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RESEARCH ARTICLE

Plant availability and leaching of ^{15}N-labelled mineral fertilizer residues retained in agricultural soil for 25 years: A lysimeter study

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Abstract

Background: A high use-efficiency of fertilizer N remains essential to sustain high crop productivity with low environmental impact. However, little is known on the long-term lability of mineral fertilizer N.

Aims: To quantify crop uptake and leaching of ^{15}N-labelled mineral fertilizer that has been retained in an agricultural soil for 25–30 years in crops with variable growing season.

Methods: A field plot received ^{15}N-labelled mineral fertilizers over a period of 5 years and was then kept under arable cropping for 12 years. After relocation to 16 lysimeters, the topsoil grew set-aside grassland for the next 13 years. Then crop uptakes and leaching losses of ^{15}N remaining in soil was tested over a 2-year period by either converting set-aside grass to production grassland, or by replacing it with spring barley (+/− autumn cover crop) or vegetation-free fallow. All treatments received unlabelled mineral N fertilizers.

Results: Crop uptake and leaching of ^{15}N were generally highest in the first test year after termination of the set-aside. The leaching of residual ^{15}N in soil declined in the order: vegetation-free soil (4.7%), spring barley (1.9%), spring barley + cover crop (0.7%) and production grassland (0.2%). Corresponding losses for the second leaching period were 2.7%, 0.9%, 0.4% and 0.06%. There was a fixed relationship between leaching losses of ^{15}N and total N.

Conclusions: After residing in soil for 25–30 years, the lability of labelled mineral N fertilizer residues appeared slightly higher than the lability of bulk soil N. Autumn vegetation was crucial for reducing leaching losses.

KEYWORDS
cover crops, residual N value, spring barley, vegetation-free fallow

1 | INTRODUCTION

In most agroecosystems, crop productivity is limited by the availability of mineral nitrogen (N) whether this is derived from mineralization of N retained in soil organic matter, in crop residues, or added in animal manure or mineral N fertilizers (Christensen, 2004). A high use-efficiency of N is essential to sustain high crop productivity with low environmental impact, and one focus of current N research remains an improved use-efficiency of soil N and of N added in fertilizers and manures. This includes the availability for crop uptake or leaching of mineral N fertilizer residues left in soil at the end of the growing season.

Mineral fertilizers labelled with ^{15}N are a useful tool for estimating the use-efficiency of added N and tracing the fate of N fertilizer residues. Early studies based on lysimeter facilities and ^{15}N-labelled mineral fertilizers applied to spring barley before the growing season demonstrated that crop recoveries of ^{15}N ranged from 46% to 57% in the first growing season irrespective of N addition rates corresponding to 54–162 kg N ha^{-1} (Dowdell et al., 1984; Kjellerup & Kofoed, 1983). The ^{15}N-recovery in crops grown the following year ranged from 1.4% to 2.4% and was <1% in the subsequent 2 years. The leaching loss after the first two crops accounted for 4%–5% of the added ^{15}N and then dropped to <1% in the following leaching season. Meta-studies, summarizing more recent studies on crop uptake of ^{15}N-labelled mineral fertilizers, confirm these findings (Gardner & Drinkwater, 2009; Smith & Chalk, 2018; Yan et al., 2020), although somewhat lower first-year crop recoveries were observed in several studies. However, studies addressing the susceptibility of ^{15}N-labelled fertilizer residues to leaching losses are extremely rare.

Even though mineral fertilizers at the time of application contain N readily available to plants, most studies show that a large fraction (typically 25%–50%) of the added ^{15}N is retained in the soil after harvest of the first crop in organic form of low availability to plants. The comprehensive syntheses of published results (Smith & Chalk, 2018; Yan et al., 2020) show that in the majority of studies, however, the fate of added ^{15}N retained in the soil has been followed only for the first 3 years after application, and there are very few studies of the long-term effects on the soil N mineralization capacity and associated potentials for crop uptake and leaching losses (Sebilo et al., 2013).

Using a comprehensive lysimeter facility, our main objective was to study crop uptake and nitrate leaching losses of ^{15}N-labelled mineral fertilizer in crops with contrasting growing periods, 25–30 years after the application of the ^{15}N-labelled fertilizer. A second objective was to compare the leaching rate from labelled N in soil with the leaching rate from total soil N (relative to the size of the topsoil N pool). It was hypothesized (1) that the lability of residual labelled N applied with mineral fertilizers many years ago is similar to that of the total soil N pool and (2) that the leaching rate from mineralized N fertilizer residues is significantly influenced by the length of the growing season and lower in crops with a long growing season.

2 | MATERIALS AND METHODS

The original field plot received labelled fertilizer N during 1989–1993 in order to grow ^{15}N-labelled plant material for use in studies outside the labelled area. The field plot was then subject to cereal crops applied with unlabelled fertilizer N until 2004 when the topsoil was relocated to lysimeters. The topsoil in the already existing lysimeters was replaced with the labelled top soil from the field plot and then subjected to set-aside grassland. A new experiment, called the test phase, was started in the lysimeters in spring 2018 as described later.

2.1 | The lysimeter facility

The used outdoor lysimeter facility at Askov Experimental Station, Denmark was established in 1973 and consists of cylindrical glassfibre-reinforced polyester tanks, 1.5 m deep and 1.03 m in diameter (surface area 0.83 m^2). Before the topsoil was replaced as described earlier, the top 1 m of the lysimeter contained soil extracted from three soil horizons (0–20, 20–40 and 40–100 cm) in a nearby field. During filling, repacking of the soil ensured that it regained its original bulk density (1.55 g cm^{-3} for 20–40 cm and 1.23 g cm^{-3} for 40–100 cm). The 20–40 cm soil layer contains 7% clay, 6% silt, 29% fine sand, 54% coarse sand and 4% organic matter; the corresponding values for the 40–100 cm soil layer are 9%, 7%, 31%, 51% and 2%. The pH of the soil layers ranged from 5.9 to 6.4. Below the soil column is a 50 cm layer of fine sand to facilitate collection of percolates. The percolate flows passively into 25-L PVC containers in a subterranean gallery situated alongside the cylinders. A water trap inserted between the PVC container collecting the percolate and the tube draining the base of the cylinders prevents air from reaching the cylinder from beneath.

2.2 | The ^{15}N-labelled topsoil

The study was based on ^{15}N-labelled soil originating from the Ap-horizon of a 20 m^2 field plot located in the B1-field at Askov Experimental Station (55°28′N, 09°06′E). During 1989–1993, this plot provided ^{15}N-labelled plant materials for various studies outside the labelled plot (Thomsen, 1993; Thomsen & Jensen, 1994; Thomsen & Christensen, 1996; Thomsen et al., 2001). In 1989, the plot was planted with spring barley and undersown with Italian ryegrass that received $K^{15}NO_3$ and $^{15}NH_4{}^{15}NO_3$ (mean atom%-^{15}N: 18.0) at a rate corresponding to 102 kg N ha^{-1}. In 1991, the plot was partly planted with oilseed rape and partly with sugarbeet applied with $^{15}NH_4{}^{15}NO_3$ (atom%-^{15}N: 5.14) equivalent to 160 kg N ha^{-1}. Finally, field peas were grown in 1993 and applied with $^{15}NH_4{}^{15}NO_3$ (atom%-^{15}N: 10.0) corresponding to 111 kg N ha^{-1} and supplemented with 14 kg N ha^{-1} of $K^{15}NO_3$ (atom%-^{15}N: 60.0). From 1994 to 2003, the plot grew mainly cereal crops occasionally applied with unlabelled mineral fertilizers and with most of the plant biomass left on the plot to recycle ^{15}N taken

TABLE 1 History of soil and outline of experimental set-up in the lysimeter experiment.

1989–1993	1993–2004	2004–2017	2018–2019 (test phase)
Application of ^{15}N-labelled mineral fertilizer to a 20-m^2 field plot	Mainly cereal cropping with unlabelled mineral N fertilizer and return of crop residues. Topsoil relocated to lysimeters in 2003	Soil kept in lysimeters under set-aside grassland, occasionally dressed with small rates of unlabelled N fertilizer	Testing the lability of ^{15}N-labelled mineral fertilizer residues by converting set-aside grassland to(1) production grassland (2) vegetation-free fallow (3) spring barley (4) spring barley, cover crop All treatments dressed with unlabelled mineral N fertilizer

up by the crops. The plot was subject to standard tillage treatments (ploughing, harrowing). The field plot was abandoned in October 2003, and the topsoil (0–20 cm) removed from the entire 20 m^2 plot and left to air-dry indoors. After sieving (<2 cm), the soil was stored in dry condition. The soil contained 11% clay (<2 µm), 8% silt (2–20 µm), 43% fine sand (20–200 µm) and 37% coarse sand (200–2000 µm) and contained 1.54% C and 0.175% N. The atom%-^{15}N of the soil was 0.4515, and the natural ^{15}N abundance measured in a soil sampled nearby the plot was 0.3683% (corresponding to 0.0832 atom% ^{15}N excess). Soil pH (0.01 M $CaCl_2$) was 5.1.

2.3 | The set-aside grassland phase

In spring 2004, the ^{15}N-labelled soil retrieved from the 20-m^2 plot replaced the 0–25 cm topsoil in 16 of the lysimeters. After removal of the original soil, each lysimeter received 365 kg of the dried and sieved ^{15}N-labelled soil, ensuring that the soil beneath remained undisturbed. The remaining labelled soil was stored dry indoors in sealed containers for later analysis. The lysimeters were sown with a grass mixture suitable for set-aside grassland. This consisted of perennial ryegrass (*Lolium perenne* L.: 16 kg ha^{-1}), meadow fescue (*Festuca pratensis* Huds.: 7 kg ha^{-1}) and Kentucky bluegrass (*Poa pratensis* L.: 5 kg ha^{-1}) and received unlabelled NPK mineral fertilizers corresponding to 100 kg N ha^{-1}, 12 kg P ha^{-1} and 44 kg K ha^{-1}. In spring 2008, 2009 and 2012, the lysimeters received small dressings of unlabelled NPK to maintain an intact grass cover. Each dressing corresponded to 50 kg N ha^{-1}, 6 kg P ha^{-1} and 23 kg K ha^{-1}. No fertilizer was applied in the other years. The grass was subject to 2–4 cuts per season during 2004–2012 with a final cut in 2015. After cutting, all plant material was returned to the cylinder in which it was grown. Table 1 gives an overview of the history.

In April 2017, we prepared for the subsequent test phase by sampling soil (2 cm diameter soil cores to 20 cm depth) and above-ground biomass cut by hand from each of the 16 lysimeters for the analysis of ^{15}N abundance. The sampling included also soil that had been stored indoors in dry condition since 2004. Soil pH (0.01 M $CaCl_2$) measured in January 2018 averaged 5.2 and each lysimeter received a surface-application of lime corresponding to 5 t ha^{-1}.

2.4 | Testing the lability of ^{15}N retained in soil

We initiated the test phase in spring 2018 by establishing four treatments each with four replicates. The treatments were (1) the continuation of grass vegetation but with N fertilization and harvest of cuttings (Grass), (2) vegetation-free fallow (Vegetation free), (3) spring barley (*Hordeum vulgare* L.) (spring barley) and (4) spring barley followed by a cover crop (spring barley CC). In 2018, the cover crop was fodder radish (*Raphanus sativus* L. var. oleiformis Pers.) planted after barley harvest, whereas in 2019, the cover crop was perennial ryegrass undersown in the barley in spring. Lysimeters with the grass treatment remained undisturbed, whereas the other treatments were subject to simulated ploughing in spring. This was done by removing the 0–25 cm soil in three successive layers. The upper third with the grass turf was returned to the exposed surface of the undisturbed subsoil in the lysimeter before adding the rest of the soil. Sowing of spring barley was on 19 April in 2018 and 6 May in 2019. At the same time, all treatments (including vegetation-free fallow) received unlabelled NPK fertilizer corresponding to 150 kg N ha^{-1}, 40 kg P ha^{-1} and 150 kg K ha^{-1}.

The grass plots were cut three times in the growing season 2018 (29 May, 6 August and 24 October) and two times in 2019 (23 May and 9 July). Harvest of spring barley took place on 3 August 2018 and 13 August 2019. The cover crop was harvested by hand cutting on 21 November 2018 and 31 October 2019. After the determination of biomass yields and retrieval of subsamples for analysis, the cover crop biomass was returned to the lysimeter in which it was grown to simulate freezing of the cover crop and then covered by a net. All lysimeters remained undisturbed from harvest 2018 until the next spring when they were again prepared for planting of spring barley. Samples of plant materials were dried at 60°C and stored for analyses.

Figure 1 shows monthly precipitation, irrigation and mean air temperature for the period January 2018–April 2020. During the growing seasons, the lysimeters were irrigated with 100 mm in 2018 and 90 mm in 2019 based on calculations of water deficit. Water percolating the soil column was collected individually for each lysimeter. Percolate collected during 13 July 2018–22 March 2019 was accumulated for eight periods, whereas five periods were adopted for the leachate collected during 8 July 2019–1 April 2020. Representative subsamples were stored at −18°C until analysis.

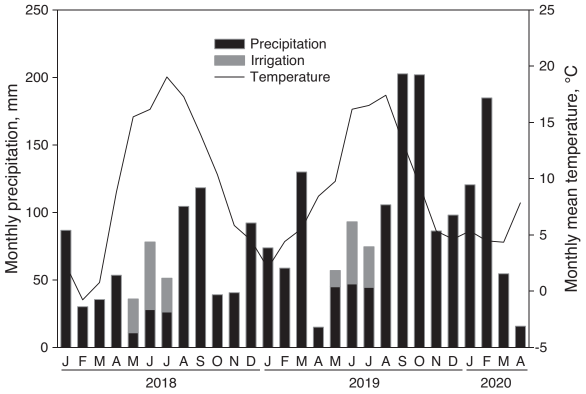

FIGURE 1 Monthly mean temperatures and monthly precipitation (including irrigation shown separately) at Askov Experimental Station during the experimental period.

2.5 | Analytical methods

Plant and soil samples were dried at 60°C and milled. Ball-milled subsamples were packed in tin capsules for the analysis of total N content and ^{15}N concentration by high temperature dry combustion using a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20–20 continuous flow isotope ratio mass spectrometer (IRMS) at UC-Davis Stable Isotope Facility (Davis, CA, USA). Concentrations of nitrate in percolates were determined by flow colorimetry on an AutoAnalyzer III (SEAL Analytical GmbH; Norderstedt, Germany). The ^{15}N content of nitrate-N in the percolate was analysed after diffusion to glass filter in Teflon traps as described by Sørensen and Jensen (1991) using an elemental analyser interfaced to an IRMS, as described above for plant and soil samples.

2.6 | Calculations

Experiments with ^{15}N-labelling generally express the ^{15}N content as an excess atom fraction ^{15}N (Chalk et al., 2015):

$$atom\%^{15}Nexcess = \%^{15}N_{sample} - \%^{15}N_{reference} \tag{1}$$

The reference (background) value used here is 0.3663% for samples of water and plant materials and 0.3683% for soil samples, based on the analysis of topsoil sampled nearby the original plot. The excess atom fraction ^{15}N was multiplied with the N pool size (in soil, plant or leachate) to calculate the amount of labelled N (excess ^{15}N), which is also termed ^{15}N in the following.

Diffusion samples containing nitrate-N were corrected for the content of N in the blank samples using the following equation (Sørensen & Jensen, 1991):

$$atom\%^{15}N\,corrected = \frac{(\mu g\,N\,measured\;atom\%\,^{15}N\,measured) - (\mu g\,N\,blank \times 0.3663)}{\mu g\,N\,measured - \mu g\,N\,blank}, \tag{2}$$

where *μg N measured* and atom% ^{15}N measured is the amount of N and the enrichment measured in the enriched sample, respectively. The *μg N blank* is the amount of N measured in a blank sample. The reference value for ^{15}N concentration was set at 0.3663%.

To facilitate interpretation, area-related results are scaled to 1 m^2 by dividing measured values with the area of lysimeters (0.83 m^2). Analysis of variance was carried out using the GLM procedure in SAS 9.4. When the treatment effects were significant ($p < 0.05$), least significant differences were used to compare means of the treatments.

3 | RESULTS

Precipitation (without irrigation) was 655 mm during August 2018–March 2019 and 1055 mm during August 2019–March 2020 (Figure 1). All treatments, including vegetation-free soil, were irrigated when dry periods occurred in May, June and July, based on water balance calculations. Most of the time during November 2018–March 2019 and November 2019–March 2020 monthly average temperatures remained between 4.5 and 5.5°C. Thus, relatively high temperatures stimulating N mineralization as well as the precipitation pattern facilitated percolation and nitrate leaching losses. Leachate collected from the treatments grass, vegetation-free fallow, spring barley and spring barley with cover crop averaged 508, 600, 515 and 463 mm, respectively, for the period 2018–2019. The corresponding percolations for the period 2019–2020 were 833, 999, 861 and 782 mm, respectively.

In April 2017, before the test phase, the 0–25 cm soil layer contained 935 g total N m^{-2} (result not shown) and 485 mg ^{15}N m^{-2} (Table 2). Figure 2 shows atom% ^{15}N excess of grass biomass and soil sampled in April 2017 and reveals insignificant differences between lysimeters allocated to each of the four crops. The average total N concentrations in grass biomass and soil were 19.6 and 1.69 mg total N g^{-1} dry matter (DM), respectively. The atom% ^{15}N excess was substantially higher in the grass biomass (average 0.0895%, standard error 0.0006%) than in the 0–25 cm soil layer (0.0659%, standard error 0.0012%).

Figure 3 shows DM yields and total N uptake in the three vegetated treatments in the test years 2018 and 2019. In 2018, the first grass cut showed considerably higher DM yield and total N uptake than in the subsequent two cuts. In the following year, the first and second cuts were almost similar in DM yield, whereas total N uptake was higher in the first than in the second cut. The uptake of N in barley grain tended to be higher in 2018 than in 2019, whereas grain DM yields differed less between years. For straw DM and total N, yields were higher in 2019 than in 2018. The cover crop was considerably higher in DM and N yields in the first than in the second year, recalling that the type of cover crop differed between years. The total biomass harvested across all crops in 2018 and 2019 corresponded to 9.1–11.2 and 6.9–10.8 t DM ha^{-1}, respectively. Total N uptakes across the crops were equivalent to 14.3–22.5 g N m^{-2} in 2018 and 12.5–18.3 g N m^{-2} in 2019. Generally, the atom% ^{15}N excess was higher in plant biomass

TABLE 2 Summary budget of ^{15}N-labelled mineral fertilizer residue (called ^{15}N) residing in soil in 2004 when the soil (0–25 cm) was introduced to the lysimeter facility (mg excess ^{15}N m^{-2}) and the crop ^{15}N uptake and leaching of ^{15}N-nitrate during the test period (2018–2020). Standard errors are shown in parentheses ($n = 4$ for crops and $n = 16$ for soil).

	^{15}N (mg excess ^{15}N m^{-2})	^{15}N (% of ^{15}N in soil 2004)
In 0–25 cm soil 2004	637 (20.1)	100
In 0–25 cm soil 2017	485 (5.1)	76
Loss per year, 2004–2017 (with grass)	11.7	1.8

Treatments from 2018	Main crop uptake	Cover crop uptake	Leaching	Sum crop uptake + leaching[b]	Crop uptake[b]	Leaching	Sum crop uptake + leaching[b]	^{15}N leaching (% of vegetation free ^{15}N leaching)[a]
2018–19	**^{15}N (mg excess ^{15}N m^{-2})**				**^{15}N (% of ^{15}N in soil 2004)**			
Grass	8.8 (0.6)		0.95 (0.24)	9.8 (0.76)	1.38	0.15	1.53	4
Vegetation free			23.0 (1.00)	23.0 (1.00)		3.61	3.61	100
Spring barley	6.8 (0.1)		9.1 (0.29)	16.0 (0.17)	1.07	1.43	2.51	40
Spring barley CC	6.3 (0.2)	6.4 (0.8)	3.3 (0.28)	16.0 (0.63)	2.00	0.51	2.51	14
2019–20								
Grass	3.8 (0.2)		0.29 (0.05)	4.1 (0.10)	0.60	0.05	0.64	2
Vegetation free			13.6 (0.37)	13.6 (0.37)		2.13	2.13	100
Spring barley	5.3 (0.3)		4.5 (0.70)	9.9 (0.73)	0.84	0.71	1.55	33
Spring barley CC	6.1 (0.2)	2.7 (0.3)	2.2 (0.36)	11.0 (0.44)	1.39	0.34	1.73	16

Note: Standard errors are shown in parentheses ($n = 4$ for crops and $n = 16$ for soil).

[a] ^{15}N leaching related to ^{15}N leaching from vegetation free soil in the same period.

[b] Including uptake in cover crop.

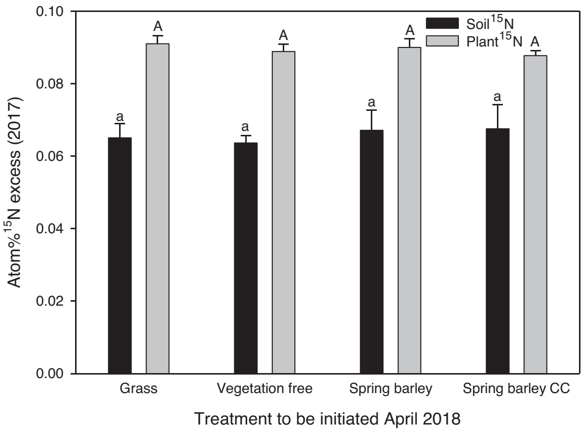

FIGURE 2 Atom% ^{15}N excess of soil and plant biomass sampled in the lysimeters in April 2017 before initiating the test phase (bars indicate standard error [SE], $n = 4$). The lysimeters are grouped according to the test treatments to be applied in April 2018: productive grassland (Grass), vegetation-free fallow (Vegetation free), spring barley without an autumn cover crop (spring barley) and spring barley with a cover crop (spring barley CC). Similar capital letters over plant ^{15}N and lower case letters over soil ^{15}N indicate no significant difference between lysimeters.

harvested in 2018 than in 2019 (Figure 4). For grass, the atom% ^{15}N excess increased from first to third cut, and in the third grass cut in 2018, atom% ^{15}N excess exceeded atom% ^{15}N excess measured in the topsoil in 2017 (Figure 2). Similarly, the atom% ^{15}N excess of the cover crops was significantly higher than observed in the preceding barley crop.

The total N loss by nitrate leaching was, as the percolation, highest for the vegetation-free treatment (Figure 5). During the period 2018–2019 and 2019–2020, the loss of N by leaching corresponded to 34.9 and 25.8 g N m^{-2}, respectively, whereas the treatment with permanent grass lost just 2.0 and 0.7 g N m^{-2} in those periods. As for the other treatments, lysimeters with spring barley showed higher nitrate leaching during 2018–2019 than during 2019–2020, presumably due to a high N release in the first year after the destruction of the grass. The use of a cover crop in the barley treatment reduced leaching from 17.2 to 5.8 g N m^{-2} in the first leaching period and from 5.7 to 3.3 g N m^{-2} in the second period.

The leaching loss of ^{15}N from the various treatments showed a similar pattern to that of total nitrate-N (Figure 5). In the period 2018–19, the leaching of ^{15}N related to residual ^{15}N present in soil in 2017 declined in the order: vegetation-free soil (4.7%), spring barley (1.9%), spring barley + cover crop (0.7%) and production grassland (0.2%). Corresponding losses for the second leaching period were 2.7%, 0.9%, 0.4% and 0.06%. Despite the very different losses associated with the widely different crops/managements and different leaching periods, the proportions of total N and of residual ^{15}N present in 0–25 cm soil in 2017 lost by leaching were closely related (Figure 6). The regression between total soil N lost by nitrate leaching and residual ^{15}N lost by nitrate

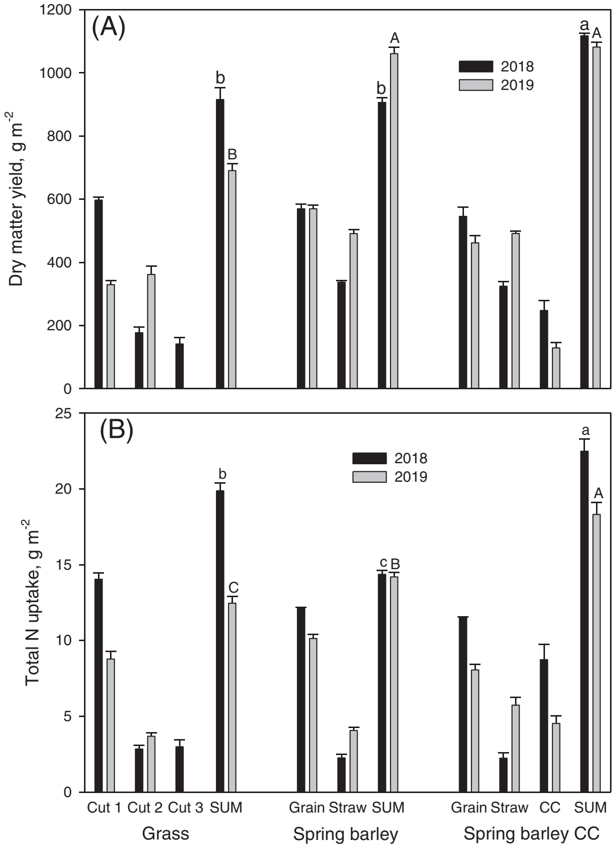

FIGURE 3 Dry matter yields (A) and total N uptake (B) of the crops grown in 2018 and 2019. Data are shown for individual grass cuts (three cuts in 2018 and two cuts in 2019), for barley grain and straw, for cover crops (CC), and for the sum of each management (bars indicate standard error [SE]; $n = 4$). See Figure 2 for crop legend. Different capital or lower case letters indicate significant differences ($p < 0.05$) for each year.

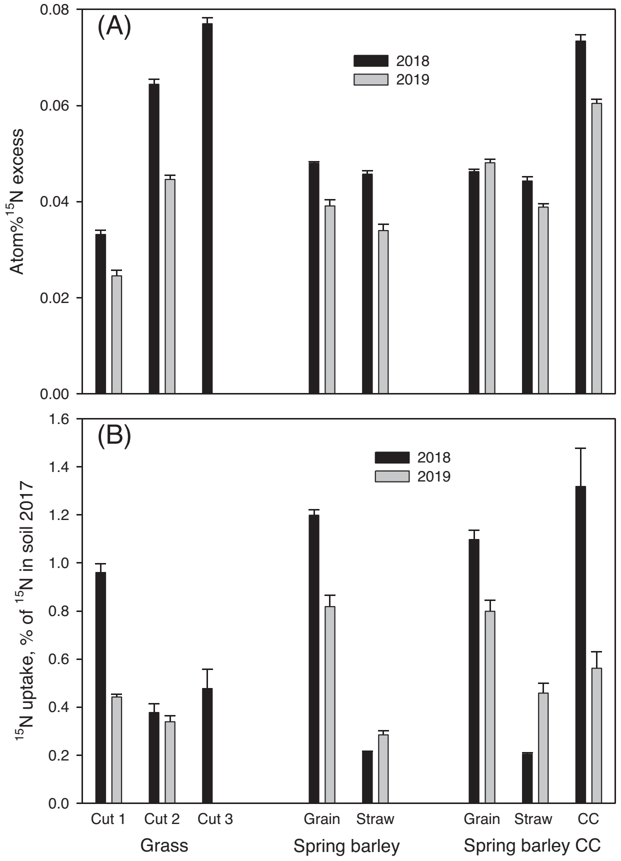

FIGURE 4 Atom% ^{15}N excess in crops (A) and uptake of labelled N (B) in crops grown in 2018 and 2019 (bars indicate standard error [SE], $n = 4$). Uptake of ^{15}N is given as % of ^{15}N in soil in April 2017. See Figures 2 and 3 for crop legends.

leaching (Figure 6) had a slope of 0.82, indicating that a smaller proportion of ^{15}N in soil was lost by nitrate leaching compared the proportion of total soil N lost by nitrate leaching. However, this could be explained by the method of calculation, as only soil N in the topsoil (0–25 cm) was accounted for in the calculation of proportion, and leaching from mineralized N in the subsoil and from the unlabelled N fertilizer addition was ignored in this calculation.

Table 2 summarizes the fate of ^{15}N residing in the 0–25 cm soil layer when it was introduced to the lysimeters in 2004 and subjected to set-aside grassland. During the period 2004–2017, the loss from the set-aside treatment was 24% of the ^{15}N residing in the soil in 2004, corresponding to an average annual loss of 1.8%. When turned under in the spring 2018 and converted to vegetation-free fallow, the loss increased to 3.6% during 2018–2019 and 2.1% during 2019–2020. When a crop was grown on the soil, the uptake of ^{15}N was 1%–2% and 0.6%–1.4% in the first and second growth periods, respectively. The proportion of ^{15}N present in soil in 2004 that was taken up by crops or leached ranged from 1.5% to 3.6% during 2018–2019 and from 0.6% to 2.1% during 2019–2020.

4 | DISCUSSIONS

4.1 | The fate of fertilizer ^{15}N in the first years after application

Our experimental set-up does not allow us to establish a budget for the ^{15}N-fertilizers applied during 1989–1993 to the 20 m^2 field plot that served as a resource for growing ^{15}N-labelled plant materials. This lack of information includes the amount of ^{15}N removed or returned in plant biomass, retained in various soil pools or lost by leaching or in gasses during the 25-year period that preceded the current study. However, it is well established from other studies that following the initial years after mineral N fertilizer application, 20%–50% the added ^{15}N is retained in soils under arable management mainly as organic-N, and that the upper 0–25 cm soil layer retains 85%–95% of the ^{15}N left in soil after 3–4 years (Glendining et al., 2001; Hart et al., 1993; Macdonald et al., 1997, 2002). Hart et al. (1993) found that a large part of the residual ^{15}N is incorporated in organic pools with intermediate resistance to mineralization, and most of the residual ^{15}N is incorporated into clay-sized (<2 μm) organo–mineral fractions (Christensen & Sørensen, 1986). A smaller part of the residual ^{15}N is present

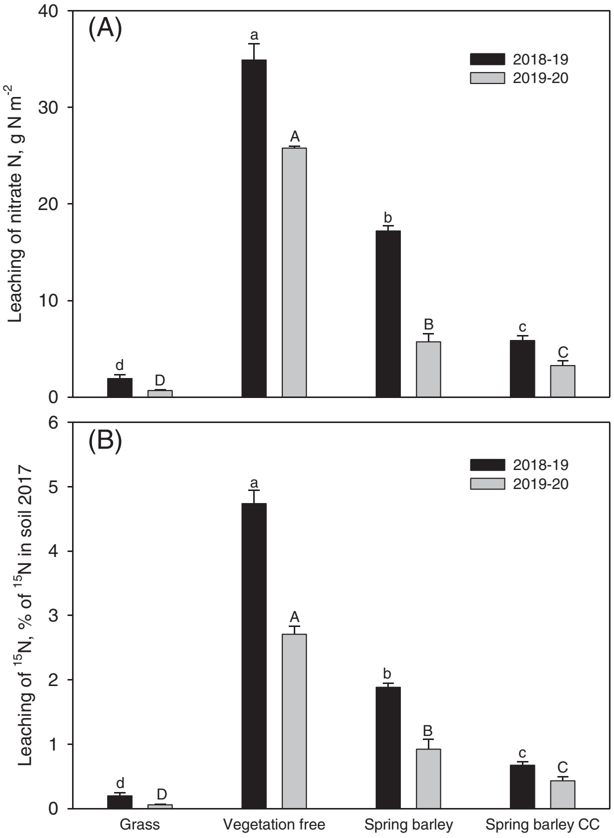

FIGURE 5 Nitrate leaching loss of N (A) and ^{15}N-labelled N (B) during the leaching periods 2018–2019 and 2019–2020 (bars indicate standard error [SE], $n = 4$). Leaching of ^{15}N is given as % of ^{15}N in soil in April 2017. See Figure 2 for treatment legends. Different capital or lower case letters indicate significant differences ($p < 0.05$) for each year.

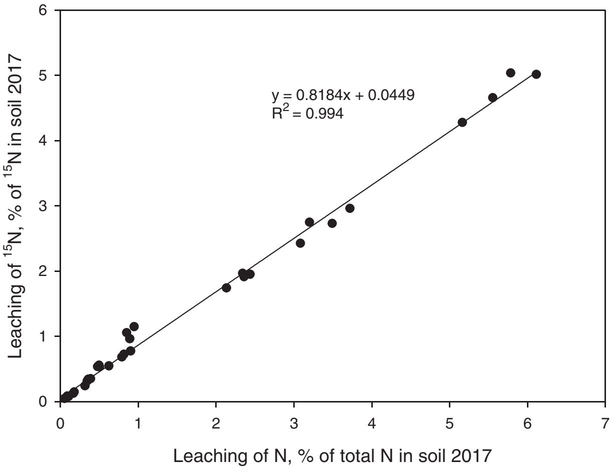

FIGURE 6 Relationship between leaching losses of labelled N in nitrate and total nitrate N estimated as % of labelled soil N and total soil N (0–25 cm in April 2017), respectively. Data points are from individual lysimeters for each leaching period and include the four treatments and two leaching periods. The linear regression line is shown.

in soil microbes, and Sørensen (2004) observed that 13% of the ^{15}N residing in soil 18 months after its application was in the soil microbial biomass. Shen et al. (1989) found that 15% of ^{15}N from fertilizer left in soil 40 months after application was in microbial biomass; this corresponded to just 1.3% of the added ^{15}N. Only a small fraction is likely to be present as mineral-fixed ammonium-N in the studied soil. For soils with 6 and 12% clay and incubated with ^{15}N-labelled ammonium for 5 years, Jensen et al. (1989) found that <1% of the added ^{15}N was present as mineral-fixed ammonium.

4.2 | Lysimeters

When relocated from the field plot to the lysimeters, each lysimeter was allocated soil equivalent to 637 mg ^{15}N m^{-2}. During the next 13 years (2004–2017) when the soil was under set-aside grassland, the amount of ^{15}N in the top soil layer decreased by 24% (Table 2). As no labelled N was removed in plant biomass, we ascribe the loss to gaseous losses by ammonia volatilization (Whitehead & Lockyer, 1989) and denitrification and nitrate leaching, as the main N loss pathways (Christensen, 2004). Before transfer from the field plot to lysimeters, the soil was subject to drying and sieving, known to facilitate mineralization of the soil organic N. We therefore expect that leaching losses of mineralized ^{15}N were highest in the initial years after soil transfer to lysimeters. The mineralized ^{15}N probably also resulted in an elevated plant uptake in the first year, and it is recalled that the plant biomass after cutting was returned to the lysimeter in which it was grown whereby the labelled N was recycled.

4.3 | The availability of residual ^{15}N compared to total soil N

When sampled in the spring 2017, the atom% ^{15}N excess was substantially higher in the grass biomass than in the top soil (Figure 2). Apparently, the labelled N, added more than 25 years ago, remained more plant available than total soil N. Similarly, the atom% ^{15}N excess was higher in the last grass cut and the cover crop in 2018 than in the top soil sampled in April 2017 (Figures 2 and 4). This is indicating that the residual ^{15}N was present in organic N pools with higher decomposability than the whole soil N pool. It has been shown that plants are able to take up simple organic N compounds such as amino acids (Neff et al., 2003; Näsholm et al., 2000, 2009), especially in low-productivity unimproved grassland (Bardgett et al., 2003). A direct plant uptake of dissolved organic ^{15}N, released from the grass roots and grass residues (Neff et al., 2003), could possibly also contribute to a higher ^{15}N enrichment in plants if the ^{15}N taken up by the grass had by-passed the soil N cycling processes and avoided dilution with mineralized soil N. The crops could also get access to unlabelled mineralized N from soil below 25 cm depth and from the new fertilizers. Despite this, the plants growing in autumn showed higher ^{15}N enrichment than N the top soil layer in 2017 indicating that the residual labelled N had a higher availability than the total soil N pool in the top soil layer.

Very few studies have examined the long-term fate of ^{15}N added in mineral fertilizers. Jenkinson et al. (2004) followed the fate of a single application of ^{15}N-labelled fertilizer to old grassland. After 19 years, successive grass cuts had removed 67% of the applied N fertilizer, whereas 15% remained in the soil and most in the top soil. In the same study, Jenkinson et al. (2004) found that less than 1% of the applied ^{15}N was in the 50–100 cm soil layer and also low amounts in the 23–50 cm soil layer. In accordance with the present study, they showed a continuous slow release of residual ^{15}N derived from the mineral fertilizer applied in the past. In the present study, we were, however, not able to relate the ^{15}N release to the original ^{15}N input. Similarly, Sebilo et al. (2013) found that after 27 years removed crops had recovered 61%–65% of the ^{15}N-labelled mineral fertilizer applied as a single dose in 1982, whereas the soil retained 12%–15%. Their study, conducted in two large outdoor lysimeters under rotating sugar beets and winter wheat crops with annual application of unlabelled fertilizers, showed that nitrate leaching to below 2 m accounted for 8%–12% of the added ^{15}N after 27 years. Sebilo et al. (2013) concluded that even after residing in soil for 27 years, the ^{15}N-fertilizer residues were still more available to plants and leaching losses than the total soil N pool. The present study similarly indicates a higher availability of ^{15}N-fertilizer residues than of the total soil N pool about 25 years after the ^{15}N application.

4.4 | The fate of residual ^{15}N after conversion of set-aside to cropping

The objective of the test phase (2018–2020) applied in our study was to elucidate the lability of ^{15}N-fertilizer residues retained in soil for 25 years, including 13 years under undisturbed and strongly N-limited set-aside grassland. The largest yields of DM and total N generally occurred in the first growing season after conversion of the set-aside grass, not considering DM and total N yields of spring barley grown without a cover crop (Figures 3 and 4). The clearest response was seen for the first grass cut in the grass treatment and for cover crops grown in the barley treatment. This pattern is ascribed to the enhanced mineralization of soil organic N induced by the soil disturbance and the killing of the grass biomass associated with termination of the set-aside grass and the ability of cover crops to retain N mineralized in the autumn after harvest of the barley crop.

Although the leaching losses of ^{15}N from vegetation-free fallow, related to soil ^{15}N in 2004, were 3.6% during the first leaching period and 2.1% during the second period (Table 2), the losses of nitrate-N, related to total N in the top soil layer in 2004, were somewhat higher (4.7% and 3.4%, respectively, data not shown). The higher loss of unlabelled N may partly reflect the addition of unlabelled NPK fertilizers that could also contribute to leaching. The 20–40 cm and 40–100 cm subsoils held 4% and 2% organic matter when introduced in 1973, and mineralization of soil N from these pools may also have contributed to nitrate leaching. Despite a higher percolation in the second leaching year (2019–2020), the leaching of both nitrate-N and nitrate-^{15}N was lower in the second year (Figure 5). The higher nitrate leaching loss in the first leaching year could be explained by a high N mineralization rate in the year following the ploughing of old grass (Eriksen et al., 2008). In the first year, percolation from the vegetated lysimeters was about 500 mm, which is high for Danish conditions, but close to the yearly average for the Askov location. The precipitation and percolation from August till March were higher than normal in the second year, but most of the N mineralized before and during autumn was probably leached in both years.

The loss of ^{15}N by leaching was substantially reduced when a crop was grown on the soil, the grass and cover crops being most effective in retaining ^{15}N from leaching. As the residual ^{15}N is mineralized during the whole year, it is critical for reducing leaching that the soil is also covered with vegetation in autumn. The differences between the loss of ^{15}N by nitrate leaching from soil under vegetation-free fallow (3.6%) and the leaching loss plus crop uptake from soil under spring barley (2.5%) during 2018–2019 may be ascribed to ^{15}N retained in the root biomass of barley. Assuming similar mineralisation potentials in all soils, the amount of ^{15}N residing in the barley root biomass may then account for 1.1% (3.6% − 2.5%) of the ^{15}N present in the soil in 2004.

We assume that the measured N leaching in the treatment without vegetation gives an approximate measure for the yearly net N mineralization, as water percolation was very high in these lysimeters, and nitrate concentration low in late winter leachates (data not shown). In Table 2, the leaching of labelled N in the treatments with vegetation is related to labelled N leached in the treatment without vegetation. This relationship gives a good indication of the proportion of mineralized N being lost by nitrate leaching with the different crop types. With spring barley, 33%–40% of the mineralized labelled N was then lost by leaching, and when including a cover crop after barley only 14%–16% was lost by leaching, whereas with the cut grass, only 2%–4% was lost, showing the very important effect of autumn and winter vegetation on leaching of mineralized N. By contrast, leaching losses from ^{15}N-labelled fertilizers in the year after application are much lower and less influenced by autumn vegetation. For instance, Thomsen et al. (1997) only found leaching losses from ^{15}N-labelled fertilizers of 3%–5% in the year after application to spring barley without a cover crop under comparable climatic conditions, showing that mineralized soil N is much more vulnerable to nitrate leaching than N in fertilizers applied in spring. This is in accordance with Frick et al. (2022) who concluded that most nitrate leaching from arable land originates from the mineralization of soil N under Swiss conditions with similar precipitation as in our study.

Relating the relative leaching loss of total soil N to that of labelled N showed the same relationship across all treatments (Figure 6). However, the relative leaching of labelled N was lower than that of soil N (slope: 0.82). This is surprising as the enrichment of plants growing in autumn had a higher enrichment than that of the labelled soil layer, and a higher enrichment than the leached nitrate (data not shown). An explanation for this could be that a larger part of the leached N derived from mineralized N (unlabelled) in deeper soil layers and from the applied unlabelled fertilizer, and apparently the cover crop and the last grass cut recovered relatively less N from these sources resulting in a higher relative contribution of unlabelled N to leaching.

5 | CONCLUSIONS

The present work confirms other studies showing that mineralized soil N including residual fertilizer N applied in the past is the major source for nitrate leaching and that plant cover in the autumn is crucial for reducing nitrate leaching from agricultural land. Crops with a long growing season can significantly reduce the leaching of nitrate derived from mineralization of soil N. After residing in soil for 25–30 years, the lability of labelled mineral N fertilizer residues incorporated in organic matter appeared slightly higher than the lability of total soil N.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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