

Nitrogen dynamics after slurry application as affected by anaerobic digestion, biochar and a nitrification inhibitor

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Abstract

Animal manures are valuable multi-nutrient fertilizers, but their short-term nitrogen (N) use efficiency (NUE) by plants is low, bearing the potential of harmful N losses to the environment, such as nitrate (NO₃⁻) leaching. To develop strategies to increase the NUE of cattle slurry, a comprehensive understanding of slurry N dynamics in the soil–plant system is needed. In a 57-day microcosm experiment in the greenhouse, we assessed the effect of different slurry treatments on slurry N turnover in the soil and its uptake by ryegrass (*Lolium multiflorum* var. *Westerwoldicum*). Employing a two-factorial design, ¹⁵N cattle slurry (SLU), ¹⁵N anaerobically digested cattle slurry (SLA), and ¹⁵N anaerobically digested cattle slurry plus biochar (SLA+) were combined with and without the nitrification inhibitor 3,4-dimethyl-1H-pyrazole monophosphate (DMPP). As references, a mineral fertilizer (MIN) and an unfertilised treatment (N0) were included. The ¹⁵N recovery, hence NUE, in plant biomass was higher for SLA than for SLU, while recovery in soil at 55 days after set-up showed an opposite trend, with over 45% of N from SLU still being recovered in soil. DMPP and biochar only marginally affected NUE and fertilizer N recovery in soil. Although ¹⁵N recovery in soil was highest for SLU, residual N leaching from SLU was low (<1% of added N). We attribute this to the limited presence of slurry N in mineral forms at this point of time, with the majority being stored in the non-microbial organic soil N pool. Leaching of residual N from MIN was significantly higher for MIN than for SLU, while SLA and SLA+ ranged in between. Overall, anaerobic digestion appeared suitable for increasing NUE of cattle slurry, but further investigations under field conditions are necessary in order to assess its potential to reduce nitrate leaching in the long-term.

KEYWORDS

¹⁵N labelling, digestate, DMPP, nitrate leaching, NUE, soil N dynamics

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1 | INTRODUCTION

Animal manures are a valuable source of nutrients for crops, but their targeted use as nitrogen (N) fertilizer is challenging. The difficulty arises from the variable proportion of mineral N directly available to plants and a considerable share of organic N (30%–75% of total N) in manure, which must first be mineralised to become available to plants (Webb et al., 2013). This makes it difficult to synchronize plant N demand with N supply from organic manures, resulting in low N use efficiencies (NUE) (Gutser et al., 2005; Webb et al., 2013). Mineral N that is not taken up by plants is susceptible to harmful losses to the environment, such as nitrate (NO_3^-) leaching (Grizzetti et al., 2011), ammonia (NH_3) volatilization (Guthrie et al., 2018), or nitrous oxide (N_2O) emissions (Eggleston et al., 2006). At the same time, the use of animal manures is crucial for closing nutrient cycles and could replace considerable amounts of mineral fertilizer (Zavattaro et al., 2017), if application amount, type and timing are optimized and losses to the environment are minimized.

Anaerobic digestion of animal manure has been suggested as a means to facilitate synchronization between N supply and crop N demand (Möller et al., 2008). During anaerobic digestion, high-molecular-weight organic compounds are broken down, producing biogas and digestate (Khoshnevisan et al., 2021). Compared to their feedstock, digestates are characterized by a lower dry matter and organic carbon (C) content, but an increased ratio of ammonium (NH_4^+) to total N (N_{tot}) and an elevated pH (Möller & Müller, 2012). These changes potentially increase the short-term N availability to crops compared to undigested slurry, facilitating a more targeted application to the plants' needs in terms of both amount and timing (Gutser et al., 2005; Möller et al., 2008). Nevertheless, estimates of the effect of anaerobic digestion on N transformation processes in soil, N availability to crops and leaching potential remain uncertain as contradictory results have been reported (Nkoa, 2014). General conclusions are only possible to a limited extent, as manures and digestates have quite variable properties depending on animal feed (Sørensen et al., 2003) and conditions during the digestion process (Möller & Müller, 2012). So far, most studies have focused on agro-industrial digestates, which are usually not produced from animal manure alone, but digested together with co-substrates, such as corn silage, green waste or sewage sludge to optimize biogas yield (e.g., Fouda et al., 2013; Nicholson et al., 2017; Svoboda et al., 2013). A mechanistic understanding of the effect of digestion on the fertilizer NUE of animal manure requires digestion without co-substrate, for which there are fewer studies (e.g., Cavalli et al., 2018; Huf & Olf, 2020; Möller et al., 2008).

Biochar, a solid by-product from pyrolysis of organic matter, has attracted considerable scientific and public interest for its potential to improve soil fertility and sequester C in soil (Bolan et al., 2022; Lehmann & Joseph, 2015). Adding biochar to digestates as a fertilizer additive might alleviate N losses by reversible adsorption of cations and anions to its highly porous structure (Sarkhot et al., 2013), and positively affect soil quality when applied repeatedly (Laird & Rogovska, 2015). In a meta-analysis, Borchard et al. (2019) found that biochar reduced NO_3^- leaching from soil by 13% on average. According to them, the underlying mechanisms may involve sorption of NO_3^- either directly to the biochar (Yao et al., 2012) or to organic coatings of the biochar (Hagemann et al., 2017), and/or biochar-induced alterations in physical soil properties such as water retention (Clough et al., 2013). However, significant reductions were only observed at high biochar application rates to the soil and were dependent on soil type (greater effects in coarse and sandy soils), pH (reduction at $\text{pH} < 5.5$) and land use (reduction only in arable, not grassland). Furthermore, the combination of biochar and different types of fertilizer affected the outcome, with organic fertilizers being clearly underrepresented in their meta-analysis. While there is ample evidence that biochar interacts with soil N transformations in various ways (Clough et al., 2013; Liu et al., 2018), the underlying drivers remain largely unresolved (Bradley et al., 2015; Fiorentino et al., 2019). ^{15}N labelling has been shown to be a suitable method to disentangle several simultaneous and interconnected processes related to fertilizer and soil N cycling that are directly or indirectly affected by biochar (Craswell et al., 2021; Schouten et al., 2012). However, to the best of our knowledge, the effect of biochar addition to ^{15}N labelled anaerobically digested cattle slurry and subsequent application to a temperate arable soil has not yet been studied.

Nitrification inhibitors (NIs), which are synthetic or biological compounds that reduce microbial nitrification in soil, have been proposed as another means to reduce N losses from agriculture. Delaying nitrification of fertilizer N added in the form of NH_4^+ could reduce N leaching, while minimizing N_2O emissions from both nitrification and denitrification. A meta-study showed the potential of synthetic NIs to reduce total N losses by on average 16.5%, while NO_3^- leaching was reduced by 47% (Qiao et al., 2015). Manufacturers of synthetic NIs claim not only lower N losses but also higher yields due to increased NUE (Sanz-Gomez et al., 2017). However, it seems that these effects depend on the form of NI (Qiao et al., 2015; Yang et al., 2016), the formulation and application method (Ruser & Schulz, 2015), the type of fertilizer (Qiao et al., 2015), the fertilizer rate (Rose et al., 2018; Rowlings et al., 2016), as well as abiotic soil conditions such as texture (Barth et al., 2019), temperature or pH (Zerulla

et al., 2001). While a broad range of potential NIs have been identified (Ruser & Schulz, 2015), only a few are currently used commercially, of which 3,4-dimethyl-1H-pyrazole monophosphate (DMPP) appears to be the most suitable as it is less phytotoxic and effective at lower application rates and over longer time spans than most other NIs (Yang et al., 2016; Zerulla et al., 2001). However, in most studies, DMPP has been combined with mineral fertilizers, while its use with different organic fertilizers has been less widely studied.

The objective of this study was to evaluate the potential of anaerobic digestion, biochar, and DMPP as well as their interactions to increase NUE, defined here as ^{15}N fertilizer recovery in plant biomass, and to reduce N losses from cattle slurry. To this end, a two-factorial microcosm experiment was set-up with the following 10 treatments: 0 N-control, ^{15}N ammonium sulphate, ^{15}N cattle slurry, ^{15}N anaerobically digested cattle slurry, and ^{15}N anaerobically digested cattle slurry plus biochar, each with/without DMPP. We measured N uptake from the fertilizers by annual ryegrass, traced N fluxes in soil, and assessed the effect of the treatments on N leaching from the residual N after 57 days of ryegrass growth. We hypothesised that (i) N uptake by plants from anaerobically digested slurry would be greater than from undigested slurry due to a higher NH_4^+ – N share in the digested slurry, (ii) the addition of biochar to the digested slurry would increase NUE by reversibly binding NH_4^+ , (iii) the addition of DMPP to the fertilizers would delay nitrification and prolong N uptake, irrespective of the fertilizer type, and (iv) consequently all mentioned slurry treatments (anaerobic digestion, biochar and DMPP) would reduce the residual N leaching after 57 days compared to untreated slurry.

2 | MATERIAL AND METHODS

2.1 | Experimental approach

A microcosm experiment with 10 treatments was established: five fertilizer treatments were combined in a two-factorial design with and without the nitrification inhibitor DMPP: 0 N-control (NO), ^{15}N ammonium sulphate (MIN), ^{15}N cattle slurry (SLU), ^{15}N anaerobically digested cattle slurry (SLA), and ^{15}N anaerobically digested cattle slurry plus biochar (SLA+), each with/without DMPP. The treatments were replicated four times.

In order to allow for repeated soil sampling during the experiment while preserving undisturbed microcosms for other measurements, the experiment was duplicated into a destructive set (D) for soil sampling and a non-destructive set (G) for gas measurements (Efosa et al., *in prep.*) and other analyses such as soil pore water

sampling. This resulted in a total of 80 microcosms (5 fertilizer treatments \times 2 nitrification inhibitor treatments \times 4 replicates \times 2 sets for destructive/non-destructive sampling). The microcosms were arranged in a complete randomized block design on movable tables in the greenhouse. Corresponding columns from the G- and D-set were placed next to each other. Tables within each block were rotated weekly, as were the entire blocks.

2.2 | Characteristics of soil, fertilizers and additives

Topsoil (0–20 cm) was collected from an organically managed field (47°35'50.5" N 8°11'57.7" E) for the experiment. The soil was a silty loam with a pH of 6.4 (Table 1). Soil was sieved field moist to 5 mm, air-dried, and stored at room temperature for about 14 months. Nine days before set-up, 400 kg of dry soil was moistened with demineralised water to about 40% of the maximum water holding capacity (maxWHC) and pre-incubated under a plastic sheet in the greenhouse to allow the microbial community to revive and adjust to the conditions in the greenhouse.

^{15}N labelled cattle slurry was produced by feeding a young heifer with ^{15}N labelled ryegrass hay for 8 days after an adaptation phase (Frick, Oberson, Cormann, et al., 2022). Faeces and urine were sampled separately and frozen daily at -20°C . Later, faeces and urine fractions with the highest ^{15}N label were recombined and diluted 1:1 with demineralised H_2O in order to achieve a representative slurry (Table 2).

A subsample of the same slurry was anaerobically digested on an Automatic Methane Potential Test System (AMPTS II, Bioprocess Control). The ^{15}N slurry was inoculated with 4% (w/w) of an external digestate from an agricultural biogas plant and split up into 500 mL Schott bottles. The slurry was fermented under mesophilic conditions (40.5°C) with regular stirring (45 s stirring every 300 s) for a period of 37 days. The process was stopped when the daily methane yield over three consecutive days had dropped below 1% of the total produced methane (Holliger et al., 2016). Batches were recombined and thoroughly mixed. Average cumulative methane yield was $369 \pm 15 \text{ L kg}^{-1}$ organic dry matter (Standard Temperature and Pressure), indicating that the digestion process was complete in all batches and comparable to fermentation of cattle slurry in an agricultural biogas plant (Achilles et al., 2013). Both slurry and digested slurry were stored frozen at -20°C until 2 days before set-up of the experiment, when they were slowly thawed and kept at 4°C .

For the mineral fertilizer treatment, a ^{15}N ammonium sulphate solution with an enrichment of 7 atom% ^{15}N abundance was prepared.

TABLE 1 Soil characteristics: texture, total N (Ntot) and organic C (Corg), pH determined in water (pH_{H₂O}) as well as maximum water holding capacity (maxWHC).

Clay	Silt	Sand	Ntot	Corg	pH _{H₂O}	maxWHC
g kg ⁻¹ dry soil					–	g H ₂ O g ⁻¹ dry soil
140	260	560	1.9	19.8	6.4	0.40

TABLE 2 Characterization of ¹⁵N slurry (¹⁵N-SLU) (Frick, Oberson, Cormann, et al., 2022) and anaerobically digested ¹⁵N slurry (¹⁵N-SLA).

	Dm ^a	Corg ^a	Ntot ^a	NH ₄ ⁺ – N ^a	NDF ^b	NDF-N ^b	pH ^a	¹⁵ N-Ntot ^c	¹⁵ N-NDF ^b
	%	g kg ⁻¹ dry matter					–	atom% excess	atom% excess
¹⁵ N-SLU	3.3	393	68.4	42.0	268	3.2	7.9	7.504	7.731
¹⁵ N-SLA	2.7	313 ^d	94.6	62.0	214	4.5	8.0	7.019	6.365

^aParameters were determined on subsamples of the fresh slurry.

^bNeutral detergent fibre (NDF), nitrogen in neutral detergent fibre fraction (NDF-N) and ¹⁵N enrichment in NDF-N (¹⁵N-NDF) were analysed in slurry dried at 60°C.

^cParameters were determined on acidified and freeze-dried subsamples.

^dCalculated based on loss on ignition.

Biochar was produced from tree and shrub cuttings at 500–600°C in a PYREG reactor (PYREG GmbH) and characterized according to the guidelines of the European Biochar Certificate (Schmidt et al., 2016). It contained 7.1 g N kg⁻¹ dry matter and 790 g organic carbon kg⁻¹ dry matter and had a pH_{H₂O} value of 8.7. Milled biochar (<2 mm) was used in order to facilitate mixing with the digested cattle slurry.

DMPP (CAS: 202842-98-6, Cayman Chemicals) was used as nitrification inhibitor. A DMPP solution containing 8.4 mg DMPP mL⁻¹ was prepared the evening before set-up of the experiment. Upon mixing the fertilizers into the soil, 1 mL DMPP solution was added to the fertilizers applying DMPP at a rate of 2% of the total N added with the fertilizers (i.e. 2 g DMPP per 100 g total fertilizer N).

2.3 | Set-up and maintenance of the microcosms

Each microcosm consisted of a cylindrical PVC tube with 15 cm diameter and 25 cm height (Figure 1). The bottom was closed with a PVC plate with a drain tap in the middle to allow for leachate collection (Bender et al., 2015). In order to avoid water-saturated conditions in the soil, a 2 cm drainage layer consisting of 400 g moist sand (0.2–0.6 mm grain size) was added to the bottom of the columns.

All fertilizer treatments were normalized to a rate of 90 mg Ntot kg⁻¹ dry soil, assuming negligible N addition by DMPP or biochar. Mixing of soil and fertilizers was done separately for each column. Immediately before mixing with the soil, fertilizers were mixed with DMPP solution, where applicable, and with demineralised water to

achieve the same amount of liquid as added with the SLU treatment. Biochar had been added to the SLA+ treatment at a rate of 2.2% (w/w) of the fresh weight of SLA 13 h before set-up to allow SLA-derived nutrients to bind to the biochar surface. Upon set-up, all microcosms also received a basal micro- and macronutrient fertilization with a modified N-free Hoagland solution that provided the following nutrient levels (mg kg⁻¹ dry soil): K 250, P 50, Ca 102, Mg 48, Zn 1, Mo 0.1, Fe 1, B 1, Mn 2, Cu 2, and Co 0.1.

Soil was thoroughly mixed with fertilizer treatments and the Hoagland solution and packed into columns at a bulk density of 1.3 g cm⁻³ and a soil height of 20 cm (split in four equal layers of 5 cm each for more homogenous compaction). In order to reach 60% maxWHC, additional demineralised water was added on top of each layer after compaction. A water content of 60% maxWHC was chosen as this was shown to represent optimal conditions for mineralisation and nitrification while denitrification was minimized (Drury et al., 2003; Linn & Doran, 1984). In the G-set, rhizon suction samplers (Rhizosphere Research Products) were installed at 5 and 15 cm soil depth for repeated non-destructive soil pore water sampling in order to assess N transformation processes in the soil at high temporal resolution. In one column per treatment of the D-set, a tensiometer (MPS6, Meter Environment) was installed at 10 cm depth in order to monitor water potential and soil temperature during the experiment. Ryegrass (*Lolium multiflorum* var. *Westerwoldicum*, Pulse) was sown at a seed density of 30 g m⁻² (i.e. 0.53 g column⁻¹) on the top of each column and covered with a thin layer of vermiculite. The columns were additionally covered with plastic wrap during the first 5 days in order to facilitate germination.

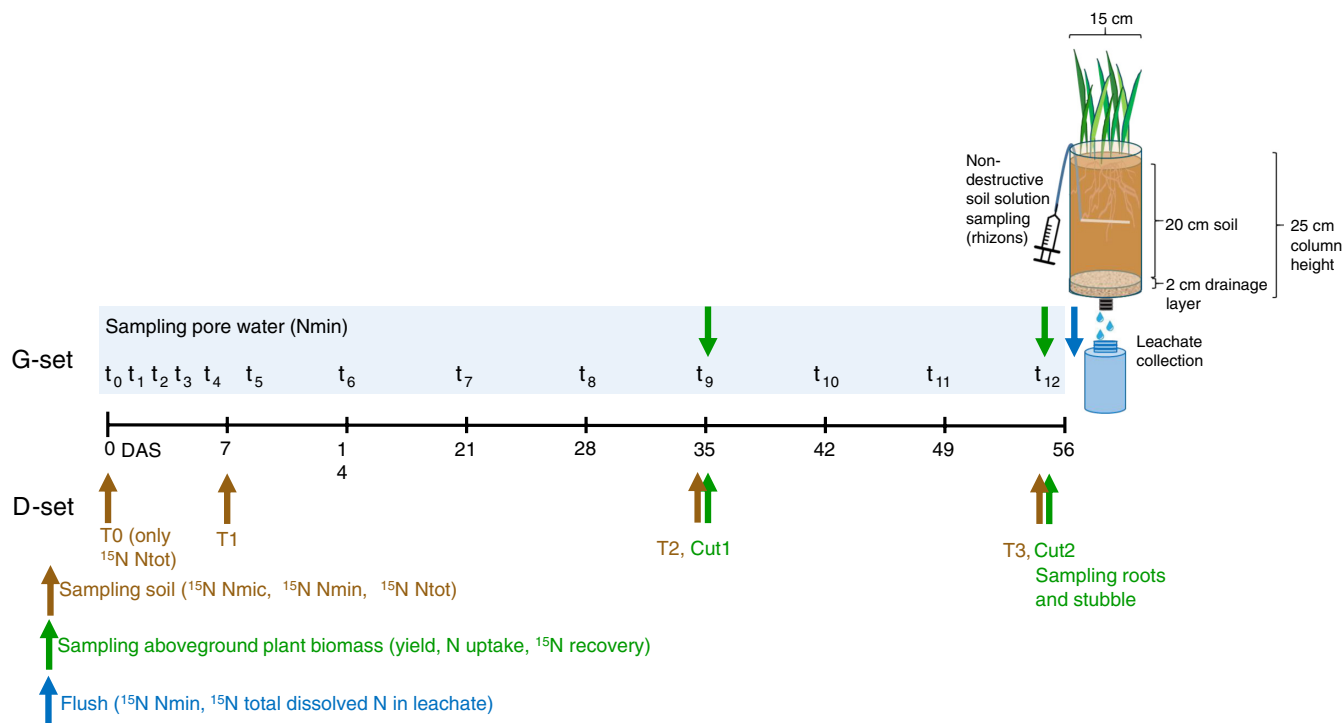


FIGURE 1 Sampling scheme and dimensions of the columns. Nmic = microbial N, Nmin = mineral N ($\text{NH}_4^+ - \text{N} + \text{NO}_3^- - \text{N}$), Ntot = total N. D-set was used for soil sampling and biomass sampling. G-set was used for non-destructive sampling of soil pore water with rhizons during the plant growth period and for leachate collection (“flush”) after the second cut.

The experiment was conducted between 5th of August and 1st of October 2020 in a greenhouse at the Research Institute of Organic Agriculture (FiBL), Frick, Switzerland (47°31′02.3″ N 8°01′35.5″ E). Average temperature was 21.5°C (range: 10.2–39.2°C, median: 20.5°C) and average relative humidity was 61% (range: 22%–89% rel. humidity, median: 63% rel. humidity). No supplemental light was provided. Columns were watered daily with demineralised water to gravimetrically adjust water content and maintain constant moisture conditions, which effectively ranged from 40% to 65% of maxWHC.

2.4 | Soil pore water sampling

Soil pore water samples were taken 1, 2, 3, 6, 9, 14, 22, 28, 36, 43, 49, and 56 days after set-up (DAS) by attaching syringes to the plug of the rhizon suction samplers and installing a vacuum by inserting a wooden retainer (Figure 1). In the beginning, syringes were attached in the late afternoon, 1 h after adjusting water content to 60% of maxWHC and left overnight. It was aimed to extract between 5 and 10 mL of soil pore water from each rhizon sampler. However, from Day 9 onwards, it was difficult to extract enough soil pore water for analysis with this procedure. Therefore, sampling was carried out during the day where vacuum could be applied repeatedly. In

addition, 100–150 mL of water (depending on the growth stage of the grass) was added about 2 h before sampling began to facilitate extraction of soil pore water. Soil pore water samples from 5 and 15 cm depth of the same column were pooled and stored frozen until analysis for mineral N (NH_4^+ and NO_3^-) (see Section 2.6). Since it was not always possible to extract soil pore water from both depth layers, in some cases, samples only consisted of soil pore water from one depth layer. However, concentrations tended to deviate from pooled samples, with lower values for the 5 cm rhizons and higher values for the 15 cm rhizons. For this reason, the rhizon data only provide semi-quantitative information.

2.5 | Soil and biomass sampling

At 7, 35, and 55 DAS, soil samples were taken from the entire soil depth of the D-set to analysis the ¹⁵N label in both Ntot as well as in the microbial (Nmic) and in the mineral N pool (Nmin) (Figure 1). At each sampling time, the soil from three cores per microcosm (2 cm diameter) was pooled, thoroughly homogenized, and stored in a cooling box until extraction. The boreholes were refilled with sealed PVC-tubes.

On the sampling day, two subsamples of 20 g dry weight equivalent from each microcosm were extracted

using the chloroform fumigation extraction (CFE) method in order to determine Nmic (Brookes et al., 1985; Vance et al., 1987). One subsample was extracted immediately with 80 mL 0.5 M K₂SO₄, while the other subsample was fumigated with chloroform for 20–24 h and then extracted. The extracts were filtered through folded paper filters and stored at –20°C until analysis. Additionally, Nmin was measured on the non-fumigated extracts. The remaining soil was air dried, pulverized in a ball mill (MM200 Retsch) and analysed for ¹⁵N-Ntot.

For analysis of ¹⁵N enrichment in the soil Nmic and Nmin pools, extracts from CFE extraction were processed using a diffusion technique adapted from Goerges and Dittert (1998). For analysis of ¹⁵N-Nmic, both fumigated and non-fumigated extracts were oxidized by autoclaving with K₂S₂O₈ (Cabrera & Beare, 1993) and afterwards diffused on acidified quartz filter traps (Whatman QM/A) by adding Devarda's alloy (0.4 g per sample), 4 mL 5 M NaCl, and 0.75 mL 5 M NaOH per 10 mL of extract (Goerges & Dittert, 1998; Mayer et al., 2003). NH₄⁺ and NO₃⁻ were diffused together on the same filter from non-fumigated extracts to determine ¹⁵N-Nmin following a similar procedure, but by adding 0.2 g MgO, instead of NaCl and NaOH (Douxchamps et al., 2011). After drying, the filters were encapsulated in tin capsules and analysed for ¹⁵N (see Section 2.7).

The aboveground biomass of all columns (D- and G-set) was harvested twice, at 35 and 55 DAS at a height of ca. 2.5 cm (Figure 1). During the final sampling (55 DAS), stubble biomass and root biomass of the D-set were sampled as well. Root biomass was quantified by washing the entire content of each column through a 1 mm sieve. The sieve residue was separated from mineral residues and exogenous organic material by combined decantation and manual sorting with tweezers (Hirte et al., 2017). Shoot and stubble biomass samples were dried at 40°C, while root biomass was dried at 60°C due to high moisture content after root washing. The dried biomass was milled in a centrifugal mill (ZM200, Retsch), and then pulverized in a ball mill and analysed for N and ¹⁵N (see Section 2.7).

2.6 | Flush

In order to assess the leachable fraction of residual fertilizer N at the end of the experiment, 2 days after the last biomass cut, the undisturbed columns (G-set) were oversaturated with demineralised water and the drain tap was opened to collect the soil leachate. Since the drain tap had been filled with glass wool during set-up, the collected leachate was already clear and not subsequently filtered (Bender et al., 2015). We aimed to slowly add demineralised water to reach 105% maxWHC. However, since infiltration varied between columns, on average only 94%

maxWHC (range 82%–103% maxWHC) was reached over a period of 12 h. After drainage of the first flush (about 12 h later), a second flush was conducted by adding another 500 mL of demineralised water at once and immediately starting the drainage. This second flush gave similar or lower concentrations in Nmin than the first flush, indicating that the concentrations of the first flush represent the kinetic equilibrium concentrations of the saturated soil extract. Water content in the columns after the first flush varied between 76% and 95% maxWHC, indicating that both infiltration and drainage did not work equally well in all columns. Therefore, the cumulated amount of N washed out across both flushes is reported.

The leachates from the first flush were diffused as described for the CFE samples to determine ¹⁵N recovery in Nmin as well as in dissolved organic N (DON; calculated as total dissolved N minus Nmin). It was assumed that the ¹⁵N enrichment did not change between the first and the second flush.

2.7 | Chemical analyses

NH₄⁺ and NO₃⁻ concentrations in the soil pore water, in the non-fumigated CFE-extracts, and in the leachate from the flushes were analysed spectrophotometrically on an automated discrete analyser (Smartchem 450, AMS Alliance) according to Keeney and Nelson (1982) for NO₃⁻ and according to Krom (1980) for NH₄⁺. Total dissolved N in fumigated and non-fumigated soil extracts was measured with a TOC/TNb-analyser (multi N/C 2100S, Analytik Jena). Nmic was calculated as the difference between fumigated and non-fumigated extracts using a conversion factor of $k_{EN}=0.54$ (Joergensen & Mueller, 1996). Non-microbial organic N (Norg) in soil was calculated as the difference between total N and the sum of Nmic and Nmin. All ¹⁵N analyses (soil samples, biomass samples, diffusion filters) were performed on an elemental analyser coupled to a continuous flow isotope ratio mass spectrometer (Pyro cube+isoprime100, Elementar). The characterization of ¹⁵N labelled slurry and anaerobically digested slurry (dry matter, pH, N, NH₄⁺, macro- and micronutrients, volatile fatty acids (VFA), heavy metals) was performed by bonalytic GmbH (Troisdorf).

2.8 | Calculations

For all ¹⁵N data, the isotopic excess was calculated by subtracting the mean ¹⁵N abundance (i.e. proportion of ¹⁵N relative to total N) of non-labelled reference samples from the measured ¹⁵N abundance. For MIN, the natural abundance of ¹⁵N in air was subtracted as reference (i.e. 0.366 atom%), while for SLU and SLA the weighted mean ¹⁵N

abundance of non-labelled faec and urine samples from the same heifer shortly before starting to feed with ^{15}N labelled feed was used as non-labelled reference (0.386 atom%). For plant biomass, soil, soil extracts and leachate, the mean ^{15}N abundance of the corresponding sample type (plant, soil, extracts, leachate) from the N0 treatment at the corresponding sampling time was used as a reference.

The ^{15}N excess was used to calculate the N fraction derived from fertilizer (Ndff_{rel} [%]) in the corresponding compartment (Hauck & Bremner, 1976):

$$\text{Ndff}_{\text{rel}} = \frac{\text{atom} \% \text{ } ^{15}\text{N}_{\text{excess sample}}}{\text{atom} \% \text{ } ^{15}\text{N}_{\text{excess fertilizer}}} \times 100 \quad (1)$$

where $\text{atom} \% \text{ } ^{15}\text{N}_{\text{excess sample}}$ is the ^{15}N enrichment of the considered sample (i.e. plant (part), soil, extracts) and $\text{atom} \% \text{ } ^{15}\text{N}_{\text{excess fertilizer}}$ refers to N enrichment of either mineral fertilizer, slurry or digested slurry.

The amount of N derived from the fertilizer (Ndff [g kg^{-1} soil]) was calculated as:

$$\text{Ndff} = \frac{\text{Ndff}_{\text{rel}}}{100} \times \text{TN}_i \quad (2)$$

where TN_i is the total amount of N in the considered sample [mg N kg^{-1} soil]. For biomass samples taken from the D-set, TN_i was corrected for the amount of soil removed from the column by soil sampling.

The ^{15}N enrichment in the N_{mic} -pool was calculated according to Mayer et al. (2003):

$$^{15}\text{N}_{\text{mic}}[\text{atom} \%] = \frac{\text{total N}_{\text{fum}} \times \text{atom} \% \text{ } ^{15}\text{N}_{\text{excess fum}} - \text{total N}_{\text{nonfum}} \times \text{atom} \% \text{ } ^{15}\text{N}_{\text{excess nonfum}}}{\text{total N}_{\text{fum}} - \text{total N}_{\text{nonfum}}} \quad (3)$$

where “fum” indicates fumigated samples while “nonfum” indicates non-fumigated samples.

The recovery, hence NUE, of the applied fertilizers in the different samples was then calculated as:

$$\text{recovery}[\%] = \frac{\text{Ndff}}{\text{N}_{\text{applied}}} \times 100 \quad (4)$$

where $\text{N}_{\text{applied}}$ is the total amount of N applied with the labelled fertilizers. The fertilizer N taken out from the D-set by soil sampling was less than 1.5 mg kg^{-1} soil and was considered negligible.

2.9 | Statistical analyses

Data preparation and statistical analysis were performed using R (Version 3.5.3) (R Core Team, 2019). A significance level of $p < .05$ was used throughout.

Statistical analyses were conducted using linear mixed effect models (lmer within package *lme4*) (Bates et al., 2015). Model validation was performed by qq-plotting and Shapiro Wilk Normality test. In case the assumptions of normal distribution or homoscedasticity of the residuals were violated, the analysis was performed with transformed data (log or square root). The *emmeans*-package (Lenth, 2020) was used for pairwise comparisons. The *p*-value adjustment for multiple comparisons was performed using the Tukey-method.

For dry matter yield, TN uptake, Ndff and recovery of shoot biomass, the mixed effect linear models included the factors *fertilizer treatment*, *DMPP*, and *cut* as well as their twofold and threefold interactions as fixed effects and *block* as random effect. Due to repeated measurements upon cuts, also *ID* was added as random factor, which specified the individual columns. The same approach was used for Ndff and ^{15}N recovery in N_{tot} , N_{mic} , N_{org} and N_{min} . Since root and stubble biomass were only sampled once at the end of the experiment, a simplified model was fitted with *treatment*, *DMPP*, and their interaction as fixed effect and *block* as random effect. The simplified model was also applied to the leached total dissolved N upon the flush. Since N_{min} concentrations in the leachate were very low (for NO_3^- , more than half of the samples below limit of detection of 0.13 mg L^{-1}), no statistical analysis was performed. In addition, due to difficulties with the extraction of soil pore water with rhizons (as described in Section 2.4), the data was not statistically analysed and must be considered semi-quantitative.

Plant biomass and soil parameters are shown for the D-set, while analysis of leachate and rhizon extracts was only possible for the G-set. Only shoot biomass was sampled for both sets and dry matter yield did not differ between D- and G-set (data not shown).

3 | RESULTS

3.1 | Biomass production, N derived from fertilizer and fertilizer recovery in biomass

Biomass dry matter yield and N uptake were highest for MIN and lowest for N0, for both the first and second cut (Table 3). The organic fertilizer treatments performed intermediate and differences between them were marginal, while SLU tended to have slightly lower yield and N uptake than SLA. The differences between the fertilizer

TABLE 3 Biomass yield, total nitrogen (TN) uptake and N derived from fertilizer ($N_{df_{rel}}$) over the two consecutive cuts.

	Treatment	Dry matter yield		TN uptake		$N_{df_{rel}}$	
		DMPP	no	DMPP	no	DMPP	no
		[g kg ⁻¹ soil]		[mg kg ⁻¹ soil]		[%]	
Shoot (Cut 1)	N0	1.06 ± 0.27	1.15 ± 0.14 a	38.5 ± 7.4	40.7 ± 9.1 a	[-]	[-]
	MIN	1.50 ± 0.21	1.23 ± 0.25 b	79.4 ± 6.5	68.6 ± 12.9 c	48.0 ± 2.8 b	48.2 ± 3.1 b
	SLA	1.20 ± 0.18	1.05 ± 0.35 ab	62.1 ± 6.5	54.5 ± 13.7 b	37.4 ± 4.0 a	42.6 ± 1.5 a*
	SLA+	1.34 ± 0.30	1.24 ± 0.16 ab	68.8 ± 12.3	63.3 ± 4.6 bc	41.1 ± 1.6 a	42.9 ± 1.5 a
	SLU	1.21 ± 0.31	1.13 ± 0.29 ab	57.0 ± 6.4	56.5 ± 11.4 b	38.0 ± 2.6 a	40.5 ± 5.1 a
Shoot (Cut 2)	N0	0.42 ± 0.07	0.38 ± 0.01 a	9.3 ± 1.3	8.1 ± 0.8 a	[-]	[-]
	MIN	0.93 ± 0.11	1.11 ± 0.13 c	34.8 ± 8.6	43.9 ± 8.7 c	45.4 ± 2.1	48.1 ± 2.0 c
	SLA	0.82 ± 0.11	0.73 ± 0.10 bc	26.3 ± 10.9	26.0 ± 11.8 b	36.3 ± 2.2	38.0 ± 1.3 b
	SLA+	0.75 ± 0.11	0.76 ± 0.07 bc	20.9 ± 5.9	20.7 ± 4.9 ab	37.0 ± 1.7	38.6 ± 1.6 b
	SLU	0.70 ± 0.14	0.63 ± 0.16 b	20.9 ± 11.0	20.0 ± 8.6 ab	32.4 ± 0.9	33.9 ± 2.9 a
Stubble	N0	0.30 ± 0.02a	0.36 ± 0.03 ab*	3.0 ± 0.6	3.4 ± 0.6 a	[-]	[-]
	MIN	0.46 ± 0.04b	0.47 ± 0.04 c	10.0 ± 2.1	10.1 ± 0.6 c	45.0 ± 2.1 c	46.8 ± 2.1 b
	SLA	0.45 ± 0.06b	0.36 ± 0.04 ab**	8.0 ± 2.9	7.5 ± 3.0 bc	35.8 ± 1.4 ab	37.8 ± 0.6 a
	SLA+	0.39 ± 0.04b	0.42 ± 0.02 bc	5.9 ± 0.9	6.2 ± 1.2 b	39.0 ± 2.2 b	39.0 ± 1.4 a
	SLU	0.40 ± 0.05b	0.34 ± 0.02 a*	6.0 ± 1.7	5.6 ± 1.2 b	32.8 ± 1.5 a	36.4 ± 2.7 a*
Roots	N0	0.50 ± 0.07	0.52 ± 0.11 ns	5.0 ± 0.5	4.9 ± 0.8 ns	[-]	[-]
	MIN	0.40 ± 0.18	0.37 ± 0.07 ns	6.0 ± 1.3	6.1 ± 0.8 ns	41.7 ± 1.6	43.1 ± 3.2 c
	SLA	0.41 ± 0.13	0.36 ± 0.18 ns	5.4 ± 1.1	4.9 ± 1.1 ns	31.7 ± 1.9	33.9 ± 1.7 ab
	SLA+	0.41 ± 0.14	0.50 ± 0.11 ns	4.9 ± 1.3	6.0 ± 0.9 ns	33.1 ± 2.4	33.6 ± 2.5 b
	SLU	0.41 ± 0.20	0.35 ± 0.07 ns	5.7 ± 1.5	4.8 ± 0.4 ns	27.9 ± 3.6	30.6 ± 3.3 a
Total biomass	N0	2.29 ± 0.31	2.41 ± 0.14 a	55.7 ± 6.9	57.1 ± 9.5 a	[-]	[-]
	MIN	3.29 ± 0.46	3.18 ± 0.35 c	130.3 ± 6.6	128.8 ± 13.3 c	46.8 ± 2.5 b	47.9 ± 2.5 b
	SLA	2.88 ± 0.29	2.50 ± 0.50 ab	102.0 ± 8.2	92.9 ± 3.4 b	36.8 ± 1.6 a	40.5 ± 1.6 a*
	SLA+	2.89 ± 0.47	2.92 ± 0.16 bc	100.4 ± 9.8	96.3 ± 3.4 b	39.8 ± 1.2 a	41.3 ± 1.2 a
	SLU	2.77 ± 0.43	2.46 ± 0.32 ab	89.6 ± 7.9	86.8 ± 7.8 b	35.9 ± 4.5 a	38.3 ± 4.5 a

Note: Stubble and roots were only sampled upon the second cut. Data represents mean ± standard deviation, $n = 4$; N0 = no N fertilizer, MIN = ¹⁵N mineral fertilizer, SLA = ¹⁵N anaerobically digested slurry, SLA+ = ¹⁵N anaerobically digested slurry + biochar, SLU = ¹⁵N cattle slurry. Letters indicate significant differences between the fertilizer treatments ($p < .05$), separately for different biomass samples. Pairwise comparisons were averaged over the levels of DMPP whenever DMPP did not have a significant effect.

Significant differences induced by DMPP (* $p < 0.05$; ** $p < 0.01$). When DMPP had a significant effect or when there was a significant interaction between fertilizer treatment and DMPP, statistical analysis over the treatments was performed separately for each level of DMPP.

treatments increased from the first to the second cut. Addition of biochar to digested slurry had no significant effect on neither yield nor N uptake. DMPP tended to increase N-uptake upon the first cut for all fertilized treatments, but the effect was not statistically significant. For stubble biomass, there was a significant interaction of DMPP and fertilizer treatment ($p = .006$), with higher stubble biomass for both SLU and SLA when combined with DMPP, but significantly lower stubble biomass when DMPP was added to unfertilised soil.

In the MIN treatment, almost half of the N taken up by the plants originated from the added ¹⁵N labelled mineral fertilizer, while the proportion of N derived from the fertilizer

($N_{df_{rel}}$) was about 40% for both the digested and undigested slurry treatments (Table 3). Differences in $N_{df_{rel}}$ between the two cuts were small, although there was a pronounced decline in absolute N uptake upon the second cut in all treatments, suggesting that the relative availability of the N from the fertilizers compared to soil N remained the same. Across all fertilizer treatments, a lower $N_{df_{rel}}$ in total biomass was observed in all fertilizer treatments when DMPP was added, but this was only significant for SLA (Table 3). Cumulated over both cuts and all plant parts, almost 70% of MIN was recovered in the plant biomass (Figure 2). As for biomass yield, the cumulative recovery of ¹⁵N in plant biomass was significantly lower for the organic fertilizers than for MIN.

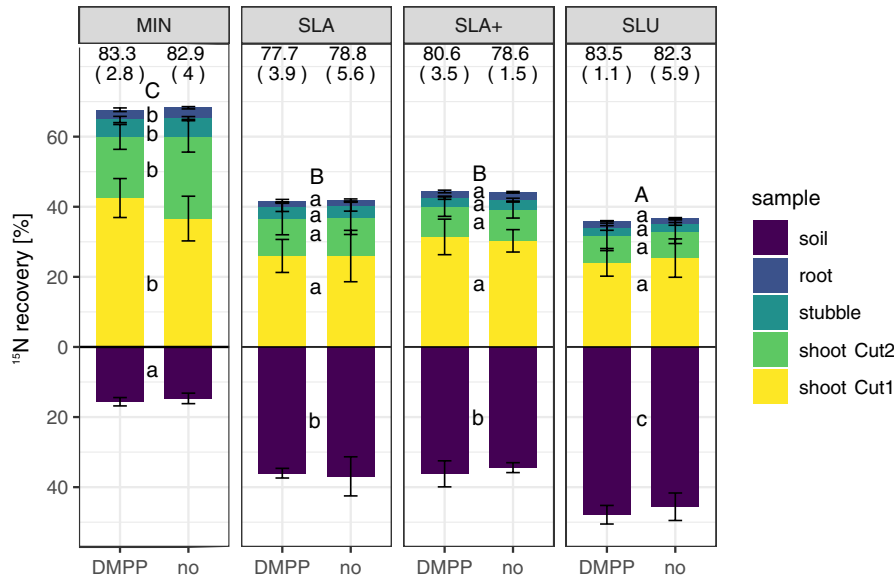
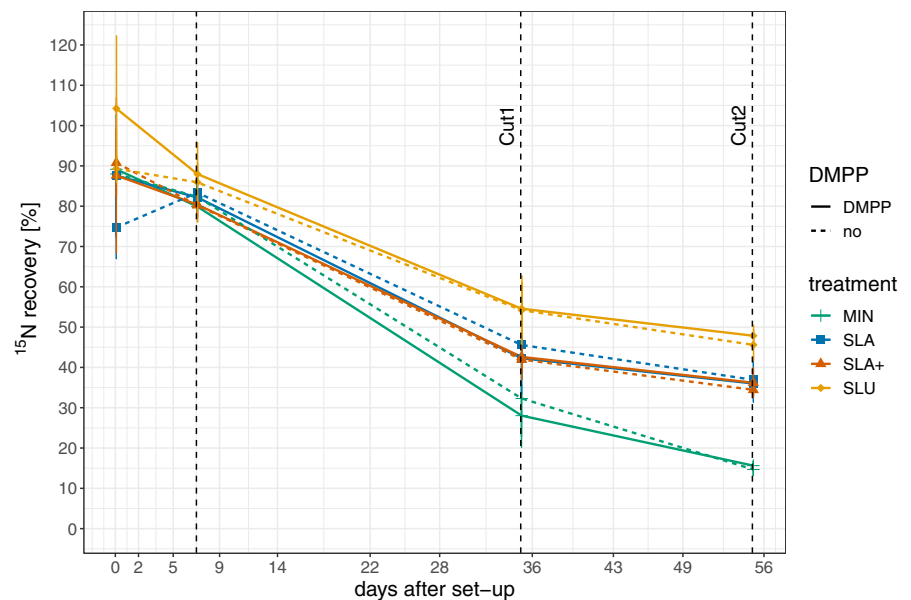


FIGURE 2 ^{15}N balance after 55 days. Soil data refers to the final sampling at 55 days after set-up (same days as Cut 2 biomass sampling including stubbles and roots). Numbers above bars indicate cumulated recovery (mean (standard deviation)). MIN = ^{15}N mineral fertilizer, SLA = ^{15}N anaerobically digested slurry, SLA+ = ^{15}N anaerobically digested slurry + biochar, SLU = ^{15}N cattle slurry. Letters indicate significant differences between fertilizer treatments ($p < .05$). Capital letters refer to the cumulated plant biomass. For pairwise comparisons, fertilizer treatments were averaged over the levels of DMPP, because DMPP did not have a significant effect.

FIGURE 3 ^{15}N recovery in total soil N; data represents mean \pm standard deviation, $n = 4$. MIN = ^{15}N mineral fertilizer, SLA = ^{15}N anaerobically digested slurry, SLA+ = ^{15}N anaerobically digested slurry + biochar, SLU = ^{15}N cattle slurry.



It reached 36% for SLU, while it was significantly increased by anaerobic digestion (42%–44% of applied N for SLA and SLA+). Neither DMPP nor biochar significantly affected ^{15}N recovery in plant biomass.

3.2 | ^{15}N fertilizer recovery and distribution in soil N pools

^{15}N recovery in soil showed a decreasing trend over time (Figure 3). The decrease was strongest between 7 DAS and 35 DAS (Cut 1) and less pronounced thereafter until 55 DAS

(Cut 2). There was a significant interaction between treatment and sampling time ($p < .001$), with differences between treatments increasing over time. At 7 DAS, the recovery of all fertilizers in soil was almost the same, ranging between 80% and 88% of the total N added. At the last sampling, recovery of MIN had declined to less than 16%, while for SLA and SLA+ 36% of ^{15}N was still found in the soil (Figure 3). For SLU, ^{15}N recovery in the soil was even higher (ca. 47% of added N) and significantly different from that of the digested slurries (SLU vs. SLA, $p = .04$, SLU vs. SLA+, $p = .02$).

DMPP did not affect the ^{15}N recovery in Ntot nor the distribution in different soil N pools. At 7 DAS, most

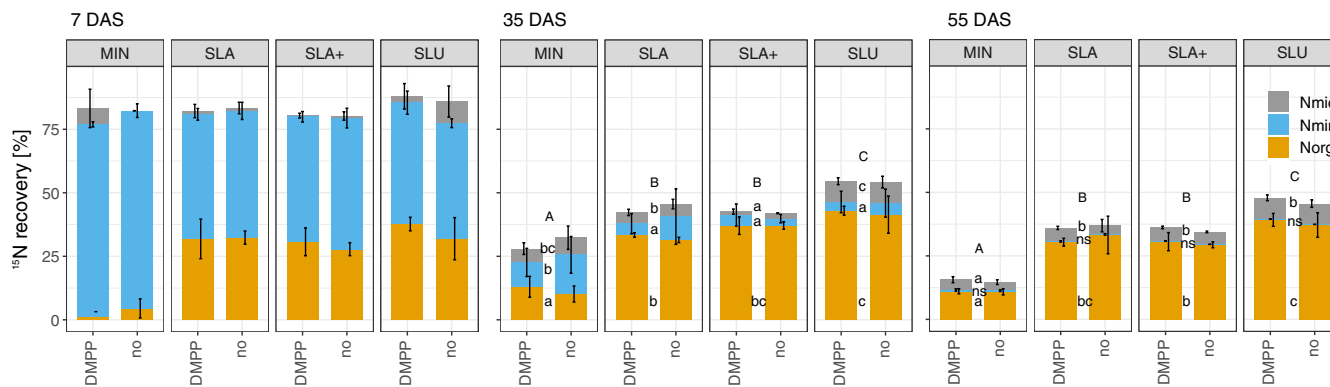


FIGURE 4 ^{15}N recovery in different soil N pools at 7, 35 and 55 days after set-up (DAS); data represents mean \pm standard deviation, $n = 4$. As DMPP did not have a significant effect, different letters indicate significant differences between fertilizer treatments within each soil N pool, averaged over the levels of DMPP ($p < .05$). Capital letters refer to ^{15}N recovery in Ntot; ns = not significant. Nmic = microbial N, Nmin = mineral N ($\text{NH}_4^+ - \text{N} + \text{NO}_3^- - \text{N}$), Norg = non-microbial organic N (calculated by subtracting Nmic and Nmin from total N in soil). MIN = ^{15}N mineral fertilizer, SLA = ^{15}N anaerobically digested slurry, SLA+ = ^{15}N anaerobically digested slurry + biochar, SLU = ^{15}N cattle slurry. Upon 7 DAS, (almost) no ^{15}N could be detected in Nmic, causing negative values for ^{15}N recovery in Nmic. For graphical illustration and for calculation of ^{15}N recovery in Norg, negative values were replaced by 0. For this reason, statistical analysis was only performed for the later time points (35 DAS and 55 DAS).

of the ^{15}N labelled mineral fertilizer was found in the Nmin pool (Figure 4). For the organic fertilizers, about one third of the ^{15}N recovered in the soil was part of the Norg pool (Figure 4 and Figure S1). Except for SLU and partly for MIN, no ^{15}N was detected in the Nmic pool at 7 DAS. Over time, recovery in Nmin decreased while it increased in Nmic and Norg. At 35 DAS, recovery in Nmic was significantly lower for SLA than for SLU ($p = .002$) and decreased further with biochar (SLA vs. SLA+, $p = .01$). Recovery in Nmin was highest for MIN and similar between the other treatments. At the last sampling, less than 0.5% of added ^{15}N was in the Nmin pool, irrespective of fertilizer type. SLU had the highest recovery in all soil N pools.

3.3 | Mineral N dynamics in soil and soil pore water

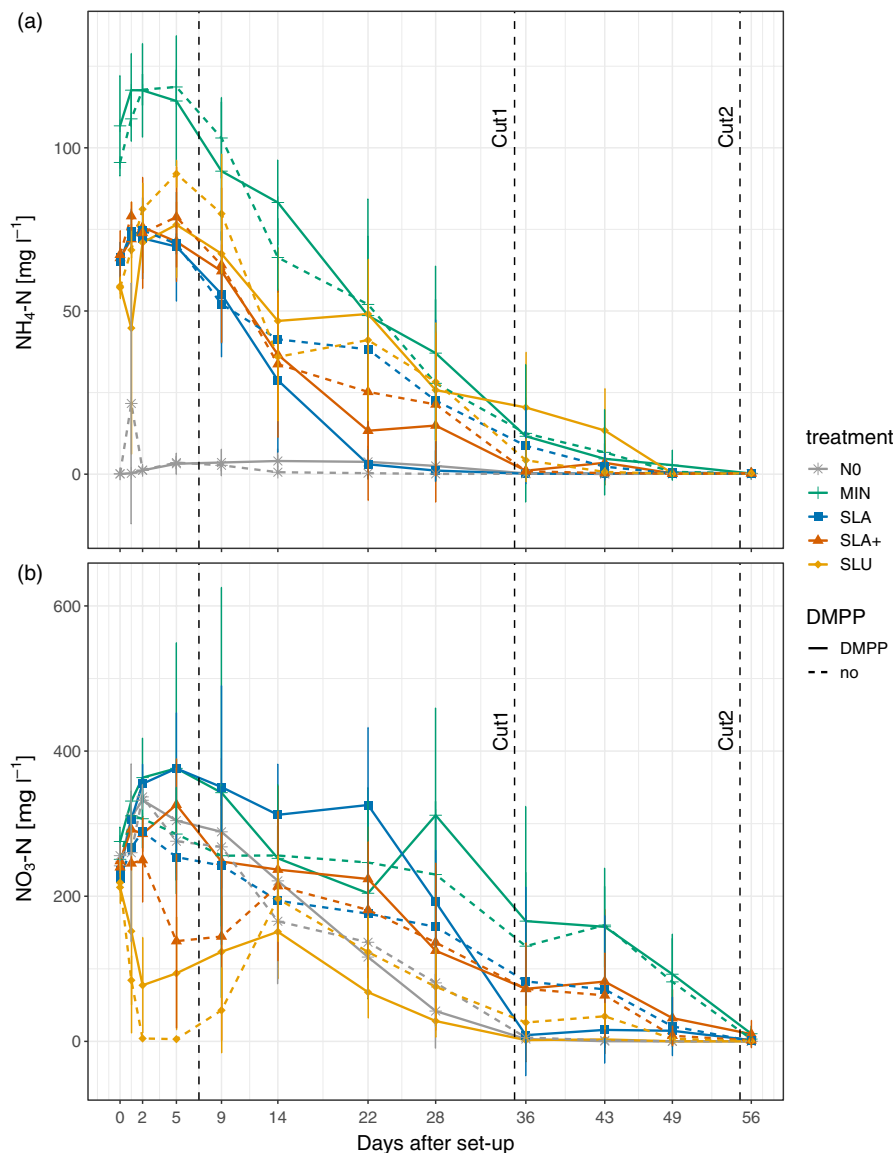
At 7 DAS, soil NH_4^+ levels clearly reflected the amounts of NH_4^+ supplied with the fertilizers, with the highest levels at MIN, intermediate at SLU, SLA and SLA+ and negligible levels at N0 (Figure S2). There were no significant treatment differences in NO_3^- levels and even N0 reached the same level as the other treatments (Figure S3). Columns treated with DMPP tended to have higher NH_4^+ levels in soil, but lower NO_3^- levels than columns without DMPP at 7 DAS, without being statistically significant (Figures S2 and S3). Soil NH_4^+ and NO_3^- contents decreased drastically over time. In contrast, Nmic increased over time ($p < .001$), but showed no significant differences between fertilizer treatments or DMPP levels (Figure S4).

In addition to the three time points of soil sampling, we sampled soil pore water at a high temporal resolution using rhizon suction samplers. Although these results are potentially influenced by the difficulty to extract sufficient soil pore water for analysis at all time points (see Section 2.4), they confirm the observed decline in both NH_4^+ and NO_3^- content in soil over time, as well as differences between the fertilizer treatments (Figure 5). They also showed that NO_3^- concentrations in soil pore water were very high in the beginning, even for N0. Unlike the soil sample extracts, the rhizon extracts revealed a sharp decline in NO_3^- for SLU during the first 2 weeks of the experiment, both with and without DMPP. In none of the fertilizer treatments the addition of DMPP had a clear effect on the NH_4^+ or NO_3^- content in soil pore water.

3.4 | Cumulative ^{15}N recovery

The cumulative recovery of ^{15}N in all biomass samples and in the soil at the last sampling ranged between 78% and 84% and was similar for all treatments (Figure 2). However, the treatments differed in the distribution of recovery in plant biomass and soil. Overall, these numbers indicate that up to 22% of added N remained unaccounted for. Likely, these amounts were lost during the first week of the experiment as the difference between ^{15}N recovered in soil at 7 DAS and the amount we originally applied ranged between 12% and 20% of applied N and was lower for SLU than for the other treatments (Figure S5).

FIGURE 5 Development of ammonium (NH_4^+) (a) and nitrate (NO_3^-) (b) concentrations in soil pore water sampled with rhizon suction samplers (G-set; mean \pm standard deviation, $n = 4$). Vertical dashed lines indicate time points for soil sampling (D-set). Cut1 and Cut2 refer to the two biomass cuts. N0=no N fertilizer, MIN= ^{15}N mineral fertilizer, SLA= ^{15}N anaerobically digested slurry, SLA+= ^{15}N anaerobically digested slurry + biochar, SLU= ^{15}N cattle slurry.



3.5 | Leaching of residual N

Total dissolved N leaching was highest for MIN, with significant differences to N0 ($p = .002$) and SLU ($p = .03$), while it was intermediate for SLA and SLA+ (Figure 6). NO_3^- leaching followed the same trend, but no statistical analysis was performed because the concentration of NO_3^- in the leachate was below the limit of detection for half of the microcosms. NH_4^+ leaching was negligible. Surprisingly, DON leaching was also highest for MIN, but similar for all other treatments. DON was in the same range as NO_3^- for MIN and tended to constitute the largest fraction of residual N leached for the other treatments. Overall, data showed high variability. DMPP did not significantly affect Ntot leaching, but NO_3^- leaching in MIN, SLA and SLA+ tended to be higher with than without DMPP.

In MIN, up to 50% of the leached residual N after 57 days of ryegrass growth was derived from the fertilizer,

while Ndff_{rel} in leachate ranged from 8% to 40% in the other treatments (data not shown). Cumulated over both consecutive flushes, about 2% of mineral fertilizer N was recovered in total dissolved N, while it was less than 1% in the other treatments, but the differences were not statistically significant ($p = .07$) (Figure 7). When leaching was expressed relative to the remaining ^{15}N in soil, differences between treatments declined, but the recovery in leached N was still highest for MIN (data not shown).

4 | DISCUSSION

4.1 | Anaerobic digestion increased NUE and reduced N recovery in soil

We expected that anaerobic digestion would increase NUE, defined in our experiment as the ^{15}N recovery in plant biomass, of cattle slurry due to its greater NH_4^+ – N

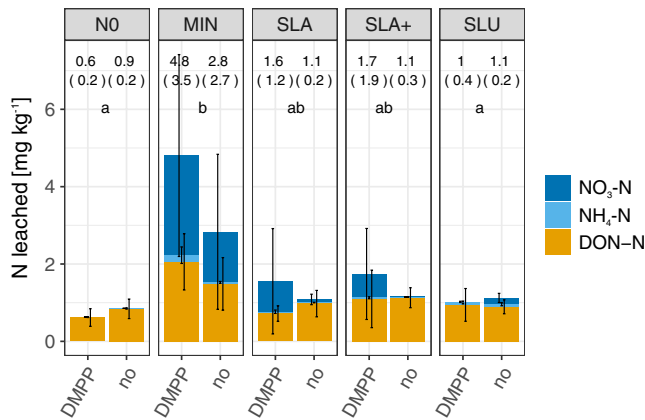


FIGURE 6 Leaching of nitrate (NO_3^-), ammonium (NH_4^+) and dissolved organic N (DON) at 57 days after set-up of the experiment. Cumulated values over both consecutive flushes are shown. Mean \pm standard deviation; $n=4$ (except MIN_no, SLA_no, SLA+_no; $n=3$). MIN = ^{15}N mineral fertilizer, SLA = ^{15}N anaerobically digested slurry, SLA+ = ^{15}N anaerobically digested slurry + biochar, SLU = ^{15}N cattle slurry. Numbers on top indicate total N leached (mean (standard deviation)). Different letters indicate statistically significant differences between fertilizer treatments in total N leached. Since DMPP did not have a significant effect, pairwise comparisons were averaged over the levels of DMPP.

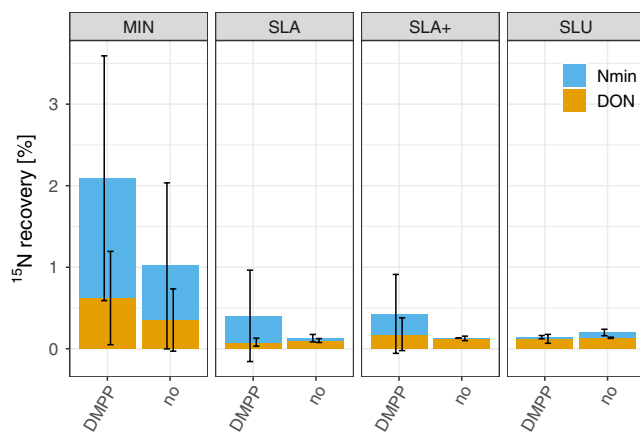


FIGURE 7 ^{15}N fertilizer recovery in leachates collected at 57 days after set-up. Cumulated values over both consecutive flushes are shown. Mean \pm standard deviation; $n=4$ (except MIN_no, SLA_no, SLA+_no; $n=3$). MIN = ^{15}N mineral fertilizer, SLA = ^{15}N anaerobically digested slurry, SLA+ = ^{15}N anaerobically digested slurry + biochar, SLU = ^{15}N cattle slurry. For recovery in total leached N, neither differences between fertilizers nor DMPP levels were statistically significant.

content compared to undigested slurry (Table 2). Indeed, the cumulative ^{15}N recovery in plant biomass was about 15% higher for digested than for undigested slurry (Figure 2). However, there was no significant difference between SLA and SLU in neither dry matter

yield nor total N uptake (Table 3). Overall, differences in NUE were less pronounced than in other studies (e.g., Messner & Amberger, 1988; Nkoa, 2014; Walsh et al., 2012). Unlike most previous studies, our digestate was produced from the same feedstock as the undigested cattle slurry and was not co-digested with other organic wastes, so any observed difference could be directly related to digestion. SLA and SLU were quite similar in their $\text{NH}_4^+:\text{N}_{\text{tot}}$ mass ratio (0.62 for SLU compared to 0.65 for SLA), which was reported to be a good predictor for N availability (Svoboda et al., 2013). Unaccounted losses during the first 7 days of the experiment were higher for SLA (17% of added N) and SLA+ (20% of added N) than for SLU (13% of added N) (see Figure S5) which may have contributed to reduced differences between fertilizers in ^{15}N recovery in biomass. It is likely that a major part of these unaccounted losses occurred as NH_3 emissions, which is in line with Möller and Stinner (2009) who reported higher NH_3 emissions from digested slurries than from undigested slurry and linked this to the higher NH_4^+ content and an increased pH in digested slurry. Overall, the NUE values were comparable to those of other pot studies conducted with ^{15}N labelled fertilizers (Langmeier et al., 2002).

^{15}N recovery in soil was significantly lower from SLA than from SLU, at least from 35 DAS (Figure 3). This could indicate a higher residual fertilizer value of undigested slurry, but might also lead to increased nitrate leaching in the long-term (Messner & Amberger, 1988; Sørensen & Jensen, 2013). In our study, we only assessed the leaching of residual slurry N after 57 days of ryegrass growth, but a longer time span (at least another one or two growth cycles) would have been necessary to evaluate differences in the residual fertilizer effects linked to the organic N in the fertilizers. After the first cut, there was still fertilizer N in the Nmin pool, and NO_3^- levels in soil remained at about half to two thirds of the N uptake in plant shoot biomass during the second growth cycle (Table 3, Figure S3). Presumably, pre-incubation of the soil had accelerated N mineralisation and nitrification in the soil, resulting in high amounts of available N in the soil, which explains why ^{15}N recovery in biomass upon the second cut was still similar for SLU, SLA and SLA+, albeit lower than for MIN (Figure 2).

The residual effect of fertilizers is determined not only by the amount, but also by the distribution of the residual fertilizer N in different soil N pools with different mineralisation rates. While the distribution of recovered ^{15}N in different soil N pools at 7 DAS reflected the original composition of the fertilizers, already at 35 DAS significantly more ^{15}N was recovered in Nmic for SLU than for the other treatments (Figure 4). Immobilization of N from SLU during the first 2 weeks of the experiment was

also indicated by reduced NO_3^- concentrations in soil pore water (Figure 5). Others also found increased immobilization with undigested compared to digested slurry (Hossain et al., 2021). This can be explained by the lower content of available C in digested slurry, reducing immobilization (Albuquerque et al., 2012). In a soil incubation study, Risberg et al. (2017) found a negative correlation between the VFA content of digestates and nitrification rate due to an inhibitory effect on ammonia oxidation. This provides another explanation for the observed lower NO_3^- concentrations in soil pore water in SLU compared to SLA, as SLA had lower VFA contents than SLU (Table S1).

Overall, the temporal development and fate of the residual labelled fertilizer N in soil was comparable to Frick, Oberson, Cormann, et al. (2022), who found a rapid decline in the recovery in N_{min} and the major fraction of residual fertilizer N in Norg under field conditions, regardless whether it originated from mineral fertilizer or cattle slurry. The residual Norg of both mineral fertilizer and cattle slurry showed a similar mineralisation rate in soil over 2 years after application (Frick, Oberson, Frossard, et al., 2022). However, this might be different for digested slurry as the remaining Norg might be more recalcitrant than that from undigested slurry (Möller, 2015; Wentzel et al., 2015). Therefore, further research on the mineralisation rate of organic N from digested and undigested slurry would be needed. In our study, ^{15}N recovery in Norg did not change significantly over time (Figure 4), but this does not necessarily mean that organic N did not start to mineralise. Instead, immobilization of $\text{NH}_4^+ - \text{N}$ was likely compensated by mineralisation of organic N from the slurries (Sørensen, 2001). An evaluation of gross N transformation rates would have been required for a clear conclusion, but this was not the focus of this study. Overall, anaerobic digestion increased NUE, even though unaccounted losses tended to be greater than for undigested slurry (Figure S5). The resulting decrease in ^{15}N recovery in soil for SLA and SLA+ compared to SLU indicated a potential for reducing both NO_3^- leaching and residual fertilizer effects.

4.2 | Minor effect of DMPP on N transformation processes and losses

We investigated the effect of DMPP on N fluxes and N forms as well as on NUE in order to evaluate its potential for reducing NO_3^- leaching through inhibiting nitrification when added to different fertilizer types. Total dry matter production and N uptake tended to be slightly higher with than without DMPP (Table 3). This finding was unexpected as yield increases were usually only reported at sites with high leaching potential,

where DMPP could reduce N losses and thus increase mineral N remaining in soil (Abalos et al., 2014; Tauchnitz et al., 2018). In our set-up with watering aimed at keeping soil moisture constant, no leaching occurred during the growth phase of the plants (until 57 DAS). Nevertheless, plants fertilized with MIN, SLU or SLA tended to produce more biomass and took up more N when DMPP was added, although the effects were mostly not statistically significant. The addition of biochar to SLA seemed to attenuate the effect of DMPP on dry matter yield and N uptake, as also described by Fuertes-Mendizábal et al. (2019). At the same time, we found that Ndff_{rel} in biomass was slightly reduced after addition of DMPP for all fertilizer treatments. Similar effects were also observed by others, who reported that Ndff_{rel} was lower with DMPP for cereals in a pot study and for pasture in a field study (Peschke et al., 2001, 2004; Rowlings et al., 2016). Lower Ndff_{rel} under DMPP treatment combined with higher biomass yield and N uptake indicate that plants grown with DMPP must have taken up more N from soil. In short-term soil incubation studies using ^{15}N labelling, increased gross mineralisation rates were observed with the addition of NIs (Ernfors et al., 2014; Shi et al., 2016). The increased mineralisation represents a non-target effect of NIs, but could explain the lower Ndff_{rel} combined with an overall higher yield and N uptake with DMPP.

Contrary to our expectations, we did not see clear effects of DMPP on NH_4^+ or NO_3^- contents in soil (Figures S2 and S3) or in soil pore water (Figure 5). The latter could be related to the aforementioned difficulties with extracting soil pore water with the rhizons at a water content of 60% max-WHC. We had chosen this soil water content to ensure nitrifying conditions, which were important for a companion study to assess N_2O emissions (Efosa et al., in prep.). Others found significantly more NH_4^+ and less NO_3^- in soil when treated with DMPP (e.g., Guo et al., 2021; Huf & Olf, 2020; Merino et al., 2005). In the past, DMPP has been reported to be effective for up to 6 weeks, depending on soil type and environmental conditions (e.g., Barth et al., 2001; Peschke et al., 2004). In our setting, the effectiveness of DMPP might have been impaired by several factors. First, the temporarily high soil temperatures due to a heat wave during the first days of the experiment might have destabilized DMPP. It has been reported that DMPP was less effective and its degradation accelerated under warm conditions (Lan et al., 2018; Zerulla et al., 2001). Second, the way NIs are applied plays a crucial role in ensuring that NH_4^+ from fertilizers and DMPP remain closely associated. All our fertilizers were in liquid form. Therefore, we mixed a DMPP solution with liquid ^{15}N labelled fertilizers, which was reported to be less effective than when formulated as granules (Ruser & Schulz, 2015). Third, at the end of the pre-incubation, NO_3^-

levels in our soil were quite high, even in the non-fertilized control (Figure 5), masking the effect of DMPP. Overall, we could not find a clear interaction between fertilizer type and DMPP.

4.3 | Biochar did not increase NUE of anaerobically digested slurry

There is some evidence suggesting that biochar can reduce N losses from liquid fertilizers by providing sorption sites for cations such as NH_4^+ (Sarkhot et al., 2013; Wang et al., 2015) (see also Figure S5). Sorbed N is protected not only from being lost through NH_3 volatilization, but also from nitrification and leaching while remaining available to plants (Bradley et al., 2015; Craswell et al., 2021; Taghizadeh-Toosi et al., 2012). We tested only the combination of anaerobically digested cattle slurry and biochar, as digestates typically have higher NH_4^+ to N_{tot} ratios than undigested slurries, making the N in digested slurry more prone to loss if not taken up by plants.

Biomass yield, N uptake and recovery were similar for SLA and SLA+, although cumulative recovery in all biomass parts tended to be slightly higher for SLA+ (~44%) than for SLA (~42%) (Figure 2). Similarly, Foereid et al. (2021) found insignificant differences in N uptake when digestates were amended with biochar.

Overall, the effects of biochar are highly dependent on feedstock and pyrolysis conditions, making them difficult to predict (Craswell et al., 2021). Furthermore, the application rate is crucial. For example, significant effects of biochar on NO_3^- leaching were observed only at applications rates of $>10 \text{ t ha}^{-1}$ (Borchard et al., 2019). In our study, we applied biochar at a much lower rate ($\sim 1.8 \text{ t ha}^{-1}$, assuming the amount applied in our columns was evenly mixed into the top 20 cm of soil), aiming to reflect realistic application amounts when used as a fertilizer amendment.

Biochar transiently reduced the recovery of ^{15}N from SLA in the soil N_{mic} pool at 35 DAS (Figure 4) without affecting the absolute N_{mic} contents in soil (Figure S4). Since fertilizer recovery in N_{mic} was reduced, it suggests that more soil N was immobilized and incorporated into N_{mic} . Thus, biochar might have induced a transient microbial immobilization of soil N, which could be explained by increased short-term gross transformation rates following biochar addition (Nelissen et al., 2015) or enhanced sorption of NH_4^+ on biochar surfaces (e.g., Knowles et al., 2011). Indeed, in a batch sorption experiment, we confirmed the biochar's capacity to effectively sorb 20%–40% of NH_4^+ added with an ammonium sulphate solution (Figure S6).

However, apparently neither sorption of NH_4^+ to biochar nor enhanced mineralisation-immobilization

turnover evoked a significant effect on yield or N losses, or the effect was masked by other processes, such as high overall fertilizer efficiency and low losses. Long-term effects of repeated biochar applications or applications of higher amounts of biochar should be further investigated.

4.4 | Only small amounts of residual ^{15}N leached, independent of fertilizer type or treatment

After the second biomass cut, the columns were oversaturated with demineralised water in order to determine the proportion of residual fertilizer N that could potentially leach. Overall, only small amounts of residual N were leached after growing ryegrass for 57 days, and the recovery of fertilizer N in leachate was less than 2% of the applied amount in all treatments (Figures 6 and 7).

Surprisingly, the amount of N_{tot} leached ($\text{MIN} > \text{SLA} = \text{SLA}+ > \text{SLU}$) followed an opposite trend to the total ^{15}N recovery in soil at 55 DAS ($\text{SLU} > \text{SLA} = \text{SLA}+ > \text{MIN}$) (Figures 2 and 6). This highlights the importance of the N pool in which the ^{15}N prevails. In fact, ^{15}N recovery in the N_{min} pool at 55 DAS was still highest for MIN and lowest for SLU, although it was relatively small overall (Figure 4). While the total N recovery in soil was highest for SLU, the leaching of residual N was lowest, indicating that the residual slurry N was stabilized in soil. This could be attributed to the observed higher microbial immobilization (Figure 4), as suggested by Sørensen (2004), and also due to the higher amounts of both organic N and organic C applied with the slurry compared to the other treatments (Table 2). Our results further indicate that within the timeframe of this study, leaching of residual N was mostly controlled by differing amounts of mineral N applied with the fertilizers.

The duration of our study was too short to evaluate the mineralisation rate of the residual fertilizer N in soil. However, this factor is crucial, especially under field conditions where fertilizers are typically applied repeatedly over the years, leading to the accumulation of residual fertilizer N in soil. Model predictions based on data from a 4-year field study demonstrated that undigested cattle slurry has a lower short-term, but higher residual fertilizer effect than digested slurry (Schröder et al., 2007). However, whether this translates into an increased NO_3^- leaching potential depends on the synchrony between plant N demand and the mineralisation rate of the residual fertilizer N, which is influenced by the degradability of the originally applied slurry, as well as soil, climate and crop (Berntsen et al., 2007). Jørgensen and Petersen (2006) simulated a decrease in soil C upon the application of digested pig slurry compared to undigested slurry, likely

due to a lower C:N ratio in digested slurry inducing soil organic matter mineralisation. In their simulation, NO_3^- leaching could only be reduced when the increased mineral N content of digestate was taken into account and the application amount was reduced accordingly. Especially with repeated applications, the residual effect should be considered for future fertilization in order to minimize the potential for leaching (Jarosch et al., 2018).

We found that equal or even higher amounts of DON than Nmin were leached, and we recovered considerable amounts of ^{15}N in leached DON, even from mineral fertilizer (Figures 6 and 7). DON leaching has been suggested as an important, though often neglected N loss pathway (Van Kessel et al., 2009). Our results highlight the importance of DON leaching as a potential pathway for N losses. However, the higher amounts of DON and ^{15}N -DON leached under MIN than under the organic fertilizers were unexpected and warrant further research, particularly under field conditions.

In our experimental setting, all tested treatments (anaerobic digestion, DMPP and biochar) had only minor effects on the leaching of residual N. Under field conditions, NO_3^- leaching was found to be driven mainly by amount and timing of inputs, whereas previous digestion of slurry had little effect (Möller, 2015; Svoboda et al., 2013). Anaerobic digestion could offer assets as the N content of the digestate is often known to the farmer, allowing for more targeted application. Overall, reduced inputs might be necessary to avoid losses. Both, anaerobic digestion and DMPP could allow for reduced N_{tot} input rates with cattle slurry, while alleviating potential negative effects on yield (Rose et al., 2018; Rowlings et al., 2016).

5 | CONCLUSIONS

In this study, we aimed to evaluate the potential of anaerobic digestion, biochar and DMPP for improving NUE and reducing residual N leaching from cattle slurry. Our findings revealed that anaerobic digestion increased plant N recovery while reducing recovery in soil. This suggests a lower residual fertilizer value of anaerobically digested slurry. It also indicates that anaerobic digestion could be a feasible way to reduce slurry N accumulation in soil and the associated potential for NO_3^- leaching, provided that the higher NH_4^+ content is considered and input amounts are reduced accordingly. Although over 45% of N from SLU was still recovered in soil at 55 DAS, this did not result in increased N leaching. This highlights the importance of whether N is present in organic or mineral form. Further research is needed to evaluate the long-term mineralisation rate of the residual N from both digested and undigested cattle slurry in soil. Biochar tended to enhance

the observed effect of anaerobic digestion with regard to increased N uptake from digested slurry, but the effects were not statistically significant. There was some evidence that biochar reduced the ^{15}N recovery in N_{mic} , likely by adsorption of the NH_4^+ applied with the fertilizer, but the effect was transient. Even though DMPP only induced small changes, the reduction in relative proportion of N derived from the fertilizers in plant biomass combined with higher absolute N uptake and dry matter yield warrants further research.

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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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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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