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Tracing nitrogen transformations during spring development of winter wheat induced by ^{15}N labeled cattle slurry applied with different techniques

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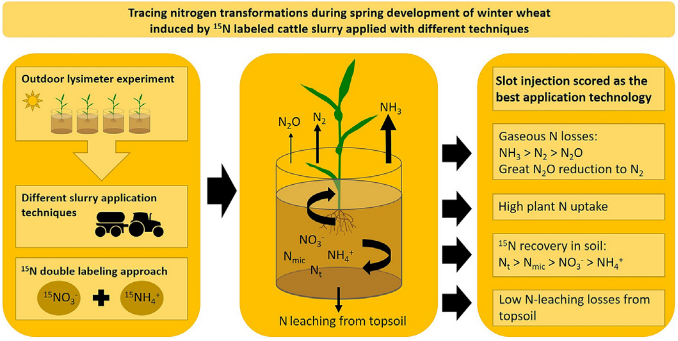
HIGHLIGHTS

- Trailing hose and open slot injection did not increase $N_2O + N_2$ emissions.
- Slurry injection reduced NH_3 volatilization and increased plant N uptake.
- Addition of a nitrification inhibitor had no impact on total N losses.
- A great share of the applied slurry was immobilized in the soil.

GRAPHICAL ABSTRACT



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ABSTRACT

Slurry application is often associated with considerable nitrogen (N) losses: ammonia (NH_3), nitrous oxide (N_2O) and a mostly unknown contribution of dinitrogen (N_2) emission, as well as N leaching. Thus, an outdoor lysimeter experiment with growing winter wheat in undisturbed soil cores was set up to follow the transformation of cattle slurry $^{15}NH_4^+$ and soil $^{15}NO_3^-$ using a double labeling approach. Slurry treatments included the following application techniques: a trailing hose with/without acidification, and open slot injection with/without nitrification inhibitor. The fertilizer application rate was 67 kg N ha^{-1}. In addition to NH_3 emissions, N_2O and N_2 emissions were measured, as well as N contents and ^{15}N enrichment of soil N pools and plant compartments. The major gaseous loss pathway was NH_3 with up to 8 kg N ha^{-1} following trailing hose application, while slot injection significantly reduced NH_3-N losses. Regardless of the application technique, N_2O emissions were low (up to 0.1 kg N_2O-N ha^{-1}), while N_2 emissions reached up to 3 kg N ha^{-1}. No effect on N leaching from topsoil was found. ^{15}N plant uptake was greater in slot injection than trailing hose treatments. An effect of the nitrification inhibitor was visible in the nitrate contents, but not in gaseous N losses or N leaching from topsoil. Impacts of the application techniques on individual soil N pools were small. The ^{15}N recovery offered a chance to map the short-term effects and was highest in the soil N_t pool (32 % to 48 % of ^{15}N applied) with a greater contribution of microbial N than mineral N at beginning of stem elongation. Indications for high N immobilization was derived from the applied N balance approach. In the present case, slot injection scored as the best application technology based on the highest NH_3 reduction, while N_2 and N_2O emissions were not enhanced.

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1. Introduction

Animal manure is a valuable resource of nutrients in agricultural systems. However, the application of manure, in particular slurry, is often associated with large N losses to air and water (Schröder, 2005). One of the largest slurry N loss pathways to air is ammonia (NH_3) volatilization. NH_3 emissions are a threat to human health and contribute to soil acidification and the eutrophication of ecosystems (Sutton et al., 2008). Furthermore, deposition of NH_3-N results in increased nitrous oxide (N_2O) emissions. N_2O is a greenhouse gas with a global warming potential (GWP_{100}) 265 times that of carbon dioxide (CO_2) (AR5, IPCC (2014)). Moreover, N_2O can be directly released following slurry application mainly via nitrification and denitrification. If denitrification is complete, slurry N is lost in the form of the non-reactive molecular nitrogen gas (N_2). This is harmless from an environmental perspective, but still of high agronomic interest in terms of N use efficiency (NUE). Nitrate (NO_3^-) leaching is another pathway by which large amounts of slurry N can be lost (Webb et al., 2010). Moreover, N leaching is known as an important source of indirect N_2O emissions (Well and Butterbach-Bahl, 2010). All pathways of slurry N slurry N loss should be addressed when evaluating different application techniques in order to avoid environmental pollution and pollution swapping, and to maximize the value of slurry as a N fertilizer.

The application technique of slurry can have a great impact on the magnitude of direct NH_3 and N_2O emissions (Fangueiro et al., 2018; Fangueiro et al., 2017; Herr et al., 2019; Wulf et al., 2002). Band application, like trailing hose application (TH) or injection techniques, are recommended to reduce NH_3 emission (Fangueiro et al., 2017; Herr et al., 2019; Seidel et al., 2017; Webb et al., 2010). However, NH_3 emission following band application of slurry can vary considerably depending on slurry composition, the width of the slurry band, soil properties controlling infiltration, and weather conditions, in particular solar radiation, wind speed, and air temperature (Sommer et al., 2003). Slurry acidification can be used to minimize NH_3 volatilization from band applied slurry (Fangueiro et al., 2015a). However, it is not clear whether acidification results in increased N_2O emission (Fangueiro et al., 2018; Fangueiro et al., 2010; Malique et al., 2021; Park et al., 2018; Velthof and Oenema, 1993). Acidification can delay nitrification and denitrification (Fangueiro et al., 2015a). It might also affect the N_2O to N_2 ratio of denitrification. Slurry injection (SI) has been shown to reduce NH_3 emissions very efficiently, since the contact area between slurry and the atmosphere is reduced significantly. The applicability of SI depends on soil texture. The best results are obtained on sandy soils where both slitting and closing the slot is much easier than on heavier textured and stony soils (Fangueiro et al., 2017; Seidel et al., 2017). However, slurry injection can stimulate denitrification by the creation of anaerobic hot spots in soils (Velthof and Mosquera, 2011; Webb et al., 2010). Adding nitrification inhibitors (NI) before injection might help to reduce both N_2O production by nitrification and denitrification, and total N losses via denitrification (Herr et al., 2020; Lin et al., 2017; Ruser and Schulz, 2015). Furthermore, NI can increase the NUE of crops (Abalos et al., 2014), when N leaching is reduced (Ruser and Schulz, 2015). However, it must always be considered that the reduction of NH_3 emissions increases the N availability in the soil and thus impacts the conditions for production and release of N_2O and N_2 (Petersen and Sommer, 2011).

Numerous studies have reported significant effects of the slurry application technique on single N fates, such as plant N uptake, NH_3 volatilization, N leaching and N_2O emission (Dell et al., 2011). However, most studies do not consider N_2 emissions as a significant N loss pathway. This is likely due to the fact that N_2 is difficult to quantify (Groffman et al., 2006), particularly because of its high atmospheric background concentration. The direct quantification of N_2 fluxes at the field scale is only possible by using the ^{15}N gas flux method (Sgouridis et al., 2016; Sgouridis and Ullah, 2015) or the acetylene (C_2H_2) inhibition technique (Yoshinari and Knowles, 1976), where the latter is no longer recommended because C_2H_2 catalyzes oxidation of NO in the presence of oxygen (Nadeem et al., 2013). We applied the improved ^{15}N gas flux method published by Well et al. (2019) in a double labeling approach with ^{15}N labeling of the soil NO_3^--N pool and the easily available nitrogen pool (urea-N) of slurry for the first time. Different slurry application techniques (trailing hose, trailing hose plus acid, slot injection, and slot injection plus nitrification inhibitor) were simulated for winter wheat growing in small lysimeters in the field. Slurry application is known to affect soil N dynamics by adding slurry N and C pools of different microbial availability and mobility in soil, but also by changing the transformation processes of soil N. Thus, we determined the effects of different slurry application techniques on transformations of easily available mineral N pools, the ammonium (NH_4^+-N) added with slurry, and the initial soil NO_3^--N pool. We followed the transformation of slurry-derived $^{15}NH_4^+$ and soil $^{15}NO_3^-$ in the soil-plant-atmosphere system in order to get more detailed information on the sources of different N fluxes, N pools, and their ^{15}N enrichment. The main objectives were:

1) to determine the impact of different cattle slurry application techniques on gaseous N losses (NH_3, N_2O, N_2), N leaching from topsoil, plant N uptake, and N transformation in soil, such as total soil nitrogen and soil microbial nitrogen;
2) to determine the effects of different slurry application techniques on the transfer of ^{15}N labeled mineral N in slurry ($^{15}NH_4^+$) and soil ($^{15}NO_3^-$) to these N pools

It was hypothesized that:

1) NH_3 emission is higher from trailing hose (TH) application compared to slot injection (SI), while acidification of slurry (TH + A) will compensate this effect.
2) N_2O and N_2 emission will be higher from slot injection (SI) compared to trailing hose (TH) application, while the addition of a nitrification inhibitor (SI + NI) will lead to a reduction of N_2O and N_2 emissions.
3) Total losses of labeled nitrogen via emissions and leaching from topsoil will be lowest for the slot-injected stabilized slurry (SI + NI). These lower N losses result in the highest ^{15}N plant uptake.

2. Material and methods

2.1. Soil properties

Undisturbed soil columns were taken in mid-October 2019 after tillage following the harvest of winter rape (crop rotation: winter rape, winter wheat, winter barley) at the research field “Goldener Acker” of the University of Hohenheim, Germany. The soil was a silty loam (loess) and classified as Haplic Luvisol (IUSS Working Group, 2015). Basic topsoil (0–25 cm) properties were 27 % clay, 71 % silt, 2 % sand, bulk density 1.4 g cm^{-3}, 6.7 ± 0.1 pH ($CaCl_2$), 1.34 ± 0.19 % organic carbon (C_{org}), and 0.15 ± 0.02 % total nitrogen (N_t). Undisturbed soil columns were taken using acrylic glass columns of 14.6 cm inner diameter and 30 cm length. The columns were driven into the soil to a depth of 25 cm, thus leaving the upper end 5 cm above the soil surface. Soil columns were transported to the Thünen Institute of Climate-Smart Agriculture in Braunschweig (Germany) and stored in the greenhouse until the start of the experimental set-up in November 2019.

2.2. Experimental set-up and treatments

The nearby weather station of the German Meteorological Service recorded daily precipitation (mm) and barometric air pressure (hPa) during the experimental period of 60 days. Air temperature (°C) and wind speed (at 2 m height) were recorded daily by a weather station at the experimental site.

In November 2019, soil columns were set up as small lysimeters in a randomized outdoor experiment (four replicates in a block design). The seeding of winter wheat (*Triticum aestivum* L., variety: *Apostel*, seed density: 350 grains m^{-2}, 6 plants per soil column) took place at the beginning of November 2019. After germination, each soil column was placed on a suction plate comprising a 0.45 μm polyamide filter membrane (ecoTech, Bonn, Germany) and connected to a 1 L glass bottle for collecting leachate via a polyamide tube (4 mm OD, 2 mm ID). The bottles were stored in a soil pit next to the lysimeters. All tubes were the same length to ensure identical drainage conditions. Four soil columns were equipped with a combined soil moisture and temperature sensor (5TM, METER group, Munich, Germany) connected to a data logger (EM60, METER group, Germany). Soil columns were buried on the experimental site, leaving the upper end 5 cm above the soil surface. They remained in this set-up for an equilibration period until the beginning of fertilization in spring. To minimize edge effects, winter wheat was also sown in the area around the soil columns.

In February 2020, treatments were established simulating four different cattle slurry (CS) application techniques and two different control treatments with and without ^{15}N labeling of the soil NO_3^--N pool. Soil columns without fertilizer and ^{15}N application (NA, natural abundance) were used as a control for ^{15}N natural abundance. This was useful for the precise calculation of gaseous ^{15}N fluxes from ^{15}N treatments. A second control without organic fertilizer (N0) but spiked with ^{15}N labeled KNO_3^- was used to determine N_2 and N_2O fluxes from soil NO_3^--N in the absence of slurry application. CS application techniques were as follows: trailing hose (TH:CS), trailing hose plus acidified CS (TH:CS + A), slot injection (SI:CS), and slot injection plus nitrification inhibitor (SI:CS + NI). 0.9 L m^{-3} of 98 % sulfuric acid (H_2SO_4) was used for acidification of the slurry to a target pH level of 6. A pH value of 6.5 was measured by pH-indicator paper immediately before CS application. However, due to the rapid buffering of the CS, the amount of H_2SO_4 was probably too low, as a pH of 7.7 was measured in the subsequent analysis (Table 1). In the SI:CS + NI treatment, the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) (trade name: VIZURA, BASF) was applied to the slurry shortly before application to the soil columns. The application rate was equivalent to the recommended 2 L ha^{-1} and did not increase the N application rate of this treatment (Table 1).

We targeted a similar ^{15}N enrichment of the initial slurry urea-N pool and the soil NO_3^--N pool to achieve similar ^{15}N enrichment in the soil NO_3^--N pool and the NH_4^+-N pool originating from slurry N after fertilization. This was done to minimize changes in ^{15}N enrichment of NO_3^- by nitrification processes in order to avoid bias when calculating denitrification rates by heterogeneity in ^{15}N enrichment of NO_3^- (Zaman et al., 2021). ^{15}N labeling of NO_3^--N of all soil columns (except NA columns) was conducted on February 21, 2020, as previously described by Buchen et al. (2016). The soil columns contained 2 kg NO_3^--N ha^{-1} and were fertilized with ^{15}N labeled KNO_3^- (98 at% ^{15}N, 66.5 mg NO_3^--N L^{-1}) at a rate of 4 kg N ha^{-1} to achieve the target enrichment of 65 at% ^{15}N. The tracer was dissolved in distilled water and then applied by injections via 12 equidistant steel capillaries. Defined volumes were injected at 5 cm, 10 cm, 15 cm, and 20 cm depth using a peristaltic pump (Ismatec BVP, Wertheim, Germany) to achieve homogenous labeling at 0 cm to 25 cm soil depth (Buchen et al., 2016). In total, 96 mL of fertilizing solution was injected into each soil column, which resulted in a 2 % increase in gravimetric water content relative to previous soil water content.

For ^{15}N labeling of the cattle slurry (in the following referred to as ^{15}N-CS), fresh faeces were collected separately from dairy cows, fed a typical diet consisting of grass silage and concentrates. These faeces were frozen at −18 °C after sampling. Artificial ^{15}N urine was prepared that contained 10.5 g N L^{-1}, 2.7 g N L^{-1} urea (CH_4N_2O), 5.3 g N L^{-1} ^{15}N labeled urea (98 at%), 0.3 g N L^{-1} hippuric acid ($C_9H_9NO_3$), 1.7 g N L^{-1} allantoin ($C_4H_6N_4O_3$), 0.1 g N L^{-1} uric acid ($C_5H_4N_4O_3$), and 0.4 g N L^{-1} creatinine ($C_4H_7N_3O$) following the recipe of Kool et al. (2006). In addition, 14 g L^{-1} $KHCO_3$, 10.5 g L^{-1} KCl, 0.4 g L^{-1} $CaCl_2$, 1.2 g L^{-1} $MgCl*6H_2O$, and 3.7 g L^{-1} Na_2SO_4 were added to better mimic real urine resulting in a urine pH closer to that of real urine (Kool et al., 2006). Faeces (49 %), artificially ^{15}N-labeled urine (27 %), and water (24 %) (Sørensen, 1998) were mixed in a container and stored for 23 days at 15 °C to achieve a complete conversion of ^{15}N labeled urea (65 at% ^{15}N) into the NH_4^+-N pool and to age the slurry in order to reduce the proportion of labile C_{org} before the start of the fertilization experiment. Gaseous losses and nitrification were avoided due to storage in a gas-tight container. Regular shaking precluded the separation of phases. Since ^{15}N labeling of CS was restricted to the urea fraction, mainly the easily available mineral NH_4^+-N pool was labeled. This is known to represent the main source contributing to N uptake but also to N losses following slurry application (de França et al., 2021). It must be noted that the large organic N fraction of the slurry was not ^{15}N labeled using the applied method of mixing ^{15}N artificial urine and natural abundance faeces. Thus, we measured the total effects of our partly artificial slurry, but our ^{15}N data reflect the transformation of slurry NH_4-N^+ and the initial labeled soil NO_3^-N. The ^{15}N enrichment of the total N (N_t) of the slurry was measured by elemental analysis and isotope ratio mass spectrometry, as described in detail in Section 2.5, since it was not possible to measure only the ^{15}N enrichment in NH_4^+-N. ^{15}N enrichment in slurry NH_4^+-N (53 at% ^{15}N) was calculated from ^{15}N enrichment in slurry N_t and the ratio of N_t:NH_4^+-N in the slurry (Table 1).

Simultaneously upon application, a ^{15}N-CS subsample (150 mL) of each treatment was refrigerated at −18 °C until analyzed at AgroLab LUFA GmbH, Hanover, Germany (Table 1). Dry matter and loss of ignition were determined gravimetrically according to DIN EN 15934:2012–11 and DIN EN 15395:2012–11; pH was measured directly according to DIN EN 12176(S5):1998–06; N_t was analyzed by the DUMAS method according to DIN EN 16168:212–11; NH_4^+-N was analyzed by distillation according to DIN 38406–5-2:1983:10; and S content was measured by aqua regia digestion according to DIN EN ISO 11885:2009–09.

Application of ^{15}N-CS to the slurry treatments (TH:CS, TH:CS + A, SI:CS, SI:CS + NI) was conducted one week later on February 27, 2020. The application rate equaled 66 kg to 68 kg N_t ha^{-1} including 46 kg to 47 kg labeled NH_4^+-N (16.2 m^3 ha^{-1}) (Table 1). To simulate the trailing hose technique, the slurry was cast from a bottle to apply a slurry band of 4 cm width, giving a ratio of slurry coverage to total surface area of 0.3. This is a typical ratio for band application (under field conditions, slurry band widths of 9 cm and 12 cm between bands are common). To simulate the open slot injection technique, a slot of 12 cm in length, 2 cm in width (equal to the width of the slurry band) and 5 cm in depth was made with a wedge, representing a common volume to surface ratio of 0.3.

The experiment was limited to 60 days to represent the N dynamics between the first and second fertilizer application. In order to protect the soil columns from substantial washout of ^{15}N label, soil columns were covered during heavy rainfall events for the first 14 days.

Table 1
Characteristics of applied cattle slurries in different treatments.

		TH:CS & SI:CS	TH:CS + A	SI:CS + NI
N application rate	kg ha^{-1}	66	68	67
Total nitrogen (N_t)	kg m^{-3}	4.1	4.2	4.2
^{15}N enrichment of N_t	at%	36		
Ammonium nitrogen (NH_4^+-N)	kg m^{-3}	2.8	2.9	2.9
^{15}N enrichment of NH_4^+-N	at%	53		
Dry matter	%	7.1	7.2	7.1
pH		7.9	7.7/6.5[a]	7.9
Loss on ignition (SOM)	%	57.9	58.5	58.3
Sulfur (S)	kg m^{-3}	0.4	0.8	0.3

[a] Measured by pH-indicator paper immediately before CS application.

2.3. NH_3 sampling, analysis, and calculation

2.3.1. Sampling

NH_3 volatilization was measured after application of ^{15}N-CS using a modified flow-through chamber system following the Dynamic Tube Method (DTM) (Pacholski, 2016). For NH_3 measurements, the headspace of the soil column was closed by an opaque PVC chamber (height: 25 cm, outer diameter: 14.6 cm, volume: 4.2 L) and was flushed with NH_3 free compressed air at a flow rate of 4.5 L min^{-1} to ensure an exchange rate of one headspace volume per minute to comply with the requirements for the application of a calibration formula for calculating absolute fluxes. The air leaving the system was connected via PTFE tubing to an automatic pump (X-act 5000, Draeger Safety AG & Co KG) and indicator tubes (types: 0.25a and 2a, Draeger Safety AG & Co KG) to obtain subsamples and subsequent determination of NH_3 concentrations. Excess air was discharged via a non-return valve. Air flow through the headspace was directed through an air stone bubble diffuser to avoid swirls on the soil surface. Measurements were conducted up to four times per day and for seven days after ^{15}N-CS application until measured values were below detection.

2.3.2. Calculations

NH_3 fluxes were calculated from the measured NH_3 concentrations, the flow rate, and the soil surface area of 167.42 cm^2, which was completely covered by the PVC chamber. The resulting uncalibrated fluxes were scaled to quantitative losses using a calibration equation including wind speed as described in Pacholski (2016). Scaled cumulative NH_3-N losses in kg N ha^{-1} were calculated by averaging the fluxes between two subsequent measurement dates, multiplying this average flux with the duration of each interval, and adding up all the losses from all the measurement intervals of a measurement campaign.

2.4. N_2O and N_2 sampling, isotopic analysis, and calculation

2.4.1. Sampling

Gas flux measurements were carried out on a daily basis for two weeks from February 21 to April 20, 2020; then three times per week for four weeks; and twice per week for the last two weeks, starting one day after ^{15}N labeling of the soil NO_3^--N pool. On each sampling day, gas samples were collected between 8 am and 3 pm. Due to differences in air temperature during the sampling, the sampling of treatments was carried out randomly. Gas fluxes were determined using the closed chamber method (Hutchinson and Mosier, 1981) in opaque PVC chambers, which were the same size as for the NH_3 measurements. Before headspace sampling, the chambers were flushed for 20 min with an artificial gas mixture with reduced N_2 content but atmospheric concentrations of Oxygen (O_2), CO_2 and N_2O (2 % N_2, 21 % O_2, 330 ppb N_2O, and 430 ppm CO_2 in Helium), prepared using a gas mixer (HovaGAS Digital G8, IAS GmbH, Oberursel, Germany) with a flow of 2.25 L min^{-1} (equivalent to 10 times the exchange of the headspace volume). The chamber atmosphere was exchanged with this mixture to reduce N_2 concentration and thus increase the measurement sensitivity for N_2 fluxes (Well et al., 2019). Headspace sampling was performed at sampling intervals of 0, 20, 40 und 60 min in crimped 20 mL vials for gas chromatographic analysis. In addition, two 12 mL septum-capped Exetainers® (Labco Limited, Ceredigion, UK) were collected after 60 min headspace closure for isotopic analysis.

2.4.2. Analysis

Measurements of N_2O, CO_2, and CH_4 concentrations in the 20 mL vials were carried out using a gas chromatograph (GC 2014; Shimadzu, Duisburg, Germany) equipped with a ^{63}Ni electron-capture detector (ECD) for N_2O and CO_2 detection, a flame ionization detector (FID) for CH_4 detection, and an autosampler (Greenhouse Workstation AS-210; SRI Instruments Europe GmbH, Bad Honnef, Germany). CH_4 was determined to evaluate the sampling procedure (see the following paragraph calculations). Analytical precision was determined by repeated measurements of standard gases (300 ppb N_2O, 350 ppm CO_2, 1.4 ppm CH_4) and was consistently <3 %.

One of the gas samples collected in 12 mL Exetainers was analyzed for isotopocule values of N_2 and N_2O using a modified GasBench II preparation system coupled to a MAT 253 isotope ratio mass spectrometer (IRMS) (Thermo Scientific, Bremen, Germany) according to Lewicka-Szczebak et al. (2013). Briefly, N_2O conversion to N_2 prior to analysis allowed the simultaneous measurement of stable isotope ratios $^{29}R(^{29}N_2/^{28}N_2)$ and ^{30}R ($^{30}N_2/^{29}N_2$) of N_2, of $N_2 + N_2O$, and of N_2O. Typical repeatability for concentration analysis was 0.1 % N_2 (1σ of four replicates), while repeatability for ^{29}R was consistently $<4 \times 10^{-7}$. The fraction originating from the ^{15}N-labeled pools with respect to total N in the gas sample ($F_{PN2+N2O}$) was calculated according to Mulvaney (1984). The fraction originating from the ^{15}N-labeled pools, i.e. the applied ^{15}N-labeled NH_4^+ of slurry and ^{15}N-labeled KNO_3^- of identical enrichment with respect to the total gas mixture ($f_{PN2+N2O}$) was calculated as $F_{PN2+N2O}$ multiplied by c_{N2}, where c_{N2} is the N_2 concentration of the gas mixture. Due to the occurrence of $f_{PN2+N2O}$ values below the detection limit, there were several gaps in the datasets. To enable the calculation of cumulative fluxes, these gaps were filled using a value of half the detection limit.

The second gas sample collected in 12 mL Exetainers was analyzed for isotopocule values of N_2O using a Delta V IRMS (Thermo Scientific, Bremen, Germany), coupled to an automatic preparation system with Precon plus Trace GC Isolink (Thermo Scientific, Bremen, Germany), where N_2O was pre-concentrated, separated, and purified and m/z 44, 45, and 46 of the intact N_2O^+ ions were determined (Lewicka-Szczebak et al., 2014). ^{29}R and ^{30}R were derived from m/z 44, 45, and 46 (Bergsma et al., 2001; Deppe et al., 2017). Typical repeatability for ^{29}R and ^{30}R were 6×10^{-6} and 2×10^{-6}. Data were normalized against background values of a breathing air laboratory standard. Fp_{N2O} and f_{PN2O} were calculated from the non-equilibrium distribution of N_2O isotopocules according to Bergsma et al. (2001) and Spott et al. (2006).

Residual N_2O from gross N_2O production from the labeled ^{15}N pools (r_{N2O}) was calculated using the following equation:

$$r_{N2O} = \frac{f_{PN2O}}{f_{PN2+N2O}} \tag{1}$$

2.4.3. Calculations

The flux rates of total N_2O ($N_2O_{fluxtotal}$) were calculated from ordinary linear regression of the four consecutive samples over time using the R package *gasfluxes* (Fuß, 2020) and the following equation:

$$N_2O_{fluxtotal} = \frac{dc_{N2O}}{dt} * \frac{V}{A} \tag{2}$$

where $flux_{N2O}$ is the flux rate in μg N_2O-N m^{-2} h^{-1}, c_{N2O} is the N_2O mass concentration in μg N m^{-3} corrected by the chamber temperature according to the ideal gas law, t is the closing time of the chamber in h, V is the volume of the chamber in m^3, and A is the covered soil area in m^2.

The reliability of the samples was evaluated for the following exclusion criteria: measurable CH_4 concentrations, which can be taken as an indicator of insufficient He flushing and/or absence of overpressure in the vial by the time of the analysis. It can be ruled out that CH_4 concentration originates from slurry treatments, since it occurs randomly and cannot be assigned to individual treatments. Based on this CH_4 check, 11 % of the samples were excluded from the dataset. An increase in CO_2 concentration was used as an additional quality parameter for reliable gas sampling.

Samples for isotopic analysis were collected after 60 min of headspace closure and N_2 and N_2O fluxes from the ^{15}N labeled pools (N_2O_{fluxL} and N_{2fluxL}) were calculated from f_{PN2O} and $f_{PN2+N2O}$ from ordinary linear regression, assuming that the increase in the emitted $N_2 + N_2O$ was also linear as shown for N_2O (Buchen et al., 2016). In the following, N_2O_{fluxL} and N_{2fluxL} from the ^{15}N labeled pools (sum of applied ^{15}N-labeled NH_4^+ of slurry and ^{15}N-labeled KNO_3^-) are referred to as applied ^{15}N. Since both mineral N pools (NO_3^- and NH_4^+) were ^{15}N labeled and a small inhomogeneity in the ^{15}N label distribution of both pools cannot be completely ruled out, N_2O from the applied ^{15}N might be underestimated by up to 25 % (Arah, 1992; Van den Heuvel et al., 1988).

2.5. N-leachate sampling, isotopic analysis, and calculation

Leachate in >25 cm soil depth was collected from the lysimeters on a weekly basis (except for the last two weeks, due to less rainfall) on seven occasions. A suction pressure of −10 Pa was applied at the bottom of the soil columns using a membrane vacuum pump (N 026.3, KNF Neuberger, Freiburg, Germany). The leachate volume was recorded by weighing, then mixed, subsampled, and refrigerated at −18 °C until analysis.

NO_3^--N and NH_4^+-N concentrations were analyzed by a photometric continuous flow analyzer (SA 5000, Skalar Analytical B.V., Netherlands). The ^{15}N abundance in NO_3^- and NH_4^+ was determined according to

Eschenbach et al. (2017) and Eschenbach et al. (2018), whereby NO_3^- was reduced to NO by vanadium chloride (V(*III*)Cl_3) and NH_4^+ was oxidized to N_2 by sodium hypobromite (BrNaO). The NO and N_2 obtained were then analyzed using a membrane inlet IRMS (Delta plus, Thermo Finnigan, Bremen, Germany) coupled to a ConFlo III interface (Dyckmans et al., 2021). The analytical precision was determined by repeated measurements of standards (1, 5, 50, and 75 at% ^{15}N) and was consistently around <0.6 at% ^{15}N (Eschenbach et al., 2017).

Mineral N leaching from topsoil (translocation to >25 cm soil depth) was calculated from the sum of NO_3^--N plus NH_4^+-N by multiplying leachate volume with concentrations.

2.6. Soil sampling, isotopic analysis, and calculation

Shortly before the beginning of the experiment in order to determine background levels, two soil columns from the NA treatment were taken out, divided into three depths (0–10 cm, 10–20 cm, and 20–25 cm), weighed, homogenized, and sieved at 10 mm mesh size for further analysis. The same procedure was used for all soil columns at the end of the experiment. For each depth increment, gravimetric water content, soil mineral nitrogen (N_{min} = sum of NO_3^--N plus NH_4^+-N), and their ^{15}N enrichment, C_{org}, N_t, and its ^{15}N enrichment, soil microbial biomass nitrogen (N_{mic}), and their ^{15}N enrichment, pH, and water-extractable organic matter (WEOM) were determined. Soil water content was determined gravimetrically after drying the soil at 105 °C for 24 h. Water-filled pore space (WFPS) was calculated from the gravimetric water content and soil bulk density, which was determined by the height and dry weight of the soil column. N_{min} was extracted by shaking 50 g soil in 200 mL 2 M KCl solution (ratio 1:4) at room temperature for 1 h. The NO_3^--N and NH_4^+-N concentrations in the extracts and their ^{15}N enrichment were determined as described in Section 2.5. Oven-dried (40 °C for 48 h) soil subsamples were milled and analyzed for C_{org}, N_t, and ^{15}N enrichment of N_t using an Elemental Analyzer (EA) Flash 2000 (Thermo Fisher Scientific, Bremen, Germany), coupled with a Delta V IRMS via a ConFlo IV interface (Thermo Fisher Scientific, Bremen, Germany). Linear regression between peak area and sample weight of different amounts of a laboratory standard were used to calculate the C_{org} and N_t (%). Precision, defined as the standard deviation ($\pm 1\sigma$) of the laboratory control standard along the run was better than ±0.5 % for C_{org}, ±0.3 % for N_t, and at 0.01 at%^{15}N for ^{15}N enrichment of N_t. For N_{mic}, fresh soil was sieved to 2 mm and refrigerated at −18 °C until analyzed. N_{mic} was determined by chloroform fumigation extraction (CFE) with 0.05 M K_2SO_4 (modified according to Brookes et al. (1985), Joergensen and Mueller (1996)) because larger quantities of salt hamper the determination of stable isotopes. An aliquot of the extract was used for N_t determination on a Total Nitrogen Analyzer (Mitsubishi TN-100, A1 Envirotech, Düsseldorf, Germany) equipped with an Auto Liquid Sampler and Auto Sample Injector (ASI-100, Dionex Softron GmbH, Germering, Germany). 10 mL of each sample were freeze dried and its ^{15}N enrichment of non-fumigated (N_{nf}) and fumigated (N_f) samples was determined by elemental analysis coupled to an IRMS as

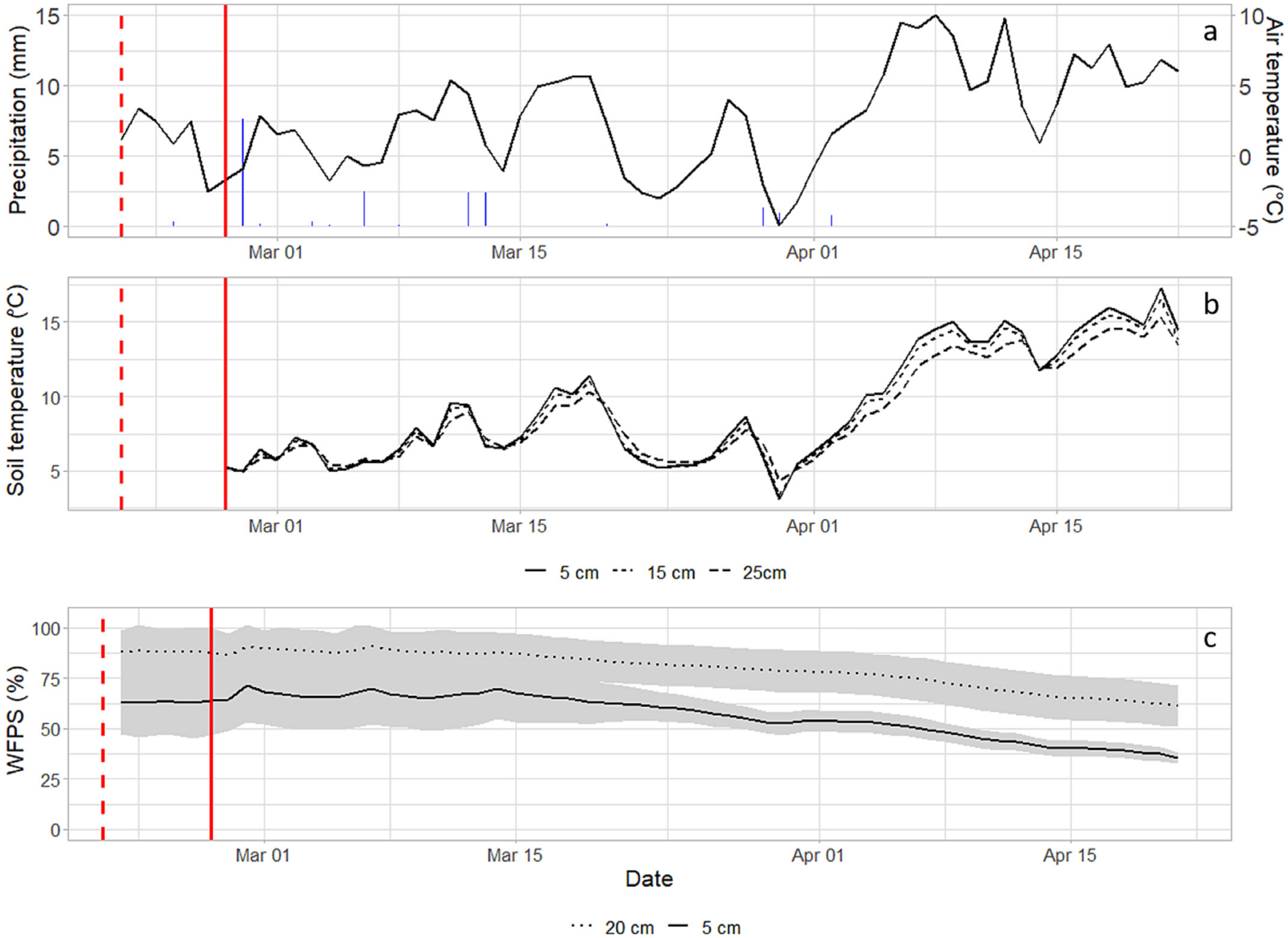

Fig. 1. Time course of (a) daily precipitation with soil column cover and air temperature, and (b) soil temperature in 5 cm, 15 cm, and 25 cm soil depth, and (c) mean WFPS values ± one standard deviation (grey shading) in 5 cm and 20 cm during the experiment. The $K^{15}NO_3^-$ application is marked by the dashed red line and ^{15}N-CS application is marked by the solid red line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

described above. The N_{mic} pool (mg kg^{-1} soil dry weight) was calculated as E_N/k_{EN} with E_N = (total N extracted from N_{nf}) – (total N extracted from the corresponding N_f) with $k_{EN} = 0.54$ (Brookes et al., 1985; Joergensen and Mueller, 1996). The ^{15}N enrichment of the N_{mic} was calculated according to Wachendorf and Joergensen (2011). Soil pH was determined by shaking 5 g air-dried soil in 20 mL 0.1 M $CaCl_2$ (ratio 1:4) solution for 1 h using a pH meter (FE20, Mettler Toledo, Urdorf, Switzerland). WEOM samples were extracted from fresh soil with deionized H_2O (ratio 1:4) and filtered through 0.45 μm polyethersulfonate membrane filters (Pall Life Science, Port Washington, NY, USA) following the procedure of Chantigny et al. (2008). WEOM extracts were measured using a Total Organic Carbon Analyzer (DIMATOC 2000, DIMATEC, Essen, Germany).

2.7. Plant sampling, isotopic analysis, and calculation

At the end of the experiment (EC30), aboveground biomass was cut from each soil column (5 cm above soil). To separate aboveground biomass (roots) from the soil, the soil was sieved at a mesh size of 10 mm. Aboveground biomass and roots were weighed, homogenized, dried at 58 °C for 48 h, and milled. Samples were analyzed using elemental analyzes and IRMS, as described in detail in Section 2.5. The apparent N recovery (ANR%) of the different slurry application treatments was calculated as the plant N uptake from the CS treatments minus plant N uptake from the N0 treatment in relation to the total slurry N input.

2.8. ^{15}N recovery

^{15}N recovery of the single pools/losses from total ^{15}N fertilizer applied (i.e. sum of applied $K^{15}NO_3^-$ tracer and ^{15}N in CS) (%) was calculated as the following (Barraclough, 1995):

$$^{15}N\ recovery = \frac{^{15}N_{recovered}}{^{15}N_{applied}} * 100, \tag{3}$$

where $^{15}N_{recovered}$ is calculated from the amount of N in the aboveground biomass, belowground biomass, and in the following N pools (N leaching from topsoil, $N_2O + N_2$ loss, N_t, N_{min}, N_{mic}) multiplied by the ^{15}N excess enrichment (at% ^{15}N measured minus the natural abundance at% ^{15}N) of the respective N fraction. 0.3663 % was used as the natural abundance of ^{15}N. Results were first scaled to the lysimeters by multiplying the calculated ^{15}N excess (mg kg^{-1} soil dry weight) by the respective soil dry weight (kg) of the soil layer sampled and then extrapolated for comparison purposes to kg N ha^{-1}.

Total ^{15}N recovery excluding NH_3-N losses was calculated as the sum of $^{15}N_{recovered}$ in $N_2O + N_2$ loss, N leaching from topsoil, N_t pool, aboveground biomass N and belowground biomass N in relation to ^{15}N fertilizer applied (%). In addition, total ^{15}N recovery was also calculated including the NH_3-N losses. We did not determine ^{15}N enrichment in NH_3-N losses. Thus, ^{15}N recovery for NH_3-N losses was calculated assuming that these losses originate from the ^{15}N-labeled NH_4^+-N pool of the slurry.

2.9. N balance approach

To derive indications for N immobilization or mineralization following slurry application, an N balance was calculated as follows:

$$N\ balance = N_{input} - N_{output} - changes\ in\ the\ soil\ N_{min}\ pool \tag{4}$$

where N_{input} = total amount of N fertilizer applied (75 kg N ha^{-1} = 4 kg N ha^{-1} from KNO_3^- fertilizer application plus 71 kg N ha^{-1} from CS application) and N_{output} = plant N uptake by aboveground and belowground biomass plus N loss (NH_3-N loss, $N_2O + N_2$ loss, N leaching from topsoil). Changes in the soil N_{min} pool were calculated as the difference between N_{min} contents at the beginning and end of the experiment. Changes in the soil N_t pool do not have to be considered in the total N balance, since the uncertainties in the measurement are superimposed on the small change in the soil N_t pool due to the short duration of the experiment. Thus, a positive value of the N balance indicates that fertilizer N accumulated in the soil as immobilized organic N. If the value is negative, this would indicate that more N was removed than fertilized, i.e. mineralization of organic soil N.

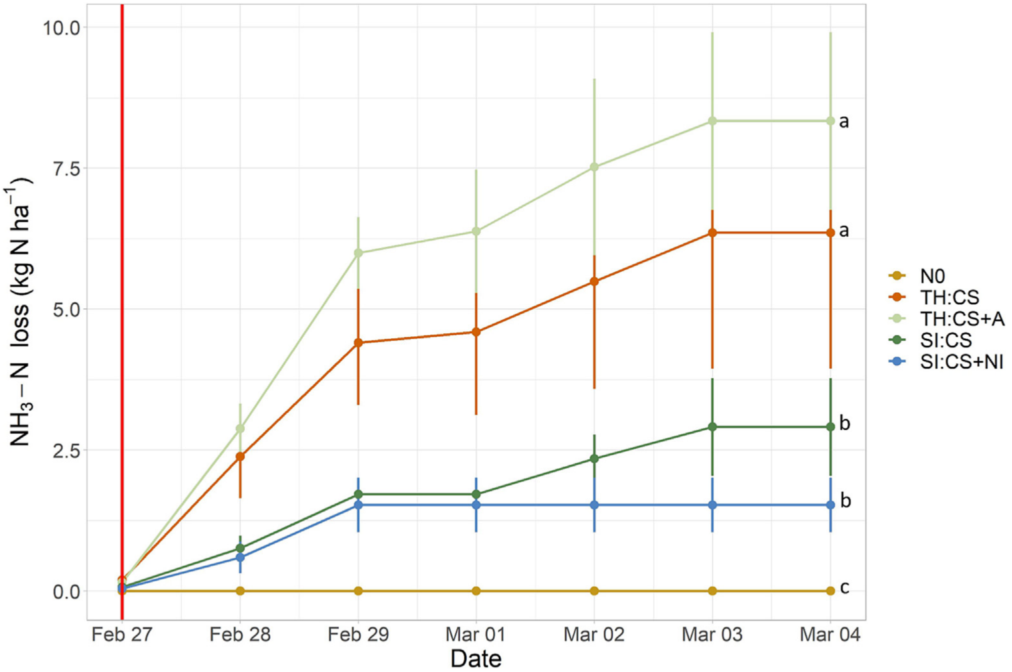

Fig. 2. Cumulative NH_3-N losses during the first days after slurry application. ^{15}N-CS application is marked by the solid red line. Different letters indicate significant differences between the treatments. Error bars indicate standard deviation of the mean (n = 4). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Mean total N_2O fluxes ($N_2O_{fluxtotal}$), N_2O fluxes from applied ^{15}N (N_2O_{fluxL}), N_2 fluxes from applied ^{15}N (N_{2fluxL}), and the residual N_2O from gross N_2O production from the labeled ^{15}N pools (r_{N2O}). Different letters indicate significant differences between the treatments. Values shown are mean of treatment replicates ± one standard deviation (n = 4, except NA where n = 2).

Treatment	N_2O flux$_{total}$	N_2O_{fluxL}[a]	N_{2fluxL}	r_{N2O}
	kg N ha^{-1}	kg N ha^{-1}	kg N ha^{-1}	
NA	0.082 ± 0.082^{a}	nd	nd	nd
N0	−0.008 ± 0.029^{a}	0.004 ± 0.003^{a}	1.63 ± 1.15^{a}	0.003 ± 0.004^{b}
TH:CS	0.095 ± 0.061^{a}	0.068 ± 0.054^{a}	1.58 ± 0.46^{a}	0.053 ± 0.029^{a}
TH:CS + A	0.004 ± 0.040^{a}	0.035 ± 0.019^{a}	1.26 ± 0.86^{a}	0.041 ± 0.037^{ab}
SI:CS	0.053 ± 0.090^{a}	0.093 ± 0.072^{a}	2.16 ± 0.62^{a}	0.026 ± 0.014^{ab}
SI:CS + NI	0.008 ± 0.020^{a}	0.023 ± 0.014^{a}	1.82 ± 0.24^{a}	0.012 ± 0.008^{ab}

nd = not determined.

[a] Individual values of $N_2O_{fluxtotal}$ were always ≥ N_2O_{fluxL}. The larger mean value of N_2O_{fluxL} in some treatments is due to the fact that there were more sampling events for $N_2O_{fluxtotal}$ and that there were fewer isotope sampling events during the later phase when fluxes were low.

2.10. Data treatment and statistical analysis

Calculations were performed using Excel 2019 and R version 4.0.2. (R Development Core Team, 2020). Each soil column (n = 4, except n = 2 for treatment NA) was used as a statistical replicate in this study. Treatment differences were tested by analysis of variance (ANOVA). Variance homogeneity and approximate normality of residuals were checked by diagnostic plots. NO_3^--N and NH_4^+-N contents at the end of the experiment were square root transformed for statistical analysis, which is a common prerequisite due to their right-skewed distribution. pH data at the end of the experiment were back-transformed for statistical analysis. If ANOVA indicated differences, a Tukey-HSD test was performed for pair-wise comparisons using the R package *emmeans*. The level of significance was set to $p < 0.05$ for all tests.

3. Results

3.1. Climatic conditions during the experiment

During the experimental period (February to April 2020), weather conditions were characterized by a daily average air temperature of 7.6 °C. Due to column cover during the heavy rain events in the first two weeks, total precipitation was reduced by about 79 % during this period. Therefore, the soil columns received a total of only about 22 mm of precipitation in the 60 days (Fig. 1a). Daily mean soil temperatures in 5 cm soil depth ranged from 3 °C to 17 °C during the experimental period (Fig. 1b). Soil moisture decreased over time after March 15. This is also reflected in the WFPS values, which showed high soil moisture values (> 70 % in 20 cm soil depth) until the middle of March, followed by a decrease of WFPS values. Wind speed during the NH_3 sampling campaign showed a day-night time scheme and ranged from a daily median between 0.21 m s^{-1} and 1.11 m s^{-1} (data not shown).

3.2. NH_3-N losses

The NH_3 emission of the application treatments TH:CS and SI:CS differed significantly. The total NH_3 emission was highest for the two treatments with band application on the soil surface (6.4 ± 2.4 kg N ha^{-1} in TH:CS and 8.3 ± 1.6 kg N ha^{-1} in TH: CS + A). We found no significant reduction of emission by slurry acidification (Fig. 2). Significantly lower cumulative emission of NH_3 occurred from the two treatments with slurry injection (SI:CS and SI:CS + NI). There was no significant difference between SI:CS with and without a nitrification inhibitor. The total emission was about 30 % lower for injected than for TH applied slurry. The losses in TH:CS and TH:CS + A accounted for 18 % and 14 % of the NH_4^+-N applied, respectively, while in SI:CS and SI:CS + N, they only accounted for 6 % and 3 % of the NH_4^+-N applied, respectively. As expected, zero NH_3 volatilization was measured in the N0 treatment.

3.3. N_2O and N_2 losses

Table 2 shows the mean values of the total N_2O emission ($N_2O_{fluxtotal}$), the N_2O emission from applied ^{15}N (N_2O_{fluxL}), the N_2 emission from applied ^{15}N (N_{2fluxL}), and the residual N_2O from gross N_2O production from the labeled ^{15}N pools (r_{N2O}) for the 60-day sampling period. No significant differences were found for $N_2O_{fluxtotal}$ nor between the CS treatments nor between CS treatments and controls (N0 and NA). N_2O_{fluxL} and N_{2fluxL} were not significantly different either (Table 2). Only r_{N2O}values differed between the treatments. In general, the total N_2O fluxes were very low during the entire sampling period. The maximum N_2O fluxes only reached up to 47 μg N_2O-N m^{-2} h^{-1} in the TH:CS treatment in mid-March about 13 days after slurry application, when daily temperatures increased and WFPS values were still high (Fig. S1). During this period of 16 days in March, the highest gaseous release was also found for N_2O from applied ^{15}N (Figs. S2 and S3). This accounted for

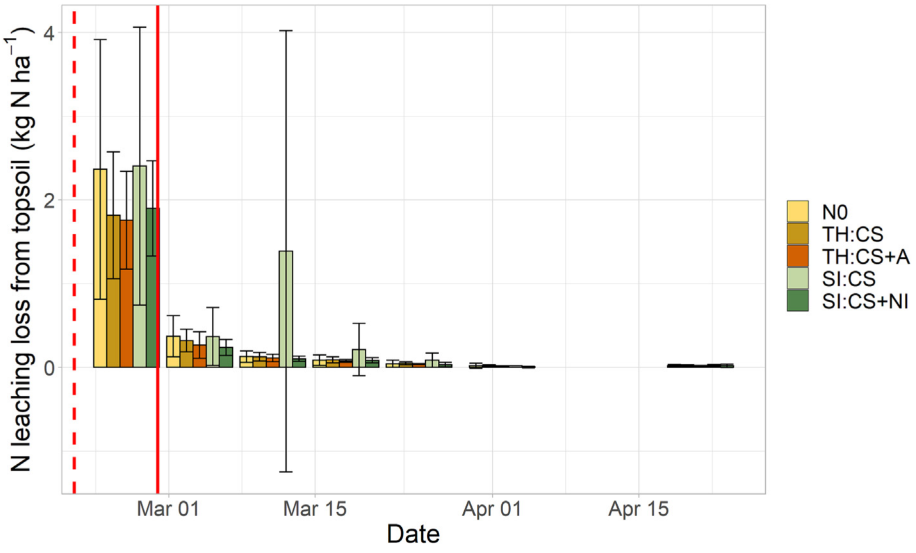

Fig. 3. Time course of the total N leaching loss from topsoil per treatment. $K^{15}NO_3^-$ application is marked by the dashed red line and ^{15}N-CS application is marked by the solid red line. Error bars indicate standard deviation of the mean (n = 4). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3

Total soil nitrogen (N_t), soil nitrate (NO_3^--N), soil ammonium (NH_4^+-N), and soil microbial biomass nitrogen (N_{mic}) in 0–25 cm soil depth at the end of the experiment. Different letters indicate significant differences between the treatments. Values shown are mean of treatment replicates ± one standard deviation (*n* = 4, except NA where n = 2).

Treatment	N_t	NO_3^--N	NH_4^+-N	N_{mic}
	Mg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}
NA	5.35 ± 0.05^a	5.07 ± 1.71^{bc}	1.53 ± 0.45^{ab}	203.77 ± 7.02^a
N0	4.98 ± 0.16^a	4.23 ± 0.26^c	1.22 ± 0.82^b	181.09 ± 19.28^{ab}
TH:CS	5.28 ± 0.23^a	5.97 ± 1.95^{bc}	2.23 ± 0.28^{ab}	163.50 ± 18.85^{ab}
TH:CS + A	5.53 ± 0.61^a	9.09 ± 1.07^{ab}	3.10 ± 1.34^{ab}	177.72 ± 17.48^{ab}
SI:CS	5.02 ± 0.50^a	11.06 ± 2.76^a	3.66 ± 2.53^{ab}	154.67 ± 10.05^b
SI:CS + NI	5.15 ± 0.09^a	6.29 ± 1.76^{bc}	4.50 ± 0.52^a	148.96 ± 2.82^b

about 12 % to 90 % of the total N_2O emission. For the entire sampling period, the N_2 fluxes were significantly higher than the N_2O fluxes, which is also reflected in the particularly low r_{N2O} values. The mean r_{N2O} values in the TH:CS treatments was significantly different from the N0 treatment, while the total CS treatments did not differ from each other. The mean r_{N2O} values ranged over time between 0.004 and 0.082 for the CS soil columns.

3.4. N leaching loss from topsoil

The time course of N leaching from topsoil is shown in Fig. 3. The greatest N leaching losses were found with 1.8 kg to 2.4 kg N ha^{-1} at the beginning of the experiment after the injection of 4 kg N ha^{-1} $K^{15}NO_3^-$ in the ^{15}N labeled treatments. Initial N leaching in the non-fertilized NA treatment was significantly lower (0.1 kg N ha^{-1}). Application of ^{15}N-CS did not lead to increased NO_3^--N or NH_4^+-N concentrations in leachate, except for one soil column (outlier) of the SI:CS treatment at the third sampling date. Also, a rainfall event shortly after CS application (7 mm) without column coverage did not increase N leached from topsoil. On the first sampling date, one week after $K^{15}NO_3^-$ application with 98 at% enrichment and before ^{15}N-CS application, the ^{15}N enrichment was 85 at% in NO_3^--N, while on the second sampling date the value declined to 32 at% and 11 at% (data not shown). Treatment differences between N0 and CS treatments were not obtained. The N leaching was dominated by NO_3^--N, with 86 % in the N0 and CS treatments (data not shown).

3.5. Soil nitrogen pools

Mean values of N_t, soil NO_3^--N, soil NH_4^+-N, and N_{mic} in 0–25 cm at the end of the experiment after 60 days are shown in Table 3, while values for the depth distribution in the soil profile are given in Fig. 5 and Fig. S5. Soil N_t did not differ between the treatments. Nevertheless, a slight increase of 1 % to 6 % in N_t values was found due to CS application when N_t values of the slurry treatments were compared to the NA treatment. The soil NO_3^- pool showed significant treatment differences after 60 days, even though NO_3^--N contents were relatively low in all treatments (3 kg to 14 kg NO_3^--N ha^{-1}). In particular, the SI:CS treatment exhibited the highest NO_3^- values with 11.06 ± 1.04 kg N ha^{-1}, while the addition of NI significantly reduced soil NO_3^--N contents. Soil NH_4^+-N contents were much lower (0 kg to 7 kg NH_4^+-N ha^{-1}) when compared to soil NO_3^-. No significant differences were found between the CS treatments. Concerning N_{mic}, the highest N_{mic} values were found in the NA treatment, while SI:CS significantly lowered the N_{mic} contents. No impact of acidification and/or the addition of a nitrification inhibitor was found.

3.6. Plant biomass and N uptake

Over the developmental period until wheat booting, dry matter (DM) production of aboveground wheat biomass was significantly higher in CS treatments, which received much more nutrients compared to the N0 (received only a small amount of $^{15}NO_3^-$-N for labeling) and non-fertilized NA treatments. The picture for DM production of belowground wheat biomass was different, since only SI:CS + NI differed from N0 and NA. The highest aboveground biomass N was found in the slot injection treatments (SI:CS and SI:CS + NI) with up to 38 kg N ha^{-1} at the end of the experiment (beginning of stem elongation, EC30). Differences in belowground biomass N were smaller and the highest values occurred in the slot injection treatments where CS was applied to 5 cm soil depth. The apparent N recovery

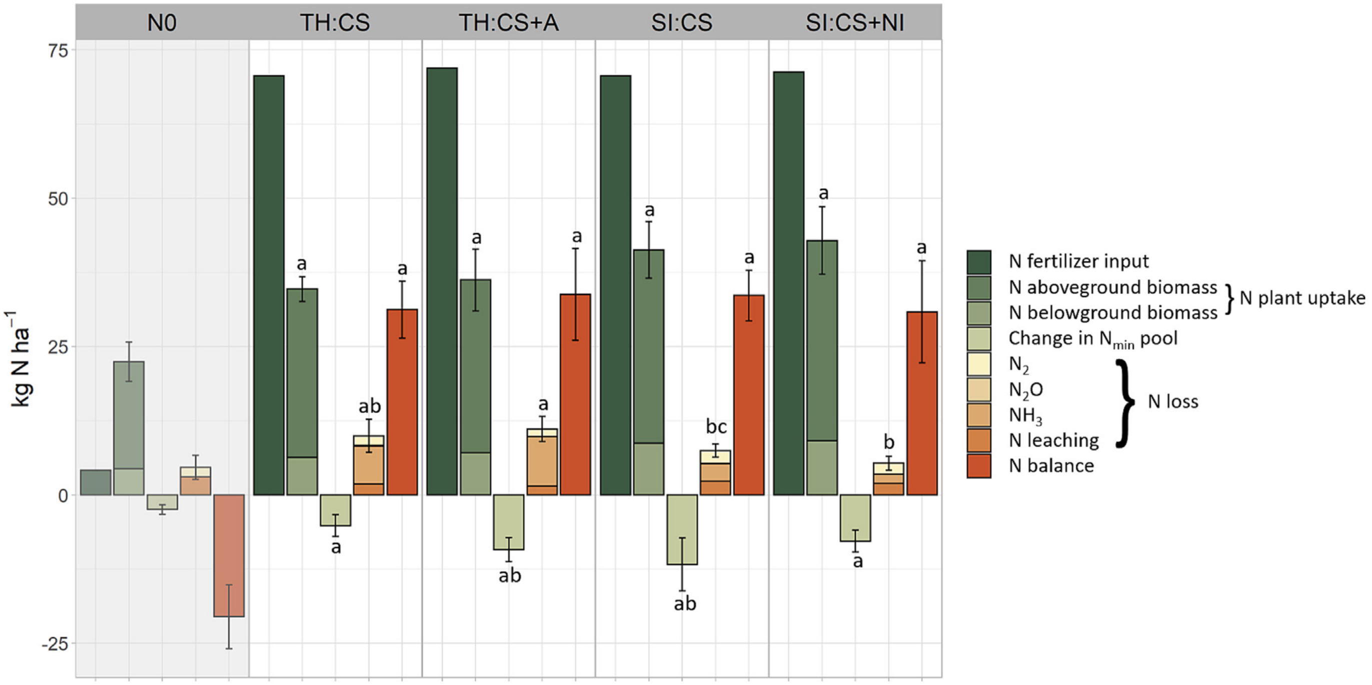

Fig. 4. Total N fertilizer input (N from $^{15}KNO_3^-$ tracer plus N from ^{15}N-CS application), plant N uptake (N_t in aboveground and belowground wheat biomass), change in N_{min} pool (difference in N_{min} before and at the end of the experiment), total gaseous, and dissolved N loss (NH_3-N losses, N_2O losses, N_2 losses, N leaching losses from topsoil) per treatment. The N balance was calculated as the difference of the total N input and plant N uptake, change in N_{min} pool, and the sum of N losses. Different letters indicate significant differences between the CS treatments. Error bars indicate the standard deviations of the mean (*n* = 4).

(ANR%) of the different slurry application treatments is 15 ± 5 % for the TH and 21 ± 6 % for the SI treatments, while neither acidification nor the addition of nitrification inhibitor showed an effect (data not shown).

3.7. ^{15}N recovery

Most of the total ^{15}N applied (24 % to 51 %) was recovered in the soil N_t pool and this was highest (47 ± 3 %) in the slot injection (SI:CS and SI:CS + NI) treatments (Table 5). N_t of aboveground wheat biomass was the second largest sink for the applied ^{15}N, showing a recovery of 16 % to 29 %. Again, the highest value was found in the SI treatments. ^{15}N allocation in belowground wheat biomass was less significant (^{15}N recovery of 3 % to 8 % only). However, for both aboveground and belowground wheat biomass, significant differences were found between trailing hose application and slot injection technique. A considerable part of the applied ^{15}N recovered in soil N_t was located in the N_{mic} pool accounting for 18 ± 9 % of the applied ^{15}N in the CS treatments (Table S1). Similar to the ^{15}N recovery in the N_t pool, no treatment differences were found. In contrast, a much smaller fraction of the ^{15}N tracer found in soil N_t was located in the N_{min} pool with 5 ± 4 % of the applied ^{15}N tracer in the CS treatments (Table S1). The SI:CS showed significantly higher ^{15}N recovery compared to the TH:CS treatment. However, this effect was not present for the treatments TH:CS + A and SI:CS + NI. The ^{15}N recovery in cumulated N_2O + N_2 emission was relatively low (2 % to 10 %) and of a similar order of magnitude as the ^{15}N recovery in cumulated N leached from topsoil (2 % to 9 %, except one extreme value in an SI:CS soil column with 17 %, see also Fig. 3).

The total ^{15}N recovery excluding the NH_3-N losses from the ^{15}N labeled slurry NH_4^+-N pool was at 93 ± 6 and 91 ± 4 % significantly higher in the SI:CS and SI:CS + NI treatments compared to the TH treatments. If NH_3-N losses are included in the total ^{15}N recovery of applied ^{15}N, the balance is almost complete.

3.8. N balance approach

An N balance approach was applied to derive indications for N immobilization or mineralization. For the CS treatments, the N balance varied between 23 kg to 43 kg N ha^{-1} (Fig. 4), indicating N accumulation in the soil. This immobilization of organic N is due to the high input of organic N with the slurry that cannot be mineralized in the short term. For the N0 treatment, the N balance value was negative (−21 ± 5 kg N ha^{-1}), which indicates that N must have been mineralized from the soil supply. However, neither N balance nor the N_t in wheat biomass was significantly different between the treatments. The sum of gaseous and leached N losses was significantly lower in the SI:CS + NI treatment than in the TH treatments (TH:CS + TH:CS + A).

3.9. Depth profiles of soil variables at the end of the experiment

In order to comprehensively examine treatment effects on soil properties, the depth profiles of soil NO_3^--N content, NH_4^+-N content, WEOM, pH (Fig. 5), N_{mic}, and WFPS (Fig. S5) were recorded at the end of the experiment. As expected, the fertilized top soil layers (0–10 cm) had the highest

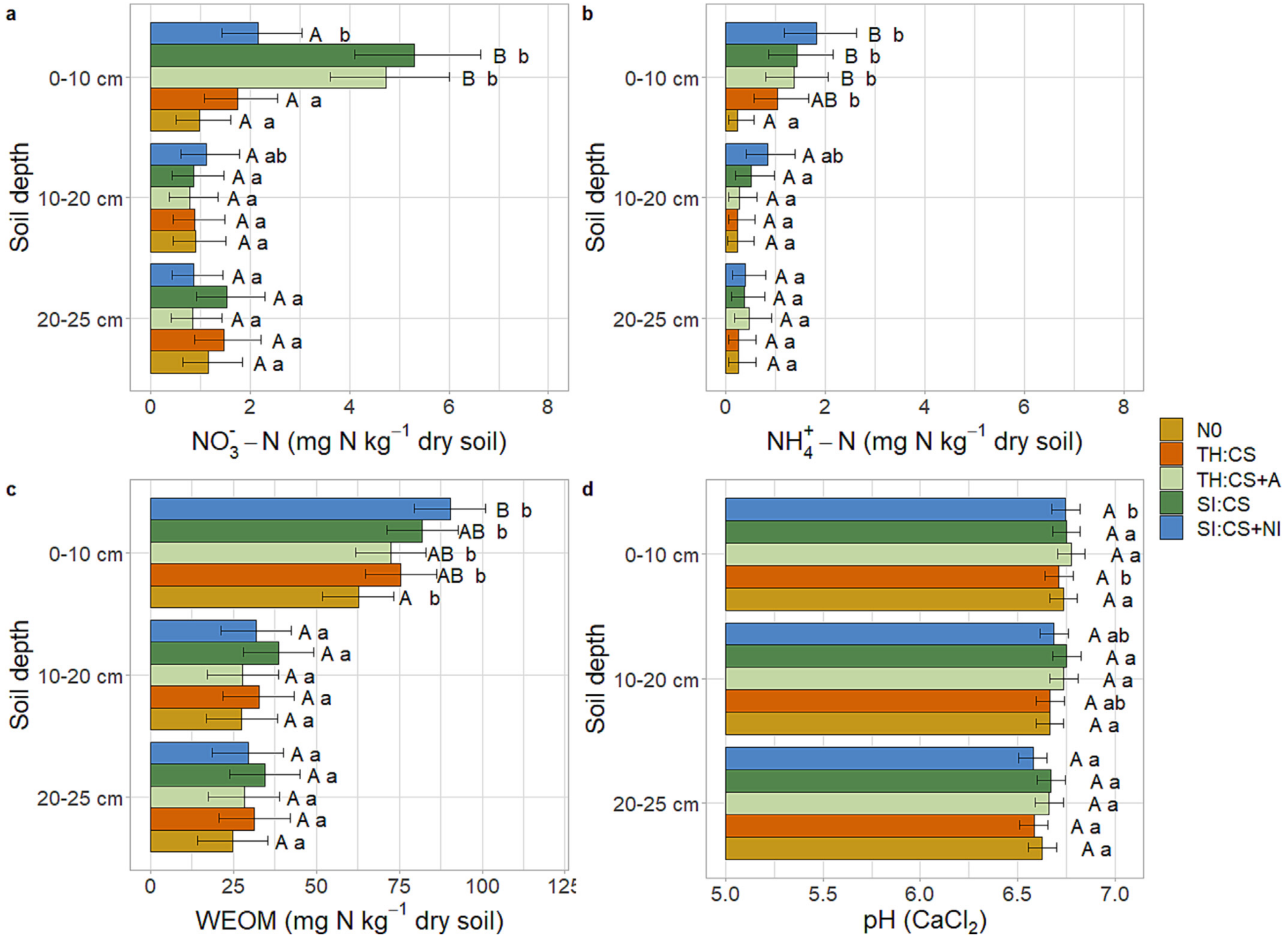

Fig. 5. Depth profiles of NO_3^--N content (a), NH_4^+-N content (b), WEOM (c), and pH ($CaCl_2$) (d) at the end of the experiment. Different letters indicate significant differences between treatment (uppercase letters) and soil depths (lowercase letters). Error bars indicate the standard deviations of the mean (n = 4).

Table 4

Dry matter production (DM) of aboveground and belowground biomass of winter wheat (EC30) along with N_t accumulation in biomass at the end of the experiment. Different letters indicate significant differences between the treatments. Values shown are mean of treatment replicates ± one standard deviation (n = 4, except NA where n = 2).

Treatment	Aboveground biomass DM	Belowground biomass DM	Aboveground biomass N	Belowground biomass N
	$Mg\ ha^{-1}$	$Mg\ ha^{-1}$	$kg\ N\ ha^{-1}$	$kg\ N\ ha^{-1}$
NA	0.88 ± 0.40^{c}	0.46 ± 0.08^{c}	17.74 ± 13.54^{b}	4.06 ± 1.22^{bc}
N0	1.35 ± 0.03^{b}	0.65 ± 0.15^{bc}	18.04 ± 2.53^{b}	4.41 ± 2.16^{c}
TH:CS	1.98 ± 0.11^{a}	0.93 ± 0.07^{abc}	28.39 ± 1.49^{ab}	6.29 ± 1.44^{abc}
TH:CS + A	2.03 ± 0.19^{a}	1.03 ± 0.16^{ab}	29.14 ± 5.10^{ab}	7.08 ± 1.09^{abc}
SI:CS	2.02 ± 0.11^{a}	1.02 ± 0.24^{ab}	32.59 ± 4.41^{a}	8.69 ± 1.76^{ab}
SI:CS + NI	2.11 ± 0.07^{a}	1.10 ± 0.25^{a}	33.75 ± 5.14^{a}	9.11 ± 2.42^{a}

nd = not determined.

N_{min} contents (Fig. 5a, b). The highest soil NO_3^--N contents were measured in the 0–10 cm layer of the treatments TH:CS + A and SI:CS. WEOM contents were also highest in 0–10 cm soil depth. Surprisingly, we found no significant difference in WEOM content in the N0 treatment and in three slurry treatments (TH:CS, TH:CS + A, and SI:CS). Only the treatment with injection of stabilized slurry (SI:CS + NI) exhibited clearly higher WEOM in 0–10 cm than any other treatments. WEOM was generally low in the two other soil depths without differences between treatments. Fertilizer application might have a significant effect on soil pH. However, we found no treatment effect on soil pH in any of the three depth increments. WFPS values followed a gradient and increased with soil depth without significant differences between treatments (Fig. S5).

4. Discussion

4.1. Effects of application techniques on emission of NH_3, N_2O, and N_2

NH_3 volatilization from slurry application mainly occurs in the first days after application (Chalk et al., 2020), while environmental factors, such as air and soil temperature, wind speed as well as solar radiation, control the emission height (Sommer et al., 2003). Also, in our study, the highest NH_3 emissions occurred within the first two days, although the general level of NH_3 emission was relatively low when compared to previous studies (Fangueiro et al., 2017; Herr et al., 2019). Total NH_3-N losses were equal to about 16 % of NH_4^+-N applied in the TH treatments and significantly lower than emissions reported in previous studies by e.g. Herr et al., 2019 at 45 % of applied NH_4^+-N. However, there are also studies with similarly low NH_3-N losses, as in Quakernack et al. (2012) with 9 % to 15 % of NH_4^+-N applied for TH application of biogas digestate to winter wheat. Our SI treatment NH_3-N losses of about 2 % to 8 % of NH_4^+-N applied were also lower than values in the literature ranging from 8 % to 28 % of NH_4^+-N applied (Fangueiro et al., 2017; Herr et al., 2019). The low air and soil temperatures, as well as one rainfall event with 7 mm after CS application, probably reduced the potential for NH_3 volatilization (Hafner et al., 2019). The early application date in mid-February is usual for slurry application to winter wheat and it appears to be favorable with respect to avoiding NH_3-N losses. Many other studies found higher NH_3 volatilization rates later in the year when warmer and sunny conditions were present (e.g. Herr et al. (2019), Quakernack et al. (2012), Pedersen et al. (2020), Fangueiro et al. (2017)).

Apart from environmental drivers, the timing of the slurry application and application rate, the application technique is known to significantly impact the magnitude of NH_3 emissions (Webb et al., 2010). Our results confirm that NH_3 emission from slurry application can be reduced by slot injection techniques. We found a reduction in NH_3 emission of 70 % compared to the band application with TH. This reduction is in line with results from a recent meta-analysis by Emmerling et al. (2020), which showed an average NH_3 reduction of 61 % (95 % confidence intervals 89 %, 31 %). Our second hypothesis that slurry acidification compensates for the effect of higher emissions from TH compared to SI was not confirmed. Although lowering the slurry pH is widely known to significantly decrease NH_3 emissions by up to 89 % (Fangueiro et al., 2017; Park et al., 2018), we have not found this effect. The most likely explanation for the missing effect of slurry acidification might be the large pH buffer capacity of slurry due to insufficient H_2SO_4 dosage. This was recently also pointed out by Wagner et al. (2021). Adjusting slurry pH by adding acid directly before application bears the risk of a relatively fast pH increase induced by this buffer capacity. If H_2SO_4 is used for the acidification of cattle slurry, a slurry pH of 6 is recommended to effectively reduce NH_3 emission (Fangueiro et al., 2015a; Sørensen and Eriksen, 2009). Thus, the stability of the adjusted slurry pH should be controlled before application or the specific pH buffer capacity of the slurry has to be determined to ensure adequate acid addition. Considering only NH_3 emissions, slot injection would score as the best application technology, but losses via N_2O and especially N_2 emissions must also be considered for a comprehensive evaluation of gaseous N losses.

N_2O + N_2 losses from slurry treated soil were generally lower than NH_3 losses during the experimental period of 60 days ($N_2O + N_2 < 2.8$ kg N ha^{-1}). Related to the total amount of fertilizer N applied (71 kg N ha^{-1}), these losses ranged from 0.6 % to 3.9 % of the total N input. 2 % to 10 % of the ^{15}N tracer added was lost via $N_2O + N_2$. Thus, $N_2O + N_2$ emission was only a minor N-loss pathway. Consequently, complete denitrification, i.e. the reduction of N_2O to N_2, prevented relevant N_2O emissions in this experiment.

Contrary to our expectations about the effect of application techniques on N_2O and N_2 emission and recent findings from a meta-analysis by Emmerling et al. (2020), total N_2O and N_2 losses were not higher from SI compared to TH application. It is assumed that SI favors denitrification and N_2O emission because of reduced NH_3 emission resulting in higher NH_4^+-N contents and the accumulation of labile C_{org} in combination with increased moisture content in the slurry slot (Webb et al., 2010). These conditions might stimulate the denitrification potential in all slurry treatments (Velthof and Mosquera, 2011). This is underlined by recent findings of Malique et al. (2021), where a tendency for higher potential denitrification rates was found for CS broadcast application with and without acidification compared to a control treatment without CS in a grassland soil under similar dry conditions in March 2020.

Table 5

Recovery of ^{15}N tracer added with $K^{15}NO_3^-$ and ^{15}N labeled slurry as total $N_2O + N_2$ emission, total N leaching from topsoil (sum of NO_3^--N plus NH_4^+-N), total soil nitrogen (N_t), aboveground, and belowground wheat biomass. Total ^{15}N recovery was calculated as the difference from 100 % ^{15}N recovery excluding and including NH_3-N losses (assuming that NH_3-N losses originate only from the ^{15}N-labeled NH_4^+-N pool of the slurry). Different letters indicate significant differences between the treatments. Values shown are mean of treatment replicates ± one standard deviation (n = 4).

Treatment	N recovered from sum of $K^{15}NO_3^-$ + ^{15}N-CS tracer applied					Total ^{15}N recovery excluding NH_3-N losses	Total ^{15}N recovery including NH_3-N losses
	$N_2O + N_2$ emission	N leaching from topsoil	N_t	Aboveground biomass	Belowground biomass		
	%	%	%	%	%	%	%
TH:CS	6 ± 2^{a}	6 ± 3^{a}	32 ± 8^{a}	21 ± 0^{b}	4 ± 1^{a}	67 ± 8^{b}	78 ± 7^{a}
TH:CS + A	5 ± 3^{a}	6 ± 2^{a}	33 ± 17^{a}	19 ± 3^{b}	4 ± 1^{a}	64 ± 19^{b}	79 ± 20^{a}
SI:CS	8 ± 2^{a}	8 ± 6^{a}	47 ± 3^{a}	25 ± 3^{a}	7 ± 1^{b}	93 ± 6^{a}	99 ± 5^{a}
SI:CS + NI	7 ± 1^{a}	6 ± 2^{a}	47 ± 3^{a}	27 ± 2^{a}	7 ± 1^{b}	91 ± 4^{a}	94 ± 4^{a}

Similar ^{15}N enrichment in N_2O emitted and soil NO_3^- in the first days following $K^{15}NO_3^-$ addition (data not shown) indicate that N_2O was mainly produced by denitrification and nitrifier-denitrification during this initial phase of the experiment before ^{15}N-CS was applied. This seems reasonable because of the high soil moisture content (< 70 % WFPS), which is known to favor N_2O reduction due to decreased O_2 availability at a high WFPS level (Weier et al., 1993). Later in the experiment, rainfall and WFPS levels decreased significantly so that nitrification likely gained increasing relevance as a source of N_2O. Following slurry application, we found just a tendency to slightly higher N_2O emissions in all treatments (Fig. S1), while previous studies mostly measured N_2O peaks directly after application (Häfner et al., 2021; Herr et al., 2019). Although the anticipated hotspot effect with high N_2O emission following slurry application was missing, nevertheless, conditions favoring N_2O reduction (low r_{N2O} values) were always present. On the one hand, this may be due to the high input of labile C by slurry and the resulting shift in the ratio between electron donors (microbe available organic C) to acceptors (N oxides) in soil, which is known to favor N_2O reduction with increasing electron donor abundance (Smith and Arah, 1990). This was supported by the low NO_3^--N concentrations in the soil. Due to a shortage of N oxides under such conditions, the relative rate of N_2O reduction (in relation to N_2O production) increases. Thus, very low $N_2O/(N_2O+N_2)$ ratios dominate (Senbayram et al., 2012). Moreover, the relatively high soil pH of about 6.7 probably supported N_2O reduction, as the synthesis of functional N_2O reductase is impeded by low pH (Liu et al., 2014). Although these drivers differed slightly among the treatments (Table 2, Fig. 5), the differences had apparently no impact on the magnitude of $N_2O + N_2$ emissions.

Up to now, no data were available on N_2 fluxes from slurry application under field conditions with growing crops. In the literature, some data can be found from laboratory incubations of soil cores in N_2-free (He—O_2) atmosphere following slurry application (Zistl-Schlingmann et al., 2019) or application of biogas digestate (Buchen-Tschiskale et al., 2020; Fiedler et al., 2017; Köster et al., 2015). r_{N2O} values of 0.01 to 0.03, calculated from the cumulative N_2O and N_2 emissions of a 2-week He—O_2 laboratory incubation experiment with intact soil cores from grassland sites following slurry application (Zistl-Schlingmann et al., 2019) show a level well comparable to the present case. However, compared to He—O_2 incubation, the lysimeter experiment shown here allows the detection of N_2 fluxes under field conditions and in the presence of growing plants, avoiding possible artefacts due to the absence of plants and light exposure.

The expected effect of the nitrification inhibitor on N_2O and N_2 emissions, i.e. the significant reduction of N_2O emissions as shown by e.g. Herr et al. (2020) under field conditions and by Dittert et al. (2001) and Wu et al. (2017) under laboratory conditions was not found in the present experiment. This might result primarily from the generally low N_2O emission level. So far, this is the first study to investigate the effect of NI on N_2 and thus r_{N2O} values under conditions comparable to field settings. Previously, only studies using oxic He—O_2 incubation (Wu et al., 2017) or anoxic He incubation (Hatch et al., 2005) found an increase of N_2 fluxes and a reduction in r_{N2O} values following the addition of NI (DCD or DMPP) on disturbed bare soil. They assumed that NI decreased r_{N2O} values by restricting nitrification and limiting NO_3^- supply to denitrifying soil microsites. Thus, r_{N2O} values decreased due to the competitive effect of N_2O and NO_3^- as an electron acceptor during denitrification (Senbayram et al., 2012). Since the study by Wu et al. (2017) applied mineral fertilizer, it is questionable to what extent this statement also applies to slurry, as the NO_3^- effect of inhibitors interacts with the effect of additional labile C supplied with slurry. Although no impact of NI on emission of N_2O and N_2 following ^{15}N-CS application was found in the present study, it is important to investigate this further under different environmental and soil conditions. It is important to note that all N fluxes were highly variable even for soil cores with the same treatment. This reflects the well-known high spatial variability of factors controlling N_2O and N_2 fluxes in soil-plant systems (Schaufler et al., 2010), which might have masked moderate treatment effects.

4.2. N leaching losses from topsoil

Generally, low NO_3^--N values and apparently low nitrification after N fertilizer application in combination with low temperatures and less rainfall due to soil coverage on heavy rainfall events lead to moderate NO_3^- accumulation until the end of the experiment (Table 3). Thus, the risk of N leaching from topsoil (< 25 cm soil depth) was reduced. N leached from topsoil was relatively low over the entire study period with 2 % to 17 % (4 % on average) of total fertilizer N applied. In addition, possibly also due to the relatively low over all N fertilizer application rate with only 71 kg N ha^{-1}. The greatest N leaching loss from topsoil was found in the first leachate sampling after $K^{15}NO_3$ application and before CS application, where 50 % of the applied ^{15}N (2 kg N ha^{-1}) was leached. This could be due to the injection of ^{15}N labeled NO_3^--N solution over the entire soil depth, which is associated with a proportion of ^{15}N tracer in the mobile soil water during the first week. This is supported by the fact, that the average ^{15}N enrichment of leached NO_3^-N (85 at%) was higher than the theoretical average enrichment resulting from complete mixing of tracer NO_3^--N and non-labeled soil NO_3^--N (60 at%). After this event, significant N leaching losses from topsoil were no longer detected due to low leachate water yield, nor did CS application have any significant effect. Even though slurry application is prone to N leaching, in particular when there is low plant N uptake, precipitation is high and free drainage is possible, e.g. on sandy-textured soils with low water retention capacity and/or high manure application rates (Chantigny et al., 2004; Kayser et al., 2015). Presumably, neither condition was met in the present study, since precipitation was low, water retention of the loamy soil is high and plant N uptake was high (Table 4). A similar low risk of topsoil N leaching on medium-textured soils, like our Haplic Luvisol, was described by Herr et al. (2020), who assumed that, due to the low N leaching risk, the positive effect of NH_4^+ conservation due to NI addition should also be low, in particular under dry weather conditions. We found no impact of the different application techniques (TH or SI) on N leaching from topsoil. This confirms findings by Kayser et al. (2015), who found similar low N leaching losses (< 75 cm soil depth) following CS application of multiple doses of 80 kg N ha^{-1} by trailing hose and open slot injection in grassland over four years. The relatively low slurry N losses by leaching are in line with the rule that slurry application in winter wheat has to be done in spring, where plant N uptake is usually high in order to minimize over-winter losses of slurry N.

4.3. Plant N uptake

Besides minimizing hazardous emissions, the main objective of improving slurry application techniques is to optimize crop N uptake and N use efficiency. Aboveground DM production of winter wheat reached 2 Mg ha^{-1} at the end of our study, which indicates a typical wheat development at the stage of beginning stem elongation (Diekmann and Fischbeck, 2005). However, average N uptake rates of the CS treatments were with 31 ± 5 kg N ha^{-1} significantly lower when compared to common N uptake rates at EC30 with 50 kg N ha^{-1} in Germany (Diekmann and Fischbeck, 2005). About 34 % to 54 % of total fertilizer N applied was found in the aboveground biomass and 7 % to 16 % of fertilizer N applied in belowground biomass (Table 4).

Considering the different CS application techniques, no significant effect on DM production and N uptake was found, although N uptake of aboveground biomass tended to be higher in the SI treatments (SI:CS and SI:CS + NI). This tendency was underlined by a higher ^{15}N recovery in the SI compared to the TH treatment. We suggest that the concentration of $^{15}NH_4^+$ from CS in the slot, very close to the wheat roots, was more readily available to the plant compared to TH, which enhanced the ^{15}N uptake. In surface application, the slurry-derived ^{15}N must first migrate and/or diffuse to the deeper root zones of the wheat plant until it is available for the roots. In addition, lower NH_3 emissions in the SI treatment might have caused higher ^{15}N uptake of the plants, which matches hypothesis 3. Comparing our results with previous studies, which investigated different application techniques with respect to ^{15}N-labeled CS, Sørensen (2004) found up to

46 % ^{15}N recovery in aboveground biomass of spring barley at maturity stage following injection to 10 cm soil depth, while only up to 13 % of ^{15}N-labeled CS was recovered following surface application. In another experiment, Sørensen and Thomsen (2005) found up to 60 % of applied N recovered in winter wheat at maturity stage after surface band-application of pig slurry, while only up to 2 % was found in roots and stubbles at the end of the entire growing period. However, previous findings are hard to compare to our findings due to harvesting at different crop development stages, differences in plant N uptake between wheat and barley, and/or differences in the utilization of pig slurry vs. cattle slurry, as well as differences in soil and climatic conditions.

Our results confirm the high value of slurry NH_4^+-N for plant nutrition and underline the importance of keeping the full fertilizer potential of slurry by minimizing gaseous and dissolved N losses. However, we can conclude that none of the application techniques investigated here present any limitation with respect to DM production and plant N uptake during the studied developmental period until wheat emergence.

4.4. Soil N pools

Differences between treatments in soil N_{min} and N_{mic} pools, and in ^{15}N recovery from labeled slurry N in these soil N fractions may provide evidence of whether application techniques alter net-mineralization and microbial immobilization of N (Dittert et al., 1998; Powlson and Barraclough, 1993). The increase of N_{min} and the net-N immobilization is known to be greatest within the first two weeks after slurry application (Sørensen, 2004). Elevated N_{min} contents after CS application in various field crops are typically found up to 50 days after CS application (Fangueiro et al., 2015b; Lin et al., 2017), which was not the case here. Presumably, because the N fertilizer rates of about 70 kg N ha^{-1} were significantly lower than in the study of Lin et al. (2017). However, with 80 kg N ha^{-1} application rates of Fangueiro et al. (2015b) were in a similar range compared to our study. Changes in the dynamic soil N_{min} pool could not be monitored in our experiment, since it was only possible to sample once at the end. At this time, overall N_{min} contents in the CS treatments were generally low (11 ± 3 kg N ha^{-1}). However, if the N_{min} contents are separated into NO_3^- and NH_4^+, NO_3^--N contents were, as expected, higher compared to NH_4^+-N contents. With respect to the application technique, the highest NO_3^--N contents were found in both SI treatments compared to the TH treatment, along with the highest plant uptake of ^{15}N when slurry was injected. Due to the significantly lower NO_3^--N contents in the SI:CS + NI compared to the SI:CS treatment, the expected NI effect (Ruser and Schulz, 2015) was confirmed here, whereas this effect was missing for N leaching from topsoil and N_2O emission data.

Although it is difficult to attribute changes in N pools to different N sources because the release coincides with mineralization processes of different slurry components (Dittert et al., 1998), the N_{mic} pool was also examined in more detail in this study. Surprisingly, the anticipated increase in N_{mic} following slurry application was not found in our study; only single treatment differences occurred (e.g. NA vs. SI in 0–25 cm soil depth). It might be that the initial flush of microbial activity, mostly occurring within the first three to four weeks after slurry application (Jensen et al., 2000), was probably not caught by sampling at the end of the experiment. In total, N_{mic} ranged from 146 kg to 211 kg N ha^{-1} in slurry fertilized and only mineral fertilized treatments were on a similar level to findings by Jensen et al. (2000). Contrary to previous findings on the soil N_{mic} pool, where the SI treatment always had the highest concentrations, the highest N_{mic} values were found in the NA treatment with a tendency to higher ones in the TH treatment as well when compared to slot injection. Nevertheless, for the ^{15}N recovery, the trend was reversed and a trend to higher ^{15}N recovery in SI compared to TH was observed.

4.5. ^{15}N recovery and the N balance

In order to comprehensively evaluate the different slurry application techniques, it is important to consider all the different N fluxes and pools. Due to the applied ^{15}N double labeling approach, it was not possible to differentiate the labeled ^{15}N derived from the $^{15}NO_3^-$-N and CS $^{15}NH_4^+$-N pool. However, total ^{15}N added originated mainly from labeled CS (85 % of total ^{15}N applied) and, thus, total ^{15}N balance was primarily dominated by labeled slurry NH_4^+-N. Up to 100 % of the sum total of applied ^{15}N was recovered in the SI and SI + NI treatments, if assumed ^{15}N losses by NH_3 emission were included. Surprisingly, ^{15}N recovery of TH and TH + A was lower, which might be due to an underestimation of NH_3-N losses and/or uncertainty in determining the different N pools. Nevertheless, the ^{15}N recovery showed for all slurry treatments that most of the sum of ^{15}N applied was taken up by the crop or retained in the N_t pool (Table 5). The importance of the N_t pool was also supported by the N balance approach applied (Fig. 4) where the positive N balance shows that about one third of the applied slurry N was immobilized in the soil, regardless of the application technique. If we account for the different N fluxes and pools in more detail, results of the N balance demonstrate that the sum of gaseous and dissolved N losses was significantly lower in the SI:CS + NI treatment than in the TH treatments (TH:CS + TH:CS + A). If the total N losses are considered in relation to the total N input through fertilizer application, the N losses only account for a share of 16 % ± 3 % in the TH treatments and 11 ± 5 % in the SI treatments. This strengthens the role of the SI application, since it increased crop productivity (DM production and plant N uptake) and undesired N losses (low NH_3-N, N_2O, and N_2 losses) were low, at least under the climatic conditions and 60 days of investigation. Concerns that higher denitrification losses and leaching could offset the benefit of reduced NH_3 reduction (Dell et al., 2011) and/or that injection could severely damage crop development were not confirmed.

5. Conclusions

A 60-day lysimeter experiment with growing winter wheat was set up to follow the transformation of slurry $^{15}NH_4^+$ and soil $^{15}NO_3^-$ using a double labeling approach. The experiment was used to evaluate the effects of different slurry application techniques (trailing hose with/without acidification and slot injection with/without nitrification inhibitor) on the partitioning of total and ^{15}N labeled N. Our hypothesis 1 was partly confirmed: open slot injection of slurry (SI) significantly reduced NH_3 emission compared to band application by trailing hose (TH). However, acidification of the slurry (TH + A) did not lead to NH_3 reduction, which was probably due to the pH buffer capacity of the slurry and increasing pH values after acidification. Hypothesis 2 was not confirmed: slurry application techniques did not differ with respect to N_2O + N_2 emission or N leaching from topsoil. Biomass dry matter production of wheat at growth stage EC30 was not influenced by the application technique. However, there was a significant effect on plant uptake of applied ^{15}N, which was greater in the SI than the TH treatments. The results indicate that injected slurry provided more plant available N than slurry applied by TH. The differences can partly be explained by lower NH_3 losses from the SI treatments. A positive effect of the nitrification inhibitor (NI) was only found through reduced NO_3^--N contents, but not on total N losses or N leaching from topsoil. Our new results on N_2 emission following slurry application show that 2 % to 10 % of the applied ^{15}N was lost by denitrification. The low r_{N2O} values indicate a low risk of N_2O, since N_2O was effectively reduced to N_2. The ratio of NH_3-N loss to N_2 + N_2O losses was higher for TH application (8.1 ± 7.9) than for SI (1.3 ± 0.6). In the present case, slot injection would score as the best application technology based on NH_3 reduction. This recommendation is supported by the N_2O + N_2 emissions in the SI treatment not being increased either. This is because it has to be kept in mind that both emission types always have to be minimized in order to optimize the fertilizing effect of cattle slurry. Impacts of the application techniques on individual soil N pools (N_t, N_{min}, N_{mic}) were small. However, the high ^{15}N recovery shows that our experiment yielded reliable data for the allocation of slurry N to soil and plant pools as well as gaseous losses. Although the study has some methodological limitations, it thus offers versatile possibilities for further use in modeling studies, since not only were the different N pathways and pools comprehensively investigated, but so were important environmental drivers. In addition, the study provides for the first time N_2 fluxes from slurry application under field conditions with growing crops.

CRediT authorship contribution statement

CBT and RW developed ideas and designed the experimental setup with great assistance from HF. CBT collected the field data and performed laboratory measurements; CBT and RW analyzed the data; CBT led the writing of the manuscript. All authors provided critical input on drafts and gave final approval for publication.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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