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Full nitrogen balances for different cattle slurry fertilization techniques in a temperate grassland

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ABSTRACT

Modern slurry application techniques have been shown to reduce ammonia losses, yet a comprehensive evaluation of their nitrogen (N)-related agronomic and ecological impacts is missing. Therefore, we utilized ^{15}N-labeled cattle slurry to examine traditional and modern application techniques regarding their effects on hydrological and gaseous N losses, plant N uptake, soil organic nitrogen (SON) formation, and total fertilizer N balances. Following the broadcast spreading of slurry, 43 % of fertilizer N was lost as gaseous emissions, irrespective of precipitation. In contrast to broadcast spreading, significant total N emission savings were achieved by the broadcast application of diluted slurry combined with a reduced N supply (47 % emission reduction). Open slot injection at depths of 5 cm and 2 cm led to even greater emission reductions of 60 % and 74 %, respectively. Recent fertilizer was typically leached in minimal amounts only, yet the application of diluted slurry elevated nitrate leaching due to increased infiltration. Overall, the high productivity and plant N uptake were hardly affected by the application method, because over 90 % of the plants' N uptake relied on mineralized SON rather than recent fertilizer. This promoted soil N mining, particularly for broadcast spreading and slurry dilution, resulting in distinctly negative N balances (17 – 37 kg N ha^{-1} deficit per fertilization-harvest cycle). Utilizing slurry injection contributed to additional SON formation, effectively offsetting the N deficit and thereby supporting the long-term maintenance of N-related soil functions.

1. Introduction

Liquid manure, also known as slurry, has become Central Europe's most important organic fertilizer for grasslands (Capriel, 2013). The traditional method of slurry broadcast spreading has long been the most common application technique and is still widely used in smaller grassland-dominated farms. Yet, this method is linked to high fertilizer nitrogen (N) losses during and after field application, causing a wide range of environmental and human health issues (Amon et al., 2006; Uusi-Kämppä and Mattila, 2010; Wyer et al., 2022). Ammonia (NH_3) is among the key N compounds lost during slurry field application, particularly when traditional broadcast spreading is used (Sommer and Hutchings, 2001). These losses cause N to cascade across ecosystem boundaries with subsequent water eutrophication, soil acidification, biodiversity loss, and air pollution (Krupa, 2003; Behera et al., 2013; Mahmud et al., 2021). Emissions of NH_3 from animal waste depend mainly on slurry pH, temperature, wind, infiltration rate into the soil, and application technique (Sommer and Hutchings, 2001; Sommer et al., 2003; Gay and Knowlton, 2005).

Measures to reduce N losses during liquid slurry application include replacing traditional broadcast spreading methods with alternative techniques such as targeted slurry application directly onto the soil surface or injection into the soil (Hou et al., 2015). Such approaches can reduce slurry exposure to the atmosphere and associated NH_3 losses, thereby increasing plant Nitrogen Use Efficiency (NUE) and soil nutrient retention (Webb et al., 2010; Nyameasem et al., 2022). Slurry injection has shown great potential in reducing NH_3 emissions by up to 80 % compared to broadcast spreading, as summarized in a review by Webb

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et al. (2010). For grasslands specifically, previous studies detected reductions from 31 % to 61 % in perennial grassland in Denmark (sandy soil) and northern Germany (marsh, clay soil) using disc injectors with varying row spacing compared to band spreading (Seidel et al., 2017). Average NH_3 emission factors were only 16 % for shallow injection compared to 74 % for surface broadcast spreading (Huijsmans and Schils, 2009).

Besides slurry injection, another approach to reduce NH_3 volatilization from slurry is to improve its infiltration into the soil. This can be achieved by reducing the dry matter (DM) content and the viscosity of the slurry, e.g., via dilution or optimized application timing immediately before rainfall events. Once the fertilizer N is in the soil, the diffusion of NH_3 is minimal due to the cohesive properties of the soil, and the sorption of ammonium (NH_4^+) to soil colloids further reduces NH_3 volatilization (Sommer and Hutchings, 2001). Mkhabela et al. (2009) applied hog slurry to forage grass on various soil types and found that simulated rainfall after slurry application reduced NH_3 losses by 45 % while diluted slurry decreased them by 41 %. For slurry dilution, the amount of water added to the slurry was shown to be linearly and inversely related to volatilization, with a water-to-slurry ratio of 0.9–1.2:1 halving the NH_3 emissions when compared to undiluted slurry (Frost, 1994). The reduced oxygen supply or increased soil water content resulting from a rainfall event shortly after slurry application can potentially enhance N_2O emissions from microbial nitrification-denitrification processes. (Butterbach-Bahl et al., 2013).

Generally, the focus of the evaluation of alternative slurry application techniques was on the abatement of NH_3 emissions, which is criticized by many authors since some measures may lead to significant increases in emissions of greenhouse gases like methane (CH_4), nitrous oxide (N_2O), or carbon dioxide (CO_2). With soil microbial nitrification-denitrification processes being major sources of N_2O (Butterbach-Bahl et al., 2013), slurry soil incorporation and infiltration can increase N_2O emissions while simultaneously decreasing NH_3 emissions (Emmerling et al., 2020). In this context, a previously often overlooked N compound is the terminal denitrification product dinitrogen (N_2), which can also strongly contribute to total slurry N losses (Zistl-Schlingmann et al., 2019; Dannenmann et al., 2024). Hence, NH_3 emissions may not be a good indicator for total N emissions. Total fertilizer N losses and full N balances have largely been disregarded, yet they must be taken into account since some recent studies indicate that negative soil N balances are a major problem of slurry fertilized grasslands (Zistl-Schlingmann et al., 2020a, 2020b; Schreiber et al., 2023). Such a deficit in the N balance poses the risk of N mining causing a depletion of soil organic nitrogen (SON). However, these studies have so far been limited to calcareous soils with a neutral pH value. The long-term positive response of improved nitrogen management practices on SOM, soil quality and productivity is well-studied (Rumpel et al., 2015; Menšík et al., 2018; Wang et al., 2020; Shi et al., 2024). Yet, research on the influence of individual organic fertilizer application techniques is scarce.

Besides gaseous fertilizer N losses, the leaching of dissolved compounds can also be problematic. There is great public awareness regarding agricultural N losses in the form of nitrate (NO_3) leaching since it can cause eutrophication in rivers and lakes or contaminate drinking water supplies, posing a risk to human health. Generally, grasslands are assumed to be less prone to NO_3 leaching losses because grass and pasture plants are assumed to be very efficient at taking up the soil mineral N (Cameron et al., 2013). In an experiment that included broadcast and shallow injection on cut grassland, Kayser et al. (2015) demonstrated that the amount of N input, rather than the application method, governs the effects of NO_3 leaching. In another study, NO_3 leaching was not promoted after slurry application on permanent grassland either, but only during intense autumn rainfall (Maris et al., 2021). Still, the agricultural input of nutrients is the main cause of high NO_3 concentrations in groundwater, which is why the European Nitrate Directive (Council of the European Communities, 1991) for the protection of water bodies against agricultural pollution was passed. However, this EU legislation is mainly based on gaseous NH_3 emissions during and after liquid slurry application, thus promoting a shift towards low NH_3 emission methods. Quantitative knowledge of how different slurry techniques impact NO_3 leaching and the full fertilizer N balance is missing.

Given that SON mining is a severe issue in temperate grasslands, a more holistic approach towards the evaluation of full fertilizer N balances of different slurry application techniques is needed, including soil N retention, plant N export, and total gaseous and hydrological N losses This is crucial to inform fertilizer ordinances and farmers decisions to facilitate more efficient N management that reduces costs as well as N pollution of ecosystems, air and water bodies, but increases soil health and fertility. To compile full N balances, ^{15}N-labeled fertilizers and subsequent tracing in the soil-plant system are required. Using stable isotopes also allows for direct tracking of fertilizer N uptake by plants and retention in the soil. So far, a more comprehensive comparison of slurry application techniques based on full N balances after fertilizer application is only available for grasslands on neutral pH calcareous soils in the pre-alpine region of Southern Germany (Schreiber et al., 2023). However, systems with a different soil pH, such as mountain ranges of Central Europe with lower pH soils (originating from silicate bedrock), need to be considered as well, given they constitute important grassland region and the dominant role of pH in regulating gaseous N losses (Butterbach-Bahl et al., 2013). Therefore, this study aims to provide a holistic evaluation of the performance of (1) traditional broadcast slurry application compared to (2) broadcast spreading of diluted slurry in combination with reduced N fertilization and (3) shallow and (4) deep slurry injection for typical grassland on silicate bedrock. A further broadcast slurry treatment followed by artificial precipitation (5) allowed to assess potential reduced N losses due to optimal fertilization timing. We used ^{15}N-labeled cattle slurry in a replicated plot-scale experiment on an extensive montane grassland to trace fertilizer N flows in the plant-soil system and different N-loss pathways. The various liquid slurry application techniques were tested for one fertilization-harvest cycle with the objective to (1) assess the potential advantages of injection compared to diluted broadcast and broadcast spreading concerning productivity, NUE, and plant N uptake and (2) compare full N-balances including total gaseous N loss and N leaching for the different application techniques. We hypothesize that (1) slurry injection increases productivity and fodder N content compared to broadcast application due to promoted plant N uptake, which is not achieved by dilution given the reduced N supply, and that (2) slurry injection and slurry dilution strongly reduce total gaseous N losses, but promote N leaching as well as N retention in SON.

2. Material and methods

2.1. Study site and experimental design

The study site is located near Süssenbach (49°06'10''N, 12°21'32''E) in the Falkensteiner Vorwald region, which is part of the Bavarian Forest, a low-mountain range in Southeast Germany. Within this hilly upland, the study site is situated in a floodplain adjacent to the Otterbach Creek at 480 m above sea level. A detailed description of the study site can be found in Lei et al. (2025). In brief, the area has a mean annual temperature of 8.9 °C with a mean annual precipitation of 875 mm. The parent material is Regensburg crystalline granite bedrock (Regensburger Kristallgranit I) (Bayerisches Landesamt für Umwelt (LfU) 2024). The floodplain is characterized by fluvial sediments, with the soil type being classified as an Eutric Endogleyic Fluvisol according to the World Reference Base for Soil Resources (IUSS Working Group WRB, 2015). In the upper 20 cm, the soil has a pH ($CaCl_2$) of 5.8, and the experimental site has a slight slope gradient of 0.86 %. Historical maps show that the floodplain was a natural grassland without active management since at least 1890, but has been under regular grassland management since 1980 and under extensive, organic management since 1995. It is normally fertilized with liquid cattle slurry at a rate of 18 m^3 ha^{-1} (ca. 50 kg N ha^{-1}) with typically two fertilization events and two cuts per year. The slurry used for the experiment is the same slurry used to fertilize the rest of the meadow, which was provided by the local farmer who manages the site.

A total of 20 plots with a dimension of 1 × 1 m^2 were chosen to investigate five different slurry treatments with four replicates each. Plots were arranged in a 5 × 4 pattern with a buffer zone of 1 m in between and a 20 cm buffer zone on plot edges which remained unsampled. Treatments were randomly assigned to the plots. Preparation of the grassland plots and application of the slurry was performed manually to simulate mechanical application techniques such as traditional slurry broadcast spreading under dry weather (B), application like (B) followed by a simulated heavy rainfall event of 30 mm per m^2 one hour after fertilization to increase slurry infiltration into the soil (BR), broadcast spreading of diluted slurry (DS), deep open slot injection ($I_{5\ cm}$, 5 cm deep, 15 cm distance between slits), and shallow open slot injection of slurry into the soil ($I_{2\ cm}$, 2 cm deep, 15 cm distance between slits). In the DS treatment, a dilution of 4:1 water to slurry ratio was conducted in order to obtain a dry matter content < 2 %, which is the legal threshold for broadcast spreading of slurry in Germany. In all treatments except for DS, an application rate of 18 m^3 ha^{-1} was used. In the DS treatment, the application rate was 8 m^3 slurry ha^{-1} plus diluting water so that the applied N equaled 44 % of the N applied in the other treatments. The chosen methods are common farming practices in the region. The labeled slurry was applied on June 22, 2022, with soil sampling and biomass harvest taking place 3 months later at the end of September 2022.

2.2. Preparation of ^{15}N-labeled slurry and rain solution

The plots were labeled by applying ^{15}N-enriched liquid cattle slurry according to Zistl-Schlingmann et al. (2020a). To achieve the desired ^{15}N enrichment of ca. 5 atom % in the slurry the stable isotope ^{15}N was added in the forms of urea ($CH_4^{15}N_2O$, 98 atom % ^{15}N, Sigma-Aldrich®, St. Louis, USA) and ammonium sulfate ($(^{15}NH_4)_2SO_4$, 98 atom % ^{15}N, Sigma-Aldrich®, St. Louis, USA) in equal N amounts to mimic fresh cattle slurry (Dittert et al., 1998; Sørensen et al., 2003; Zistl-Schlingmann et al., 2020b). Immediately before fertilization, the ^{15}N tracer was added to the slurry in a barrel and mechanically stirred with a paint mixer attached to a cordless screwdriver to ensure a homogenous dispersion within the slurry. Each plot was fertilized with 1.8 L of liquid manure, resulting in an addition of 263.72 or 116.88 mg ^{15}N m^{-2} respectively in the case of the diluted slurry treatment. The cattle slurry was analyzed for N compounds by a commercial laboratory (Raiffeisen-Laborservice, Ormont, Germany) containing 2.61 kg m^{-3} total N on average, consisting of 1.19 kg m^{-3} (45.6 %) NH_4^+-N and 1.42 kg m^{-3} (54.4 %) organic-N including urea. The dry matter content of the slurry was 7.11 %. The ^{15}N addition marginally increased the N content of the slurry to 2.73 kg m^{-3} with a ^{15}N excess enrichment of 5.21 atom %. In total, 49.14 kg N ha^{-1} was applied with this amendment or 21.78 kg N ha^{-1} in the case of diluted slurry.

For treatment BR, a standard rain solution was prepared for each plot by dissolving calcium chloride dihydrate (11 mg L^{-1} $CaCl_2$ * 2 H_2O, Honeywell Fluka™, Muskegon, MI, USA), potassium chloride (24.4 mg L^{-1} KCl, Merck KGaA, Darmstadt, Germany), and sodium sulfate (18.6 mg L^{-1} Na_2SO_4, Merck KGaA, Darmstadt, Germany) in 30 L deionized water (Breuer et al., 2002). The solution was then spread evenly over the plots and mesocosms from a height of 1.5 m with a watering can.

2.3. Plant and soil sampling and analysis of N pools and ^{15}N enrichment

Aboveground biomass was harvested from the entire 1 m^2 plot 92 days after fertilization by cutting the vegetation at ground level and then dried at 60 °C for 48 h to determine the dry weight. A representative subsample was ground with a ball mill (Mixer Mill MM400, Retsch® GmbH, Haan, Germany) and 2 mg were weighed into 5 × 9 mm tin capsules (IVA Analysentechnik, Meerbusch, Germany) for ^{15}N enrichment and total nitrogen (TN) concentration analyses via elemental analysis (Flash EA, Thermo Scientific™, Waltham, USA) coupled to an isotope ratio mass spectrometer (Delta V™, Thermo Scientific™, Waltham, USA) (EA-IRMS) according to Dannenmann et al. (2018). Five soil cores with a diameter of 5.1 cm were taken from each plot 99 days after fertilization and divided into three depths (0 – 5, 5 – 15, 15 – 25 cm). The respective depths were mixed to form one homogenous composite sample. To determine the gravimetric soil water content, roughly 20 g of fresh soil was dried at 105 °C for 24 h, and ca. 10 g was dried at 60 °C for two weeks for N isotope and TN analysis. Additionally, roots were picked from the corresponding soil samples, washed with tap water, and dried at 60 °C for 48 h to determine the dry weight. Dried soil and root samples were then milled, weighed into tin capsules (10 mg for soil and 4 mg for roots), and prepared for EA-IRMS analysis identically to the shoots.

2.4. Nitrogen leaching

Nitrogen leaching was measured with small soil mesocosms made of stainless-steel cylinders with an inner diameter of 16.5 cm and a height of 25 cm that were installed at the edge of each plot. The cylinders were pushed into the soil by an excavator arm, applying consistent pressure to minimize compaction and disturbance of the soil. A polyester fabric bag containing cation and anion ion-exchange resin (75 g of Amberlite™ IR120 Na^+-Form and 75 g of AmberChrom™ 1 ×8 Cl^--Form, Sigma-Aldrich®, St. Louis, USA) was installed at the bottom of each cylinder at a depth of 25 cm to quantify the amount and N species of leachate. The steel cores received the same fertilization treatment as the adjacent plots. Resin bags were excavated from the ground 99 days after fertilization and adherent soil was rinsed off with distilled water before extracting them for 30 min in a 1 M NaCl solution. The extract was then analyzed for NH_4^+-N and NO_3^--N concentrations and ^{15}N enrichment using the SPINMAS technique at the “Bundesanstalt für Geowissenschaften und Rohstoffe (BGR)” laboratory (Stange et al., 2007). The measurements were carried out in an automated sample preparatory (SPIN unit; InProcess, Bremen, Germany) coupled with a mass spectrometer (GAM 400; InProcess, Bremen, Germany).

2.5. Calculation of fertilizer ^{15}N recovery

The amount of ^{15}N excess $m^{15}N_{pool}$ [mg] was calculated for all investigated pools using the following equation according to Zistl-Schlingmann et al. (2020b).

$$m^{15}N_{pool} = mN_{pool} * \left(\frac{^{15}N_{pool} - 0.3663}{100}\right) \quad (1)$$

mN_{pool} is the amount of ^{14}N and ^{15}N [mg] in the plant or depth-specific soil N pool. $^{15}N_{pool}$ is the enrichment (atom % ^{15}N) of the respective N pool and 0.3663 [%] is used as the ^{15}N natural abundance. Errors induced by possible slight variations of ^{15}N natural abundance were negligible due to the high enrichment obtained from ^{15}N slurry labeling. Dividing $m^{15}N_{pool}$ in the analyzed pools by the ^{15}N addition through slurry fertilization on the respective plot revealed the ^{15}N excess recovery [%]. Following the mass balance approach, including ^{15}N leaching losses, unrecovered ^{15}N was attributed to total gaseous N losses (NH_3, N_2O, N_2). Based on the sampled volume and soil dry weight, the bulk density was calculated for each soil layer (0 – 5 cm, 5 – 15 cm, 15 – 25 cm).

2.6. Nitrogen balance

The N balance contains fertilizer-N, atmospheric N deposition, and Biological Nitrogen Fixation (BNF) as inputs and plant-N harvest exports, and total slurry-N-loss (gaseous + leaching) as outputs. Total slurry-N-loss was calculated based on ^{15}N recovery multiplied by the N input through slurry application. N inputs via atmospheric N deposition were obtained by the "Bayerisches Landesamt für Umwelt" (Bayerisches Landesamt für Umwelt (LfU), 2021). The most recent dataset from the closest measurement station to the experimental site in Eining, Neustadt an der Donau (ca. 50 km distance to the study site) indicates a mean bulk deposition of 8 kg N ha^{-1} a^{-1} from 2013 – 2015. For the duration of the experiment, this results in an estimated bulk deposition of 2 kg N ha^{-1}. BNF was estimated for N-fixing red clover (*Trifolium pratense L.)* that occurs with a mean coverage of 5 % on the experimental site of this study. Assuming that clover coverage is proportional to its contribution to dry matter, BNF rates can be estimated using only the legume dry matter yield (DM_{legume}, kg ha^{-1} a^{-1}) of the pasture via the formula BNF (kg N ha^{-1} a^{-1}) $= 0.026 * DM_{legume} + 7$ as proposed by Carlsson and Huss-Danell (2003). This estimation results in an annual BNF rate of 11.2 kg N ha^{-1} a^{-1} when using the DM average of all treatments. However, this value is reduced by more than half to 4.4 kg N ha^{-1} when considering the duration of the experiment of 92 days and an average duration of grassland growth of 233.6 days (Deutscher Wetterdienst (DWD) 2024).

Two different fertilizer N mass balances were calculated, as the polymeric N compounds in the slurry were not labeled with ^{15}N, but only the NH_4^+ and urea fractions. Firstly, we multiplied the percentage of unrecovered ^{15}N by the total fertilizer N amount. This approach assumes that polymeric N in the slurry behaves similarly to ^{15}N-labeled urea and NH_4^+, thus providing an upper estimate of fertilizer N losses. In a second approach, we assumed that all losses originate from ammoniacal slurry N only, while all polymeric N of the slurry is retained in the soil. Hence, this approach results in a lower boundary estimate of slurry N losses.

2.7. Statistical analysis

Each plot was used as a statistical replicate with a total of four replicates per treatment. The requirements for parametric tests, i.e., normality and homogeneity of the variances, were tested with the Shapiro-Wilk and Levene's test. In the case of normal distribution and homogenous variances, a parametric Two-sample *t*-test was used to identify the differences between the groups. If the variances were not homogeneous, a Welch Two-sample *t*-test was performed. In the event that the data was not normally distributed, a Mann–Whitney *U* test was chosen. We chose those tests due to the high variance within the groups and the low number of replicated plots of N = 4, which limits the statistical power of ANOVA to detect differences across treatments. Statistical analysis and graphical display were done with R version 4.3.2 (R Core Team, 2023). The packages "tidyverse", "ggplot2", "plotrix", "patchwork", "readxl", "plotly", "rstatix", "car", and "psych" were used besides the already implemented functions in R.

3. Results

3.1. Plant biomass yield and N export

Yields three months after fertilization varied between 3.05 (DS) and 3.57 t dry matter (DM) ha^{-1} (BR), with no statistically significant differences between the slurry application treatments (Fig. 1 A). Nitrogen concentrations in the shoots ranged between 1.79 (DS) and 1.94 % (BR) with a significant difference only between BR and DS (Fig. 1 B). With about 55 – 70 kg N ha^{-1}, plant N export via biomass harvest was larger than the total fertilizer N addition of 22 kg N ha^{-1} (DS) and 49 kg N ha^{-1} (all other treatments) (Fig. 1 C). The ^{15}N recovery in shoot biomass revealed that, despite this large plant N export, recent fertilizer contributed only 1.79 (DS) – 3.23 (BR) kg N ha^{-1}, i.e. 3.32 % (DS) – 5.30 % (BR) to plant N export and therefore to plant nutrition.

3.2. ^{15}N recovery in plant and soil pools

Large parts of the applied ^{15}N excess were recovered in the soil (ca. 43 % - 79 %), with the unrecovered ^{15}N – reflecting gaseous N losses – being particularly important in the broadcast treatments (Fig. 2). ^{15}N recovery in aboveground and belowground plant biomass was multiple times lower, while in leachate it was negligible under N mass balance considerations (max. 0.26 %). The fertilizer ^{15}N recovery patterns and the resulting relative gaseous fertilizer N losses strongly differed between slurry application techniques, thereby clustering into two main groups. The first distinct group consists of the two broadcast application methods with and without rainfall, where only 56.8 % (B) and 52.9 % (BR) of the fertilizer ^{15}N were recovered. Correspondingly, they showed the highest gaseous N losses (B: 43 %, BR: 47 %) meaning that simulated rainfall did not reduce slurry N losses. The second distinct group was

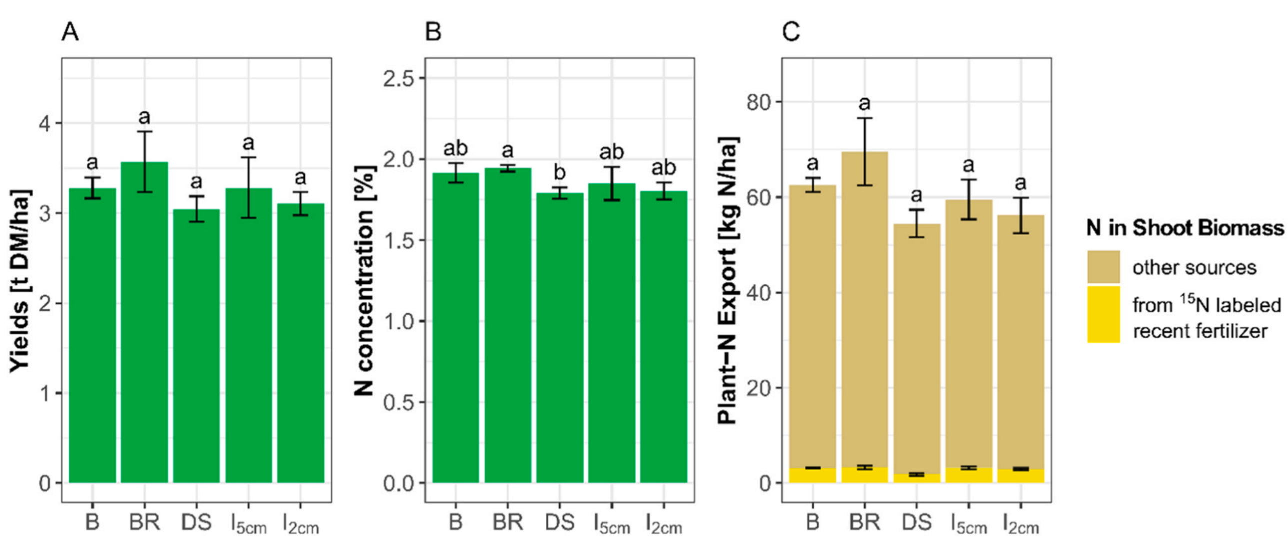

Fig. 1. Biomass yields, N concentration in the shoots, and plant N export via harvest three months after fertilization. B: Broadcast slurry application, BR: Broadcast + rainfall, DS: Diluted slurry combined with reduced N application rate, $I_{5\ cm}$: Deep injection, $I_{2\ cm}$: Shallow injection. Application rates: B, BR, $I_{5\ cm}$, $I_{2\ cm}$: 49.1 kg N ha^{-1}; DS: 21.8 kg N ha^{-1}. Error bars indicate the standard error of the mean (n = 4) and letters indicate significant differences between treatments ($p < 0.05$).

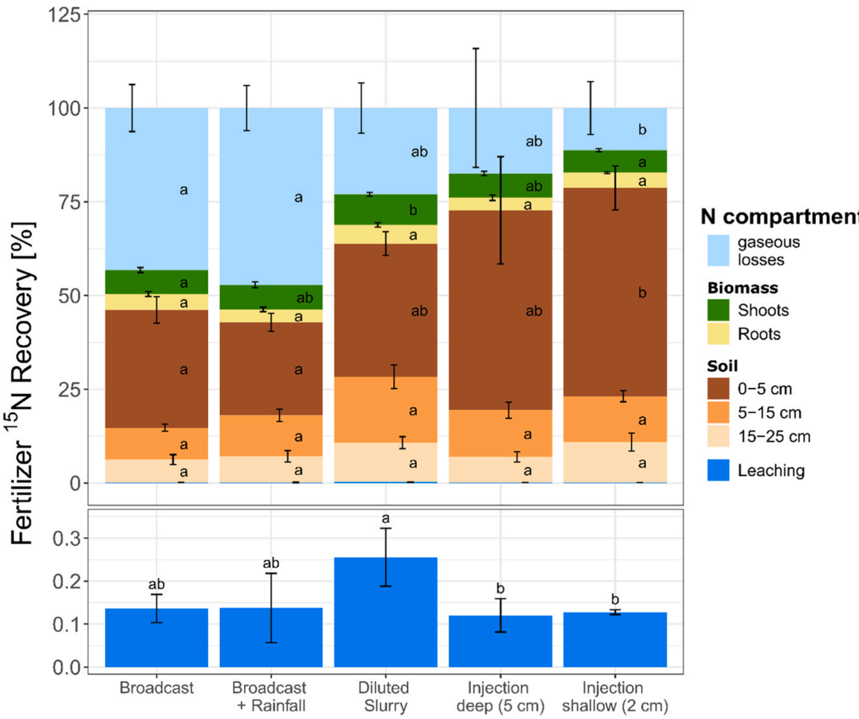

Fig. 2. Recovery of fertilizer ^{15}N excess in shoot and root biomass, soil, and leachate. Unrecovered ^{15}N was assumed to equal total gaseous N losses (NH_3, N_2O, N_2). Error bars indicate the standard error (n = 4) and letters indicate significant differences between the parts of the different treatments ($p < 0.05$).

formed by the broadcast application of diluted slurry and the two slurry injection treatments. Compared to regular broadcast spreading, applying diluted slurry resulted in a substantially higher total recovery of 77 % of the applied ^{15}N in the soil and plant compartments with only 23 % lost as gaseous N emissions. The highest overall recovery was observed after slurry injection ($I_{5\,cm}$: 82.5 %, $I_{2\,cm}$: 88.7 %), resulting in the overall lowest gaseous N losses ($I_{5\,cm}$: 17 %, $I_{2\,cm}$: 11 %). Deep and shallow injections performed comparably in terms of minimizing total N losses, but only $I_{2\,cm}$ displayed significant differences from the broadcast treatments due to the high variability within the topsoil of $I_{5\,cm}$.

Among the measured ^{15}N fates in different N compartments, the largest discrepancies between the application techniques were observed in the soil, especially in the top layer (0 – 5 cm), which was most apparent when comparing broadcast spreading and slurry injection. Yet, the high total recovery in DS predominantly results from the large quantities of ^{15}N found in the deeper soil layers (5 – 15 and 15 – 25 cm), indicating higher vertical ^{15}N translocation in the DS treatment due to improved infiltration. This is also confirmed by the increased leaching of ^{15}N below the main rooting zone, which is significantly higher in DS compared to the two injection treatments (Fig. 2). Recovery rates for the roots were very similar and remained unaffected by the slurry application techniques with mean values ranging from 3.4 ($I_{5\,cm}$) to 5.0 % (DS). For the shoot biomass, the ^{15}N recovery rates were also comparable across all treatments with means from 6.0 ($I_{2\,cm}$) – 8.2 % (DS). However, there was a statistically significant difference between the highest values in DS compared to B and $I_{2\,cm}$. Regardless of the differences between the application methods, ^{15}N recovery in leached mineral N (NO_3-N + NH_4-N) was very low for all treatments (max. 0.26 % of applied fertilizer ^{15}N excess). In contrast to gaseous N losses, this makes leaching of recent fertilizer N an insignificant component of the N mass balance within the first fertilization-harvest cycle of 3 months.

3.3. Nitrogen balance

Two calculation approaches were used to compose the N balances of the investigated fertilization-harvest cycle, while N inputs by biological N fixation remained unconsidered (Table 1). Calculating N losses by multiplying unrecovered ^{15}N excess with total applied slurry N provided an upper limit, whereas calculation based on ammoniacal fertilizer N provided a lower limit estimate of total gaseous slurry N losses. The calculated N balance is negative for all treatments in both scenarios. The deficit is largest for BR and DS, followed by B. Slurry N losses are approximately 3 – 4 times higher in B and BR compared to the other treatments. Despite DS showing relatively low N losses and high soil fertilizer N retention, its N deficit is among the largest with negative N balances of > 29 kg N ha^{-1}. This was because of the high plant N export, which equaled that in other treatments, while the fertilizer N application rate was much lower. The slurry injection treatments showed the least negative N balances and the much lower N deficit goes along with the smallest N losses.

4. Discussion

4.1. Effects of application techniques on plant parameters

Despite the large differences in slurry infiltration capacity and soil disturbance across treatments, the tested slurry application techniques had no significant impact on dry matter yields three months after fertilization. This is partly contradictory to earlier studies, which reported contrasting effects of the application method on plant productivity and plant N uptake when comparing surface application to injection for both single and multiple growing seasons (Webb et al., 2010). Many reports indicate slight increases in yield (Schils and Kok, 2003; Huijsmans et al., 2016) or no significant effect (Seidel et al., 2017). On the contrary, several studies also observed decreasing yields (Misselbrook et al., 1996; Mattila et al., 2003; Schreiber et al., 2023). A possible explanation for these diverging results is the large variability of manure composition, application rate, background soil fertility, and weather, all of which can influence grassland productivity (Webb et al., 2010). Many studies refer to grass sward and root damage caused by slurry injectors as the main reason for the lack of yield benefits (Misselbrook et al., 1996; Mattila et al., 2003; Bittman et al., 2014). This

Table 1
Nitrogen balance (± SE) for total fertilizer N and ammoniacal fertilizer N (NH_4-N + urea). B: Broadcast spreading, BR: Broadcast + rainfall, DS: Diluted slurry, $I_{5\ cm}$: Deep injection, $I_{2\ cm}$: Shallow injection. Slurry N losses include gaseous losses and leaching. Lowercase letters indicate significant differences between treatments for the total fertilizer N balance and capital letters for the ammoniacal N balance ($p < 0.05$).

	Treatment	N Input			N Output		Total N Balance	
		Total slurry N	Atmospheric Deposition	BNF	Plant N Export	Slurry N losses		
Slurry losses calculated via total slurry N [kg N/ha]	B	49.1	2	4.4	62.6	21.2	−28.2 ± 4.1	ab
	BR	49.1	2	4.4	69.5	23.2	−37.1 ± 7.0	a
	DS	21.8	2	4.4	54.5	5.6	−31.9 ± 4.2	a
	$I_{5\ cm}$	49.1	2	4.4	59.5	8.6	−12.5 ± 7.9	ab
	$I_{2\ cm}$	49.1	2	4.4	56.2	5.5	−6.2 ± 7.0	b
Slurry losses calculated via slurry NH_4-N + Urea N [kg N/ha]	B	49.1	2	4.4	62.6	10.4	−17.4 ± 2.7	AB
	BR	49.1	2	4.4	69.5	11.3	−25.3 ± 6.9	AB
	DS	21.8	2	4.4	54.5	2.7	−29.0 ± 4.4	A
	$I_{5\ cm}$	49.1	2	4.4	59.5	4.2	−8.1 ± 4.9	B
	$I_{2\ cm}$	49.1	2	4.4	56.2	2.7	−3.3 ± 5.3	B

implies that despite the higher amount of NH_4-N left after slurry injection due to reduced ammonia volatilization, the grassland productivity does not significantly increase in the short term compared to surface spreading (Rodhe and Etana, 2005; Rodhe and Halling, 2014). On the other hand, it has been shown that the roots can quickly recover from the mechanical disturbance of the injection due to the increased, readily available N supply after directly applying the slurry into the soil (Chen et al., 2001).

In this study, injection, like all tested treatments, neither increased nor decreased biomass yields and N uptake compared to broadcast spreading. Root biomass tended to be slightly but not significantly higher in the broadcast treatments, indicating that possible root damage due to injection had been quickly compensated (Figure S1). Yields only tended to be slightly higher after simulated rainfall (BR), which could result from increased initial water availability. However, this did not apply to the DS treatment, probably due to the lower addition of water, but in particular to the reduced amount of slurry N (44 % compared to the other treatments). A similar pattern emerged in the plant N concentrations with a minor but significant decrease in DS compared to BR, which may be related to reduced N addition. At the same time, the uptake of faster-infiltrating fertilizer N was still relatively efficient compared to the other treatments, as indicated by the highest fertilizer ^{15}N recovery in the shoots in the DS treatment (Fig. 2). The combination of yields and N concentration results in the highest plant N export in BR and the smallest in DS. In contrast to this short-term study, Mattila et al. (2003) found higher N concentrations in shoot biomass after slurry injection but no higher dry matter yield in a 3-year field study. However, all assessments of the effects of changes in grassland management on productivity and N export must account for the particular fate of fertilizer N in grassland, i.e. the fertilization primarily of the soil with a minor contribution of < 5.3 % of recent fertilizer N to plant nutrition. Plant N nutrition hence relies predominantly on the depolymerization and mineralization of SON (Schimel and Bennett, 2004; Zistl-Schlingmann et al., 2020b). Considering this, it becomes very plausible that management changes like a shift from broadcast application to slurry injection will result only in a very slow response of plant productivity and N content. This is because the plants will only substantially benefit from the increased N supply after the fertilizer N has completed the cycle through SON and remineralization, which can take years to a few decades (Han et al., 2025). This also suggests that the agronomic benefits of slurry injection, such as stabilizing or increasing productivity and forage quality, are likely to occur with a significant delay after implementing such management changes.

4.2. Fertilizer N cycling and grassland soil N balances

Our study revealed negative N balances over one fertilization-harvest cycle of 17 – 37 kg N ha^{-1} for broadcast slurry application and application of diluted slurry with reduced N supply. Slurry injection in contrast only resulted in negative N balances of ca. 3 – 13 kg N ha^{-1} for one fertilization-harvest cycle (Table 1). Biological N fixation as an input component can provide substantial amounts of N via the symbiotic relationship of nitrogen-fixing bacteria and legumes. The magnitude of BNF is particularly affected by legume biomass but also by environmental and management factors including the large spatial heterogeneity of chemical and physical soil properties and N addition via fertilization (Carlsson and Huss-Danell, 2003; Zheng et al., 2019). The N deficit exists for all treatments but is very small and not statistically different from zero in the case of $I_{2\ cm}$. In grass swards with higher BNF rates, e.g. due to larger proportions of legumes, the remaining deficit for slurry injection could then potentially be offset. We assumed BNF as constant across the treatments for the N balance calculation. This is however not necessarily realistic as BNF rates might respond to altered fertilizer N input (Burchill et al., 2014; Kristensen et al., 2022). Thus, higher N retention following slurry injection could have suppressed BNF, whereas higher N losses in broadcast applications could have maintained or even increased BNF rates. With a mean clover coverage of 5 % at the study site and the low estimated importance of BNF for the total N input, we don't expect that such potential variability in BNF substantially influenced our N mass balance considerations. Nonetheless, we suggest to consider direct BNF measurements in future studies. Similar to BNF, we also considered atmospheric N deposition to be constant across treatments, which appears less problematic due to lower N input rates, the proximity of the plots, and their randomized arrangement.

The more closed N balances in the slurry injection treatments result from the enhanced stabilization of fertilizer N in SON due to reduced gaseous N losses. In contrast, the largest N loss component of the balance, plant N export, remained unaffected. Similar results were reported for slurry application on calcareous grassland soil, with the largest fertilizer-derived SON formation occurring after slurry injection compared to acidified slurry and broadcast slurry spreading (Schreiber et al., 2023). However, in the latter study, N balances remained negative even after slurry injection. This might be explained by the much higher SOM contents of these soils compared to the soil in this study, which enables high N mineralization rates (Wang et al., 2016) and thus supports higher productivity and N export. While the short duration of this study (92 days) limits predictions about the long-term soil N depletion risks of the different application techniques, the observed predominant fertilization of the soil instead of the plants emerges as an unexpected but important role of slurry. This implies that plants largely rely on SON mineralization to meet their N demand, which was recently detected on calcareous soil with neutral pH values in pre-alpine grasslands of Southern Germany (Zistl-Schlingmann et al., 2020b, 2020a; Schreiber et al., 2023). The strong dependence of plant N nutrition on SON mineralization but not on recent fertilizer explains why many grasslands remain highly productive even after decreased fertilizer N addition, as mineralization might continue at high rates until a critical depletion of SOM stocks. Consequently, the main role of fertilizer N is to refuel SON stocks to an extent that prevents negative N balances and N mining, which can be associated with SOC mining as well (Wang et al., 2021). Such a decline in SOM negatively affects major economic and ecological soil functions like productivity, nutrient and water retention, filter function, pH regulation, and structural stability (Cotrufo and Lavallee, 2022; Hueso-González et al., 2018; Krull et al., 2004). Maintaining SOM levels consequently therefore plays a crucial role in supporting the long-term fertility and the provision of ecosystem services of soils (Napoletano et al., 2025).

4.3. Effects of application techniques on fertilizer N losses

Leachate amounts of recent fertilizer N were generally small during the three months of this study irrespective of application technique, which is consistent with previous studies (Kayser et al., 2015; Maris et al., 2021; Zistl-Schlingmann et al., 2020b). However, the higher infiltration capacity of diluted slurry still promoted NO_3 leaching below the main rooting zone of 25 cm. Gaseous losses of fertilizer N, on the other hand, strongly contributed to the N balances, with 43 % of the recent total fertilizer N lost during broadcast application. This compares well to the median NH_3 emission factor for surface broadcasted manure of 48 % of total ammoniacal N (TAN) given by Hou et al. (2015). Besides the undesired environmental consequences, such high N losses are also economically unviable considering the nutrient value of the lost manure N.

Optimizing the timing of slurry application has been presented as a rather simple solution to reduce N losses since emission pathways like NH_3 volatilization are known to depend largely on meteorological processes (Sommer et al., 2003; Jones et al., 2013). Previous studies simulating precipitation events of 30 mm or more shortly after slurry application observed considerable reductions in NH_3 emissions of up to 70 % (Malgeryd, 1998; Smith et al., 2008; Mkhabela et al., 2009). Conversely, a heavy rainfall event of 30 mm in the present study, applied one hour after fertilization, did not reduce total fertilizer N losses compared to broadcast spreading without subsequent rainfall. The vertical distribution of ^{15}N in the soil profile of this study did not support the idea of increased infiltration into deeper soil layers after rainfall. We therefore suppose that a reduction of NH_3 emissions due to improved infiltration in the top few centimeters of soil was ultimately compensated by enhanced denitrification emissions of mainly N_2 (Zistl-Schlingmann et al., 2019; Dannenmann et al., 2024). Denitrification might have been triggered by anaerobic soil conditions together with organic C supply from slurry (Butterbach-Bahl et al., 2013). Moreover, such heavy rainfall events increase the risk of surface runoff of slurry (Laurenson and Houlbrooke, 2014), which could have laterally transported fertilizer N out of the plot area.

In contrast to the rainfall event, diluted slurry clearly improved infiltration, as indicated by the ^{15}N distribution in the deeper soil layers and leachate, and reduced total gaseous N losses of labeled fertilizer N by 47 %. Mkhabela et al. (2009) reported a decline of total average NH_3 losses by 41 % at a 1:1 water to slurry ratio, with lower dilution rates leading to a lower reduction in emissions. These findings emphasize that diluting manure before field application efficiently reduces N losses and that a greater dilution increases emission savings. A major advantage of diluted slurry is that no new machinery is required since a broadcast spreader can be used for application. However, if the same amount of fertilizer N is to be applied, more trips need to be made to spread the desired volume and N amount, which increases the expenditure of time and costs and enhances soil compaction. Despite the positive effect of DS on slurry N losses, our data clearly show that SON mining might still be the consequence when diluted slurry application coincides with reduced N fertilization. Further, the increased infiltration promoted NO_3 leaching, and the elevated soil moisture could promote N_2O losses via denitrification (Wallenstein et al., 2006). The leaching losses of diluted slurry in particular should therefore be investigated in long-term studies.

Injection achieved the most substantial reduction of total gaseous N losses compared to broadcast application, with a 60 % decrease in the case of deep and a 74 % decrease in shallow injection. It showed the highest increase in fertilizer N retention in the soil, almost closing the N balance. These values agree well with the results of two meta-analyses by Hou et al. (2015) and Emmerling et al. (2020) reporting reductions in NH_3 emissions by 80 % and 61 % respectively after slurry injection. Interestingly, in our study, deeper injection at a depth of 5 cm did not provide any advantages over shallow injection at a depth of 2 cm. This is probably because increasing NH_3 abatement with injection depth (Hansen et al., 2003) is counteracted by increasing denitrification N losses, such as N_2O (Duncan et al., 2017) and N_2. The good performance of shallow slit injection makes slurry injection more attractive for farmers, as a shallower working depth significantly reduces the required tractive force and therefore saves fuel (Huijsmans et al., 1998; Rodhe et al., 2004). Although there are no differences in shoot and root biomass visible between $I_{5\ cm}$ and $I_{2\ cm}$ in this study, less physical disturbance of the soil potentially decreases any damage to the grass sward and roots. While the associated investment in new machinery for slurry injection is relatively high, the cost difference to broadcast spreading decreases with farm size, and the overall expenses should be corrected for the potential savings by the nutrient value of N (Huijsmans et al., 2004; Hadrich et al., 2010; Rotz et al., 2011). Here we show that slurry injection can close N balance gaps of ca. 25 kg N per ha and fertilization event. Grassland N balance gaps are typically closed by use of increasingly expensive mineral fertilizer N, with costs meanwhile reaching up to 1 € kg^{-1} N (European Commission, 2025). Therefore, investments in slurry injection machinery need to be put in perspective with increasing direct N cost savings.

The overall positive effects of injection compared to the different tested application methods reported here could not be observed in an earlier study also using ^{15}N-labeled slurry, but applying it on calcareous soil with neutral pH (Schreiber et al., 2023). The natural slurry pH value of approx. 8 was probably only slightly lowered by those neutral pH values of the calcareous soils. Given the importance of soil and slurry pH in regulating NH_3 volatilization (Sommer et al., 2003; Kim et al., 2021), the more acidic pH in our soils might explain the higher NH_3 emission reduction. Furthermore, neutral pH values of ca. 7 in calcareous soil might promote nitrification and denitrification of injected N, resulting in large gaseous N_2 and N_2O emissions (Butterbach-Bahl et al., 2013; Zistl-Schlingmann et al., 2019). This may explain why lowering the pH via slurry acidification in calcareous soil was more effective in reducing total gaseous N losses than slurry injection (Schreiber et al., 2023). For this reason, injection might be particularly useful for soils derived from silicate bedrock, since the acidic soil pH can effectively reduce slurry pH, thereby lowering both NH_3 and denitrification emissions (Bremner and Shaw, 1958; Wallenstein et al., 2006). However, reduced manure exposure to wind and atmosphere and cooler temperatures in the soil remain the decisive mechanisms to abate NH_3 emissions after injection (Sommer and Hutchings, 2001; Duncan et al., 2017; Siman et al., 2020).

5. Conclusion

This study highlights that plant N nutrition of the investigated grassland is primarily based on SON instead of recent fertilizer N. In the case of broadcast slurry application, this results in negative N balances and N mining caused by the large gaseous N losses. This jeopardizes essential soil functions, such as productivity, nutrient and water retention, filter capacity, pH regulation, and structural stability over the long term. In that regard, the major role of advanced slurry fertilization techniques is not to directly fertilize plants but to reduce N losses while refueling SON stocks to maintain soil fertility in the long term and to avoid SOM mining. Slurry injection – irrespective of injection depth – performed particularly well in this respect, virtually eliminating negative N balances without negatively affecting yields. The substantial reduction of total N losses due to slurry injection allows farmers to save the costs for ca. 25 kg N ha^{-1} additional fertilization per harvest cycle to close the N balance gap. The use of diluted slurry similarly reduced N losses. However, our findings point out that slurry dilution should not be accompanied by reduced N application, as otherwise the risk of N mining persists. For agricultural practice, this means that – while costs for open slot injection equipment can be saved and existing machinery can still be used – more fertilization trips are needed when using slurry dilution with associated fuel costs, a higher risk of soil compaction, and nitrate leaching due to increased infiltration. Based on our N mass balance considerations, we therefore recommend slurry injection in shallow slits when farm size enables its economic feasibility.

CRediT authorship contribution statement

C. Florian Stange: Writing – review & editing, Methodology, Formal analysis, Data curation. **Ingrid Kögel-Knabner:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Jörg Völkel:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Michael Dannenmann:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Sebastian Floßmann:** Writing – original draft, Resources, Investigation, Formal analysis, Data curation, Conceptualization. **Kaiyu Lei:** Investigation, Data curation. **Sigrid van Grinsven:** Writing – review & editing, Project administration, Investigation, Conceptualization. **Ulrike Ostler:** Writing – review & editing, Methodology, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dannenmann, Michael; Völkel, Jörg; Kögel-Knabner, Ingrid report financial support was provided by the Bavarian state ministry for environment and consumer protection. If there are other authors, they declare that they have not 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. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2025.109843.

Data availability

Data will be made available on request.

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