\text{N}$  labeled cattle slurry under on-farm conditions to gain a more realistic view of the NUE of cattle slurry. They found a lower fertilizer value of cattle slurry compared to mineral fertilizer under on-farm conditions, with a significantly higher recovery of applied N in the plant biomass for mineral fertilizer than in the slurry when applied at the same rate of mineral N. But despite these initial differences they also found a similar distribution of  $^{15}\text{N}$  labeled cattle slurry and mineral fertilizer in soil after one year. In grasslands broadcast surface application is the most common application technique for liquid cattle slurry, but involves a range of environmental issues resulting from the low NUE and high N losses induced by this fertilization technique (Zistl-Schlingmann et al. 2019, 2020). Schreiber et al. (2022) used  $^{15}\text{N}$  labeled cattle slurry to study the impacts of slurry acidification and injection on fertilizer nitrogen fates in grassland. They show how alternative slurry application techniques can increase NUE and/or promote soil organic N formation from applied fertilizer to a remarkable extent, but emphasize that the additional refueling of SON stocks promoted by slurry injection is still not sufficient to prevent soil N mining mostly resulting from large plant N exports that even exceed total fertilizer N inputs.

A better understanding of N supply from crop residues and their utilization by the subsequent crop can help to improve NUE in crop production and reduce N losses to the environment. Available N for plants in soil does not only derive from fertilizer inputs, but also from the decomposition and mineralization of plant residues and litter, as well as from rhizodeposition. Those can be important pathways for satisfying the N demands of crops and grasses, but our knowledge is inadequate. Three papers in this special issue used  $^{15}\text{N}$  tracer methods to study those pathways.

Whittaker et al. (2023) studied the contribution of shoot and root residues from forage crops to the subsequent crop, in their case potato, by use of  $^{15}\text{N}$  enriched residues from different forage plants. In two experiments they showed that less than 5% of the  $^{15}\text{N}$  in residues were recovered in the potato plants, while the majority was recovered in soil. However, the study also demonstrated that an equal fraction of  $^{15}\text{N}$  was recovered in the potato plants from residue roots compared to residue shoots, highlighting that future studies need to consider the root fraction.

Introducing cover crops during fallow periods is a good practice for improving soil health, reducing off-farm inputs and protecting natural resources. Cover crops that are able to scavenge and assimilate large amounts of soil N during periods of high loss potential increase N retention in agroecosystems and can supply N to subsequent cash crops (White et al. 2017). However, knowledge on the decomposition and fate of N sequestered within the cover crop biomass and its utilization by the following crop is still limited. Lacey et al. (2022) studied the fate of cereal rye N and utilization by subsequent corn and soybean over three site-years at two locations. They could show that N from cereal rye residues does not provide a meaningful amount of N to the subsequent cash crop but is more likely to be recovered in the soil. Roth et al. (2022) used  $^{15}\text{N}$  enriched cereal rye residues to track N in root and shoot biomass amongst different soil N pools following decomposition. They found that even under ideal mineralization conditions, the bioavailability of N from cereal rye residues is low, indicating limited benefit in the form of N supply to the following crops. Thus, the value of cereal rye as cover crop lies in the build-up of soil organic matter rather than a potential N supply to subsequently grown cash crops.

### Identification of nitrogen cycle pathways and process rates

The rhizosphere is a hotspot for biogeochemical dynamics, and nutrient cycling (Frank and Groffman 2009; Phillipot et al. 2013). This is fueled by rhizodeposits, that prime microbial activity. Can  $^{15}\text{N}$  leaf-labelling be used to quantify rhizodeposition in nodulated legumes? That is the question Araujo et al. (2022) asked. They conducted a series

of experiments, comparing two widely used methods and testing several assumptions, with a focus on the role of nodules and their  $^{15}\text{N}$  enrichment. Overall, Araujo et al. (2022) concluded that the rhizodeposition of nodulated legumes could not be estimated reliably, despite attempts to correct for immediate  $^{15}\text{N}$  exudation/leakage into the soil and differences in  $^{15}\text{N}$  content between tissues (shoot, root, and nodules). The main issues were difficulties to obtain representative samples of the root-nodule system, calling for future studies to refine methodologies.

Conducting studies in soil with an intact rhizosphere is also important when quantifying gross N cycling dynamics that should be representative for in situ conditions (Templer et al. 2008; Rütting et al. 2011). While previous methods were conducted in the  $\text{cm}^2$  scale, Stange et al. (2022) developed and tested a new technique for quantification of gross nitrification at the  $\text{m}^2$  scale in the field. The technique is based on the  $^{15}\text{N}$  pool dilution approach (Kirkham and Bartholomew 1954), and a newly-developed irrigation system was used to distribute the  $^{15}\text{N}$  tracer homogeneously. Stange et al. (2022) observed low gross nitrification rates across all their experiments, which were at least one order of magnitude lower than the global average rates reported for croplands based on laboratory experiments (Elrys et al. 2021). Interestingly, one of the few other studies on in situ gross nitrification rates in a cropland (Laine et al. 2018) reported rates similar to the ones by Stange et al. (2022). That raises interesting questions for future studies: Are in situ gross nitrification rates in general lower than laboratory-based studies; and if so, why?

Different soil N transformation processes can produce the powerful greenhouse gas  $\text{N}_2\text{O}$  and there is an increasing interest in source partitioning of  $\text{N}_2\text{O}$  production. This can be achieved by both  $^{15}\text{N}$  tracer studies (Friedl et al. 2021) as well as using  $^{15}\text{N}$  natural abundance (Lewicka-Szczebak et al. 2020). The latter approach was used by Berendt et al. (2022) to study the sources of  $\text{N}_2\text{O}$  in different peatland types. Several noteworthy observations were done: under all environmental conditions, nitrification and denitrification contributed to  $\text{N}_2\text{O}$  production and the relative contribution of the two processes was not affected by season nor by soil moisture. Moreover, under all conditions  $\text{N}_2\text{O}$  reduction took place, and this happened prior to the mixing of  $\text{N}_2\text{O}$  from different sources. The quantification of total denitrification still poses

a great challenge to scientists, making the magnitude of total denitrification losses a major uncertainty for N-budgets from terrestrial ecosystems (Groffman et al. 2006). One of the few methods that can be applied in the field is the  $^{15}\text{N}$  gas flux method that Friedl et al. (2023) used to study the effects of crop residue management and a nitrification inhibitor on total denitrification in a sugarcane cropping system. The nitrification inhibitor was not only extremely effective in reducing overall  $\text{N}_2\text{O}$  and  $\text{N}_2$  losses but also in promoting complete denitrification of  $\text{N}_2\text{O}$  to environmentally benign  $\text{N}_2$ , recommending its use in cropping systems with banded fertilizer where localized zones of high  $\text{NO}_3^-$  concentration around the fertilizer band can result in elevated  $\text{N}_2\text{O}$  emissions. They conclude that the use of nitrification inhibitors in sugarcane cropping systems is an effective approach to minimize N losses, while keeping the benefits of crop residue (cane trash) retention.

### Methodological advances

All studies employing  $^{15}\text{N}$  as a tool are depending on accurate and precise determination of the stable isotope ratio in environmental samples. Many methods exist for analyzing the  $^{15}\text{N}$  abundance of inorganic N species (ammonium and nitrate) in liquid samples (Biasi et al. 2022), all with their own limitations and advantages. Jia et al. (2022) conducted an extensive literature review on those methodologies as a base for developing a decision support tool as a guideline for research to select appropriate analysis methods for their samples. This will certainly be of benefit both for  $^{15}\text{N}$  novices as well as experienced researchers for planning future studies.

### Concluding remarks

The stable isotope  $^{15}\text{N}$  remains widely used as tracer and is still one of the most powerful tools to study N flows in agroecosystems, including determination of NUE, N losses via denitrification and biological  $\text{N}_2$  fixation. We would like to thank all authors whose contributions have helped to advance our knowledge on N cycling in agroecosystems and hope that this SI will have a stimulating effect on future N research.

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