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Increasing grassland productivity and reducing environmental N losses – Multiple benefits of advanced cattle slurry separation

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ABSTRACT

Reducing the high nitrogen (N) losses during fertilization with cattle slurry is key to reduce environmental impacts of grassland farming. We tested the hitherto unknown potential of separated versus regular unseparated slurry (control) to mitigate total N losses in a three-year experiment using ^{15}N-labelled slurry. Slurry separation was enhanced using starch, clay minerals, and centrifugation, yielding an organic-rich solid fraction and a liquid fraction with low dry-matter content and ca 70 % ammonium-N. The use of separated slurry significantly increased plant productivity (+12 %), plant N uptake (+21 %), and total biomass harvest N export (+20 %) compared to the control. Additionally, fertilizer N retention in topsoil organic N (SON) increased by 8 %. Due to higher plant uptake, and higher soil storage of fertilizer N, total gaseous N losses from separated slurry were lower (33.5 % of added N) than from regular slurry (57.6 %). Leaching of fertilizer N remained negligible in both treatments. However, this did not apply for N_2O emissions, which were of low relevance for N balance considerations, but tripled after the addition of the liquid phase of separated slurry in summer. This undesired effect however might be prevented if the solid phase is applied in summer and the liquid phase in spring when soil microbial activity is still low. In summary, separated slurry reduced N losses, increased productivity, fodder quality, and fertilizer N retention, thereby mitigating N deficits and soil N mining. Thus, with appropriate application timing, use of separated slurry can enhance both ecological and economic soil functions and ecosystem services.

1. Introduction

Reducing dry matter (DM) in liquid organic fertilizers such as cattle slurry is a legal alternative for farmers to surface-close application of slurry using open slot injection technologies or horse-shoe techniques. For example, in the German State of Bavaria with its vast grassland regions, farmers are still allowed to use their broadcast-spreading machinery in the year of 2025 when slurry has a DM content below 4.6 %, according to a general decree regulating the authorization of exceptions to §6 Section 3 of the German Fertilizer Ordinance (Bundesministerium für Ernährung und Landwirtschaft, 2017). Typical cattle slurry has a dry matter content of larger than 6 %, with considerable variation (S.I. No. 113/2022). This exception to the fertilizer ordinance was authorized since water diluted slurry with a low DM content infiltrates more rapidly into the soil, thereby reducing ammonia volatilization and total N losses (Bourdin et al., 2014; Floßmann et al., 2025). However, diluted slurry

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involves the need for application of higher volumes to achieve the same N application rate, which increases fuel costs and the risk of soil compaction. In contrast, slurry separation into liquid and solid phase minimizes the DM content and maximizes the content of ammoniacal N content in the liquid phase. In this regard, slurry separation techniques are superior to slurry dilution with water. As a consequence, liquid phase separated slurry (LPS) has received significant attention due to its high proportion of readily plant available nitrogen (NH_4^+-N), favorable mineral N-to-total N ratio (Beauchamp, 1987), and greater availability of decomposable carbon compared to unseparated slurry (Peacock et al., 2001).

The composition of slurry depends on multiple factors, including livestock, fodder types, the presence of bedding materials and dilution by cleaning water, storage duration and conditions, and pre-application treatments (Sørensen, 1998; Reijs et al., 2007). In cattle slurry, although over half of the N is present in inorganic forms readily available to plants, organic forms still constitute a substantial proportion, which cannot be directly utilized by plants until mineralization has taken place. This reduces the immediate effectiveness of slurry as a fertilizer and leads to greater variability in N availability compared to synthetic fertilizers (Schröder, Uenk, and Hilhorst, 2007). Following solid-liquid separation, LPS typically constitutes 90–95 % of the initial slurry mass (Chadwick et al., 2011). Although the readily available N content in LPS (mainly NH_4^+-N) is lower than that in inorganic fertilizers (Polglase, Comerford, and Jokela, 1992; Beauchamp, 1983), the presence of urine-derived urea significantly increases the proportion of ammoniacal available N, often strongly exceeding 50 % of total N. In contrast, solid phase slurry (SPS) contains only about 9 % readily available N (Beauchamp, 1986). Consequently, LPS is generally considered a more plant-effective organic fertilizer at comparable application rates (Beauchamp, 1986; Sutton et al., 1986). The N transformation in, and loss mechanisms from LPS remain poorly understood, contributing to uncertainties in its fate once applied to the soil. These processes are influenced by several interacting factors, including soil properties and weather conditions. Compared to unseparated slurry, LPS contains a higher proportion of immediately available NH_4^+-N, which can significantly enhance grassland productivity (Kruczyńska and Seniczak, 2011). Some studies reported that when applied at recommended N rates equivalent to those of inorganic fertilizers, LPS can achieve comparable productivity (Kaffka and Kanneganti, 1996; Matsi, Lithourgidis, and Gagianas, 2003; Lithourgidis et al., 2007). Additionally, organic N in LPS undergoes gradual mineralization, providing a retarded nutrient supply to plants over time (Burger and Venterea, 2008).

Ssolid phase separated slurry (SPS), with its higher DM, has a more pronounced long-term fertilization effect and supports soil organic carbon (SOC) and N accumulation (Chang, Sommerfeldt, and Entz, 1991; Eghball, 2002). In contrast, LPS, with its lower DM content, exerts a relatively short-term fertilization impact. However, at long-term continuous application at rates equivalent to recommended organic fertilizer levels, also LPS can significantly enhance SOC levels (Culley et al., 1981; Antil et al., 2005; Nikoli and Matsi, 2011). For example, Nikoli and Matsi (2011) observed substantial increases in SOC and dissolved organic carbon in soils after 9 years of LPS application at rates equivalent to recommended organic fertilizer levels (Nikoli and Matsi, 2011). In addition to N and C, LPS can also improve soil phosphorus and potassium availability. These findings underscore the potential agronomic benefits of separating organic N-rich SPS from inorganic N-rich LPS to optimize nutrient availability and crop uptake (Bittman et al., 2011; 2012; Neufeld et al., 2017). When LPS is applied to soils, fertilizer N undergoes various transformation processes, including, microbial assimilation with subsequent necromass stabilization in SON, mineralization, adsorption to clay minerals in exchangeable forms, plant uptake, and leaching (Bechini and Marino, 2009).

In temperate grasslands rich in soil organic matter (SOM), N mineralization promotes high productivity despite high gaseous N losses from organic fertilizers and low plant N use efficiency (NUE) (Wiesmeier et al., 2013, Schlingmann et al., 2020a, Andrade-Linares et al., 2021). Thus, these practices have led to negative N balances and soil N mining particularly under stimulated plant growth under climate change conditions (Schlingmann et al., 2020; Schreiber et al., 2023; Han et al., 2025). To mitigate soil organic N (SON) mining and related losses of soil functions, as well as environmentally harmful gaseous and hydrological N losses, application techniques increasing the NUE of organic fertilizers are crucial. This is even more important, given the recent legal restrictions limiting organic fertilizer application rates to e.g., a maximum of 170 kg N ha^{-1} yr^{-1} in Germany. However, such legal changes have been largely based on effects of application techniques on ammonia losses, while effects on other N gases such as nitrous oxide (N_2O) and dinitrogen (N_2), as well as hydrological losses, and effects on total N balances and associated effects on SON and soil organic carbon (SOC) mining have been rarely considered (Garcia-Franco et al., 2024; Schreiber et al., 2023; Schlingmann et al., 2020). For the application of separated slurry, such a holistic assessment of the effects on all N-related soil functions is still lacking.

Studies conducted in SOC-rich and productive Southern Germany's pre-Alpine grasslands revealed biomass harvesting to be the largest component of the N balance. Additionally, the high soil SOM content provides excellent nutrient filter capacities that strongly limit N leaching to only ca 0.8–6.9 % of total N losses (Fu et al., 2017; Schlingmann et al., 2020a; Schreiber et al., 2023; Han et al., 2025). Compared to arable crops, grasslands are perennial and thus have a longer N uptake period (Di and Cameron 2002a,2002b). Besides plant N uptake, also heterotrophic microbial assimilation, promoted by the continuous input of C by root exudates and root turnover enhances microbial N retention significantly and reduces the risk of N leaching (Dannenmann et al., 2025; Schlingmann et al., 2020a; Premrov et al., 2012; Kunrath et al., 2015). However, N losses from LPS, especially ammonia (NH_3) volatilization, still represent a significant environmental concern. Peak NH_3 emissions occur within hours to days following LPS application, particularly if broadcast methods are applied (Pfluke et al., 2011; Webb et al., 2010). The higher proportion of urine in LPS increases its urea content, resulting in greater NH_3 volatilization after decomposition by urease activity compared to SPS (Whitehead and Raistrick, 1993). Additionally, LPS infiltration into soil layers with depleted oxygen levels may promote denitrification, leading to elevated N_2O and N_2 emissions. As shown by Zistl-Schlingmann et al. (2019) dinitrogen emissions can even dominate total gaseous N losses from grassland, even exceeding ammonia emissions and thus significantly contribute to low fertilizer NUE. To mitigate these adverse environmental impacts, it is essential to balance LPS application rate to the plant N demand, thereby also considering N supply from SOM mineralization. Since LPS is well suitable for online on-site nutrient analyses with techniques such as near infrared spectroscopy (Tangorra et al., 2024), the use of precision application technologies can enhance agronomic performance while minimizing the ecological footprint of N fertilization.

Because of the missing holistic evaluation of the use of separated slurry based on full N balances, here we conducted a comprehensive three-year experiment using ^{15}N fertilizer isotope tracing in soil and plant compartments of grassland lysimeters together with measurements on N leaching and automated N trace gas exchange. Thus the objectives of our study were to assess the impact of the use of separated slurry on (1) plant productivity, plant N concentration, and biomass harvest N exports, (2) fertilizer N fates in the plant-soil-system including total gaseous N losses as well as N_2O emissions, (3) full ecosystem N balances. We hypothesized that the use of separated instead of regular slurry would through enhanced infiltration and availability of ammoniacal N (1) increase productivity, plant N content and total plant harvest N export; (2) reduce total gasous N losses but eventually increase N_2O emissions; and (3) due to this shift from gaseous N losses to plant N exports overall not change total N balances.

2. Materials and methods

2.1. Study sites and experimental setup

This study was conducted at the Fendt site of the TERENO Pre-Alpine Observatory (47.8329° N, 11.0607° E, 595 m) using weighable lysimeters (1 m^2 area, 1.4 m depth) and continuous automated measurements of soil-atmosphere exchange of N trace gases (Fig. 1). The location is characterized by flat terrain and in 2021–2023 had an annual average air temperature of 9.80°C and annual rainfall of 1001 mm (2021–2023). 2022 was a rather dry year, with only 855 mm of precipitation (supplementary Figure 1). For further details on the TERENO Pre-Alpine Observatory see (Kiese et al., 2018). We used six grassland lysimeters which were sampled in 2011 at a distance of 13.4 km in Rottenbuch, Germany (47.7299° N, 10.9690° E, 769 m) and since that managed with 3–5 organic fertilization application and cutting events. The slurry separation experiment started in 2021 with the lysimeters being divided in one control group and one treatment group of three lysimeters each. The control group received unseparated regular cattle slurry as in the years prior to this experiment, while the treatment group received the same slurry but separated into solid (SPS) and liquid phase (LPS). The same slurry was used for all applications, with storage at 4℃ in closed barrels to minimize changes in chemical composition over the course of the experiment. Slurry analyses were conducted immediately after each application to quantify N application rates. In 2021, there were three fertilization and four harvesting events that increased to five fertilization and harvesting events in 2022. In 2023, the third year of the experiment, four fertilization and harvesting events took place (Fig. 1). Fertilizer was manually applied with a measurement beaker from a height of 1 m, simulating broadcast application. In the first year (2021), the unseparated slurry treatment (control) received 314 kg N ha^{-1}, while the slurry separation treatment amounted to 386 kg N ha^{-1} (LPS was 316 kg N ha^{-1} and stable, slow release SPS was 70 kg N ha^{-1}). In the second year (2022), the control fertilization amounted to 176 kg N ha^{-1} and the slurry separation treatment to 186 kg N ha^{-1} (LPS was 106 kg N ha^{-1} and SPS was 80 kg N ha^{-1}); in the third year (2023), control and slurry separation treatment were both fertilized with 176 kg ha^{-1} (Table 1). The high rate of slurry N addition in the first year is comparable to typical slurry N application rates in the region and for the investigated soil before the fertilizer ordinance limitation to 170 kg N ha^{-1} became active in the year 2016.

2.2. Slurry N composition and ^{15}N application

The fresh slurry used for both control and slurry separation treatments in this experiment originated from a farm in lower Bavaria, and either remained untreated or underwent immediate separation using centrifugal separation after adding clay mineral and starch additives to enhance separation. The three slurry products (unseparated, SPS, LPS) were analyzed in a commercial laboratory (Raiffeisen Laborservice, Ormont, Germany) for elemental concentrations and NH_4^+-N concentration. For the separated slurry (fresh matter), the N content of the SPS

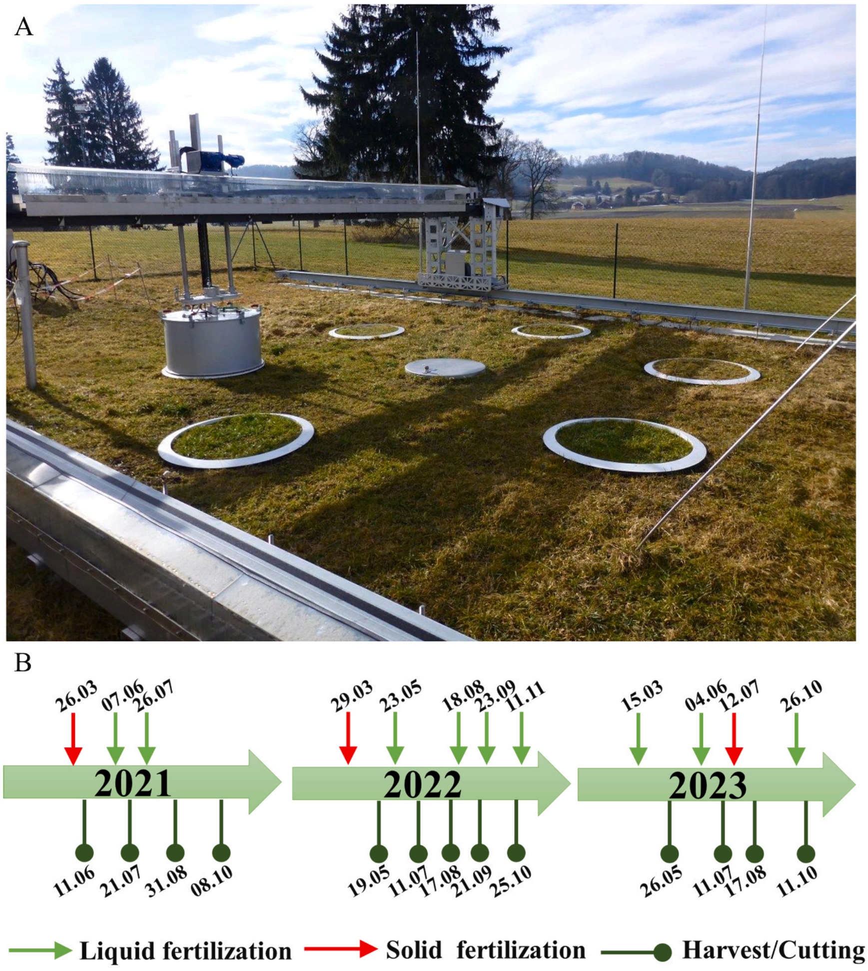

Fig. 1. A. Lysimeters and automated GHG Measuring system Fendt (47.8329° N, 11.0607° E, 595 m) (Photo by Rainer Gasche KIT/IMK-IFU). B. Grassland management in the years 2021–2023, i.e. timing of fertilization and cutting events. The control treatment received unseparated slurry at any event. The separated slurry treatment received SPS in the first application in 2021 and 2022, and in the third application in 2023 while other events were fertilized with LPS.

Table 1
Annual total N application rates for the two treatments.

		Broadcast Fertilization (Control)	Slurry Separation Fertilization
2021	Total Fertilizer Input kg N ha^{-1}	314	386
	Solid slurry Input kg N ha^{-1}	0	70
	Liquid slurry Input kg N ha^{-1}	314	316
2022	Total Fertilizer Input kg N ha^{-1}	176	186
	Solid slurry Input kg N ha^{-1}	0	80
	Liquid slurry Input kg N ha^{-1}	176	106
2023	Total Fertilizer Input kg N ha^{-1}	176	176
	Solid slurry Input kg N ha^{-1}	0	70
	Liquid slurry Input kg N ha^{-1}	176	106

was 8.6 %, with 85 % as organic N (including urea), whereas the N content of the LPS was 4.9 %, with 70 % of it in the form of NH_4^+-N. The unseparated slurry had an N content of 5.6 %, with 60 % as NH_4^+-N (Table 2). ^{15}N labelling was carried out by enriched ammonium (NH_4^+) and urea additions immediately before fertilization. For the unseparated slurry, we followed the approach described by Schlingmann et al. (2020) and added equal N and ^{15}N amounts of 99 % ^{15}N-enriched $(NH_4)_2SO_4$ and urea, thereby labelling the majority of slurry N. Addition of ^{15}N label increased the N content of slurry by 4.2 % on average (Zistl-Schlingmann et al., 2020). To LPS, only ^{15}N-enriched $(NH_4)_2SO_4$ (99 % ^{15}N) was added, thereby increasing the N content of the slurry by 2.2 %. The ^{15}N-labeled tracer was added to polyethylene barrels containing the fertilizer immediately before its application, followed by thorough mixing with electric stirrers. We could not label the SPS with ^{15}N-additions because of the dominating heterogenous polymeric organic N components. Consequently, the first fertilization event in 2021 and 2022 in the separated slurry treatment was not labeled with ^{15}N. In 2023, both control and separated slurry treatments did not receive any ^{15}N label, because we focused on greenhouse gas emissions in this year.

Table 2
Slurry Analysis. Chemical properties of the used slurry variants related to fresh mass. Due to slight variations induced by slurry storage, the content in each barrel differed; the data presented here represent the average of all analyses conducted. LPS-liquid phase slurry; SPS-solid phase slurry.

		Unseparated	LPS	SPS
pH		7.5 ± 0.1	7.7 ± 0.2	4.3 ± 0
C: N ratio		7.0 ± 1.3	3.8 ± 0.7	9.5 ± 0.3
Organic Matter	kg/t fresh matter	69.3 ± 2.4	32.9 ± 3.2	203.9 ± 16.3
Total N		5.6 ± 0.9	4.9 ± 0.5	11.5 ± 1.2
Organic N		2.1 ± 0.2	1.5 ± 0.2	9.8 ± 1.4
NH_4^+ - N		3.5 ± 0.8	3.4 ± 0.6	1.7 ± 0.3
Effective N		3.7 ± 0.7	3.4 ± 0.5	4 ± 0.2
Phosphate (P_2O_5)		1.6 ± 0.2	0.7 ± 0.1	5.9 ± 0.7
Potassium (K_2O)		4 ± 0.2	3.4 ± 0.3	4.9 ± 0.2
Magnesium (MgO)		2.4 ± 0.2	1.9 ± 0.1	6.9 ± 0.2
Calcium (CaO)		2.4 ± 0.4	1.5 ± 0.2	9 ± 0.8
Sodium (Na_2O)		0.5 ± 0	0.5 ± 0	0.6 ± 0
Sulphur (S)		0.6 ± 0.1	0.4 ± 0.1	2 ± 0.7
Cobalt (Co)	g/t	0.3 ± 0.3	1.0 ± 0.8	15.6 ± 0.8
Iron (Fe)		87.5 ± 33.0	306.5 ± 130.4	4842.9 ± 81.9
Copper (Cu)		4.2 ± 0.5	3.1 ± 0.3	7.7 ± 0.7
Manganese (Mn)		18.6 ± 2.6	15 ± 1.8	176.8 ± 21.9
Molybdenum (Mo)		0.8 ± 0.6	0.5 ± 0.4	2.4 ± 0.4
Zink (Zn)		18 ± 2.1	12.3 ± 1.6	56.1 ± 4.9

2.3. *Sampling of aboveground biomass, roots, and soil*

Harvesting of aboveground plant biomass was performed for the entire 1 m^2 lysimeter area as described in detail by Zistl-Schlingmann et al. (2020). Biomass was cut with scissors at a height of 5 cm, placed into paper bags and transported to the IMK-IFU for further processing. The biomass was turned every two days and dried for one week, then transferred to an oven set and dried at 60°C until weight constancy. The dried biomass was weighed, cut into small pieces with scissors, homogenized, and then a representative subsample was ground into a fine, uniform powder for three minutes at a rate of 30 revolutions per second using a ball mill (Retsch, MM301, Germany). Soil was sampled at the end of the first year of the experiment, before the second-year fertilization in March 2022, and in March 2023, at the end of the second year of the experiment. At each sampling, three soil cores (5 cm diameter, 25 cm depth) were taken from each lysimeter and divided into three subsamples (0–5, 5–15, and 15–25 cm). The subsamples of each lysimeter were pooled and sieved through a 2 mm mesh net, dried to weight constancy at 60°C, and ground with a ball mill (Retsch, MM301) as described above. To sample roots within a specified volume, additional soil cores of 100 cm3 volume were taken at depths of 0–5 cm, i.e., the main rooting zone. The roots were manually removed from the soil, washed with distilled water, dried and ground to a fine powder as described above. The N/C content and $^{15}N/^{13}C$ abundance of aboveground biomass and roots were analyzed with an element analyzer (Flash EA, Thermo Scientific, Waltham, Massachusetts, USA) coupled to an isotope ratio mass spectrometer (Delta PlusXP, Thermo Scientific, Waltham, Massachusetts, USA) as detailed by Guo et al. (2013).

2.4. *Soil-atmosphere exchange of N trace gases*

Soil-atmosphere exchange of CO_2, N_2O and CH_4 was measured using the static chamber approach, with a robot system moving on rails from lysimeter to lysimeter and placing an opaque chamber (0.7 m height) on the collar of the lysimeter. The chamber collar was equipped with a rubber sealing to ensure tightness and remained on the lysimeter for 15 min. The chamber is connected to a Quantum Cascade Laser Absorption Spectrometer, QCLAS (Aerodyne Research, Billerica, MA, USA), through a 1/8-inch PTFE tube. The QCLAS instrument was located in an air-conditioned housing next to the lysimeter field and continuously takes in chamber headspace air at a rate of 0.3 l min^{-1}. From the continuous change in GHG gas concentration over time after chamber closure, the flux rate was calculated using the R-package gas fluxes (Fuss and Hueppi, 2024, CRAN repository). Please note that CO_2 fluxes represent ecosystem respiration (Reco) as an opaque chamber was used. The temporal resolution was about four measurements per lysimeter per day as the entire lysimeter field comprises a total of 18 lysimeters but only six of them were used in this study.

2.5. *Nitrogen leaching*

To assess N leaching from the weighed lysimeters, we followed the approach described by Fu et al. (2017) and Yankelzon et al. (2024) for the same lysimeter type and instrumentation. Soil water was extracted via suction lysimeters (models SIC20&VS PRO from METER Group, set at 200 hPa) in 140 cm soil depth and were collected every two weeks and transported for analysis of nitrate (NO_3^-) concentration in the laboratory of KIT IMK-IFU. For a detailed description of the setting of the hydrological lower boundary conditions of the lysimeters with a suction ray and the water balance required for quantitative leaching calculation, we refer to Fu et al. (2017) and Yankelzon et al. (2024).

2.6. ^{15}N fertilizer balance

For the calculation of ^{15}N fertilizer fates and the ^{15}N fertilizer balance, we followed the approach described for the same lysimeter type set up by Yankelzon et al. (2024). Briefly, we calculated excess ^{15}N amount using the following Equation 1 for every analyzed soil, root and aboveground biomass N pool:

Equation 1. ^{15}N Isotopic Excess Nitrogen Formula

$$^{15}N\ excess = \frac{APE}{100} * N_{pool}$$

Where N pool is the amount of N per aboveground biomass, roots, or depth-specific soil in mg; APE (atomic percent excess) is the ^{15}N excess enrichment (atom%15 N measured minus the natural abundance atom% ^{15}N) of the respective N pool. The ^{15}N natural abundance was standardized to 0.3663 %. The recovered ^{15}N excess amount was divided by the cumulative ^{15}N excess addition with the fertilizer applied until the time of sampling to calculate the ^{15}N recovery rate in % at the level of individual lysimeters. ^{15}N recovery in plant biomass was cumulated, considering all earlier plant ^{15}N excess exports. In view of insignificant ^{15}N leaching, we considered unrecovered N to represent total gaseous N losses.

2.7. Total N balances

We calculated total ecosystem N balances for the year 2021, and combined for 2021 plus 2022. Nitrogen inputs included the measured application of fertilizer N, thereby considering each single event (including SPS), biological N fixation (BNF) and N deposition. Estimates of N deposition rates of 33 ± 7 kg N ha^{-1} year^{-1} for both dry and wet deposition were taken from (Zistl-Schlingmann et al., 2020). Biological N fixation (BNF) was estimated using the measured legume contribution to total plant biomass yield, along with established relationships between legume abundance and BNF rates reported in previous studies from similar grassland sites in Switzerland (LfL, 2023; Jacot et al., 2000). In this study, a legume abundance of 5–15 % resulted in a contribution of N fixed by BNF to plant biomass N ranging from 9 % to 16 %, corresponding to 1–26 kg N ha^{-1} . Based on these relationships, the observed 2 % legume proportion in total DM yield translated to an estimated BNF of 15 kg N ha^{-1} year^{-1} (Zistl-Schlingmann et al., 2020). For N outputs, we considered the measured N export with mowing, as well as fertilizer N losses, which were calculated by multiplying unrecovered ^{15}N excess with applied fertilizer N amount of regular slurry or LPS. Total N losses thus are not directly measured but indirectly using a mass balance approach, which has been shown to be particularly feasible for lysimeter studies (Yankelzon et al., 2024). This approach also assumes that there were no significant losses from the unlabeled SPS, which is likely because of its polymeric N which is further stabilized by separation additives and acidic pH as well as high C:N ratio of ca 10.

2.8. Statistics

Each of the three replicated lysimeters per treatment served as a distinct statistical replicate in this study. All data were analyzed using OriginLab 2021 software (OriginLab Corporation, Massachusetts, USA) and Python packages Numpy, Pandas, and Matplotlib (Hunter, 2007; Harris et al., 2020). For single-timepoint datasets (e.g., final soil and root samples), due to the small sample size (N = 3 per treatment), descriptive statistics were used. Data were summarized as mean ± standard deviation, and all individual data points were plotted to visualize variability and potential trends. For time-series datasets (e.g., GHG flux measurements and biomass samples repeatedly collected from the same lysimeters over time), a repeated measures ANOVA was conducted to account for dependencies of measurements conducted at the same lysimeters. This approach ensures that the within-subject variability of repeated measurements is properly considered, rather than treating each measurement as an independent observation.

3. Results

3.1. Fertilizer and soil properties

Unseparated slurry, LPS, and SPS showed significant differences in chemical properties (Table 2). On a fresh mass basis, SPS had the highest organic matter content (203.9 ± 16.3 kg/t), approximately three times that of unseparated slurry (69.3 ± 2.4 kg/t) and six times that of LPS (32.9 ± 3.2 kg/t). TN was also highest in SPS (11.5 ± 1.2 kg/t), nearly double that of unseparated slurry (5.6 ± 0.9 kg/t), with a higher C:N ratio (9.5 ± 0.3 vs. 7 ± 1.3 in unseparated slurry and 3.8 ± 0.7 in LPS). N in LPS (4.9 ± 0.5 kg/t) was predominantly inorganic (~70 %), with NH_4^+-N at 3.4 ± 0.6 kg/t, while SPS nitrogen (9.8 ± 1.4 kg/t) was mainly organic, with lower NH_4^+-N content (1.7 ± 0.3 kg/t). The pH of SPS was notably acidic (4.3 ± 0.0), whereas unseparated slurry (7.5 ± 0.1) and LPS (7.7 ± 0.2) were near neutral. SPS also had significantly higher nutrient concentrations than unseparated slurry or LPS, including phosphate (5.9 ± 0.7 kg/t vs. 1.6 ± 0.2 in unseparated slurry and 0.7 ± 0.1 in LPS), as well as MgO (6.9 ± 0.2 kg/t), CaO (9 ± 0.8 kg/t), and Zn (56.1 ± 4.9 g/kg). Fe content was especially high in SPS (4842.9 ± 81.9 g/kg) compared to unseparated slurry (87.5 ± 33.0 g/kg) and LPS (306.5 ± 130.4 g/kg).

Soil was characterized during sampling in March 2022 and March 2023. The results showed that there were no significant differences in SOC content, inorganic carbon content, TN content, and C:N ratio between the two treatments. The mass distribution of aggregates also exhibited the same pattern supplementary table 2,3). Additionally, at both sampling events, the SOC content, TN content, and C:N ratio in particulate and mineral-associated organic matter did not differ between the two treatments. This shows that there were no significant differences in the general soil properties between the two treatments (supplementary table 2,3).

3.2. Aboveground plant biomass productivity, biomass N export and biomass N content

The annual plant dry biomass productivity of individual lysimeters ranged from 6.8 to 14.0 t ha^{-1}, while the productivity per single harvest event ranged from 0.7 to 4.9 t ha^{-1} (Fig. 2). Compared to the annual productivity in 2021 (13.0 t ha^{-1}), plant dry biomass productivity tended to be lower in both, 2022 and 2023 with values ranging only between 6.7 and 8.2 t ha^{-1}. Over the three years, cumulative plant dry biomass in the slurry separation treatment was 11.5 % higher than in the control, yet a statistically significant difference was observed only in 2021 ($p = 0.049$). For single harvests, significant increases in productivity for the slurry separation treatment were noted at the second harvest event in the years 2021 and 2022 ($p = 0.015$ and $p = 0.012$, respectively). No significant differences were detected in subsequent harvests despite a persistent pattern of numerically higher values in the slurry separation treatment than in the control. The annual cumulative N export in harvested plant biomass ranged from 110.4 to 343.9 kg ha^{-1}, and from 7.0 to 87.3 kg ha^{-1} for individual harvests. Compared to the control, fertilization with separated slurry led to a marginally significant 23.3 % increase in plant N export over the three-year period ($p = 0.098$), while annual biomass N export was significantly increased in 2021 ($p = 0.038$). During the first harvests—immediately following the application of the SPS in the slurry separation treatment—differences in plant dry biomass productivity and N export were low, and the control even slightly exceeded the slurry separation treatment. For all subsequent harvests in the same year – after LPS fertilization -, productivity and N export showed a consistent pattern of increase under the slurry separation treatment, but these differences were not statistically significant.

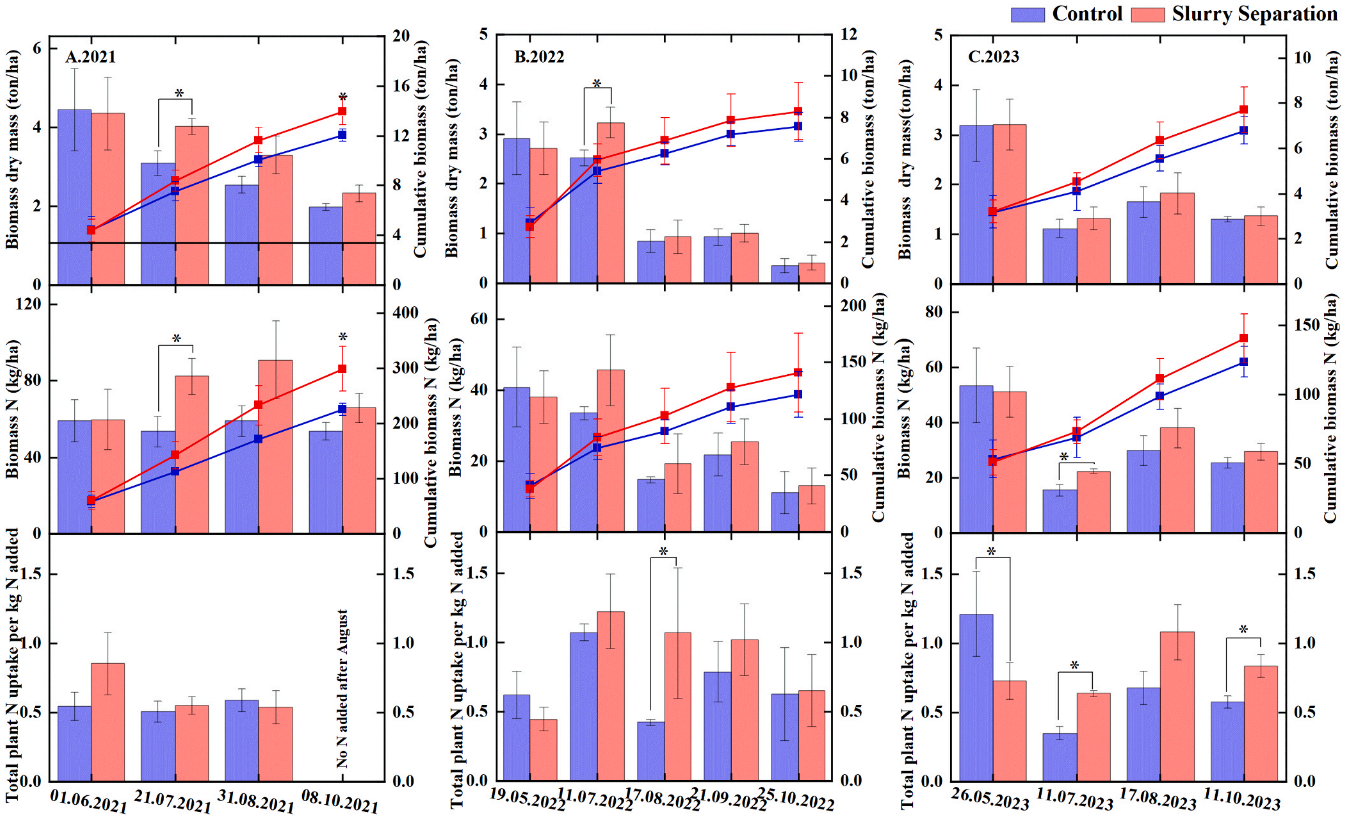

Fig. 2. Productivity and N export via mowing (for single harvests in columns and cumulative as dots), and plant N export with harvest for the years 2021, 2022 and 2023 for application of regular slurry (blue) and separated slurry (red). Note that for separated slurry, the SPS was applied through the first fertilization in 2021 and 2023, but in the third fertilization in 2023. In order to account for the differences in N addition between treatments, we express plant N uptake and harvest export also in relation to added fertilizer N in the third panel.Asterisks represent significant differences between regular slurry and separated slurry treatments ($p < 0.05$).

In order to account for differences in fertilizer N addition across the treatments, we expressed plant harvest N exports also in relation to the amount of added fertilizer N (Fig. 2, lowest panel). This slightly weakened the pattern of increased N export in the slurry separation treatment in 2021, but resulted in a more pattern of increased plant N uptake and export in 2022 and 2023, with the exception of the first harvest in 2023.

Differences in plant N concentrations were small, averaging at 2.0 % in the control and 2.1 % in the slurry separation treatment ($p = 0.38$).

3.3. Fertilizer ^{15}N recovery in aboveground biomass

The cumulative biomass ^{15}N recovery related to the cumulative amount of ^{15}N excess added with fertilizer until the respective sampling event was persistently increased when separated slurry was used compared to regular slurry (Fig. 3). For the first fertilization in 2021, the slurry separation treatment used SPS without ^{15}N labeling, resulting in no ^{15}N recovery at the first-time harvest. The control treatment showed a consistent recovery rate of around 20 % in both 2021 and 2022. In contrast, the slurry separation group demonstrated a recovery rate of about 30 % in both years, being significantly higher than in the control group ($p = 0.039$) (Fig. 3).

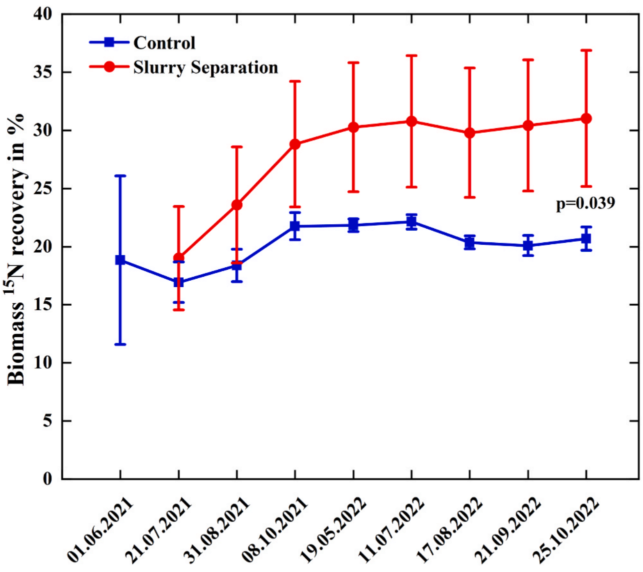

Fig. 3. Fertilizer ^{15}N recovery in aboveground biomass (% of added ^{15}N excess). ^{15}N recovery for the single sampling points is calculated by dividing the cumulative ^{15}N excess amount harvested at the given sample date by the amount of cumulative ^{15}N excess added with fertilizer until the respective sampling. Note that no ^{15}N was added during the addition of SPS, i.e., for the first harvest in 2021 and 2022 each.

3.4. ^{15}N recovery in soil

Soil ^{15}N recovery was analyzed following the final harvests after the first year (2021, sampling March 2022) and second year (2022, sampling in March 2023) of the experiment and also refer to the respective cumulative ^{15}N addition rates at the time of soil sampling (Fig. 4). Generally, ^{15}N recovery declined with depth so that the lowest soil layer had only minor contribution to the total soil recovery. At the end of the first year, total soil ^{15}N recovery in 0–25 cm ranged between 25.6 % and 32.1 %, with no significant difference observed between the control and slurry separation treatment. However, by the second year, the 0–25 cm

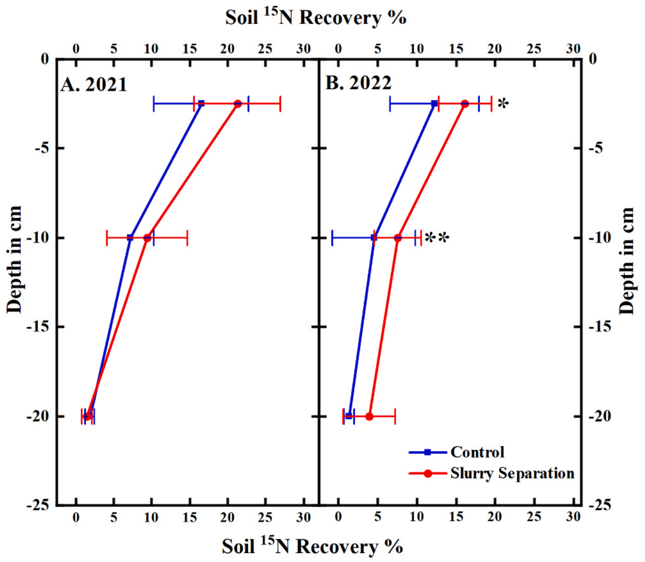

Fig. 4. Soil ^{15}N recovery at different depths (0–5 cm;5–15 cm;15–25 cm) based on the cumulative ^{15}N excess addition at the time of sampling. Fig. A represents soil ^{15}N recovery after the first experimental year, sampled in March 2022; and B represents soil ^{15}N recovery after two years, sampled in March 2023. Asterisks represent significant differences between control and separate slurry treatments at the same depth ($p < 0.05$).

soil ^{15}N recovery ranged between 18.1 % and 27.7 %, with the slurry separation treatment exhibiting higher recovery rates than the control, particularly in the 0–15 cm layers. Both in the 0–5 cm and in the 5–15 cm soil layers, the ^{15}N recovery rate of the applied fertilizer N in the soil was by 4 % higher for the slurry separation treatment compared to soil of the control treatment ($p = 0.043$, and $p = 0.008$). After two years, a trend towards increased ^{15}N recovery at 15–25 cm depth was recorded for the separated slurry treatment (Fig. 4).

3.5. Belowground biomass and belowground biomass ^{15}N recovery

Root biomass ranged from 4.1 to 7.4 t ha^{-1}. In 2021, slurry separation produced higher root biomass than the control, averaging 7.2 ± 2.1 t ha^{-1} compared with 5.6 ± 1.7 t ha^{-1}, while in 2022 biomass was similar between treatments at around 6 t ha^{-1}. Root N content showed no significant difference between treatments in either year, being about 0.8–0.9 % in 2021 and around 1.3 % in 2022. Root N storage increased from 45.9 ± 12.4–75.9 ± 9.2 kg ha^{-1} in the control and from 64.9 ± 17.5–75.1 ± 9.3 kg ha^{-1} in the slurry separation plots, without a significant treatment effect. In contrast, root ^{1} ^{5}N recovery was significantly higher under slurry separation in both years, rising from 3.5 ± 1.1 % compared with 1.6 ± 0.4 % in 2021, and from 5.5 ± 2.5 % compared with 3.4 ± 0.1 % in 2022 (Table 3).

3.6. Soil microbiome and soil-atmosphere exchange by GHG emissions

We did not observe significant differences in the abundance of fungi, bacteria, archaea, or archaeal and bacterial ammonia oxidation functional genes (*AOA-amoA, AOB-amoA*) across the years sampled (2021–2023) (see supplementary results and figures S2-4). Furthermore, the application of separated slurry did also not significantly affect denitrification-related genes and gene ratios (Supplementary figures S5-S8).

The N_2O, and CH_4 fluxes, were continuously analyzed and compared between the control and slurry separation treatments from 2021 to 2023. Peak emissions of N_2O occurred within a few days following fertilization. This applied particularly for 2021, when the total annual fertilizer application exceeded 300 kg N ha^{-1} and soil moisture was high throughout the year, thereby leading to significantly higher cumulative N_2O emissions than in 2022 and 2023 particularly under the slurry separation treatment ($P < 0.05$) (Fig. 5). We observed short-lived net CH_4 emissions mainly after fertilization events either LPS or unseparated slurry, with no significant effect of LPS on CH_4 emission peaks. During other periods there was rather net CH_4 uptake in the soil which overcompensated CH_4 emissions so that soils were a net CH_4 sink at annual scale. The annual net CH_4 sink strength increased over the increasingly dry years. In the wet year 2021, the cumulative flux was −1.2 kg C ha^{-1} in the control treatment and −0.9 kg C ha^{-1} in the slurry separation treatment. By 2023, it had decreased to −1.8 kg ha^{-1} (control) and −1.7 kg ha^{-1} (slurry separation).

3.7. Leaching

Cumulative NO_3^- leaching remained at very low levels across all lysimeters throughout the two-year period (2021–2022), with final values ranging from 2.2 to 5.6 kg N ha^{-1} . These differences were not statistically significant. Seasonal fluctuations were minor, and no clear peaks in leaching were observed following fertilization events. Notably, periods without detectable leachate at 140 cm depth occurred intermittently across treatments, indicating limited drainage and low leaching potential under prevailing climatic conditions. NO_3^- leaching remained negligible across all treatments, and that slurry separation had no significant effect on NO_3^- leaching in this study (Fig. 6).

3.8. Fertilizer N balance

The fertilizer N balances determined for 2021 and the combined 2021&2022 period demonstrate a clear influence of slurry separation on fertilizer N fates and loss (Fig. 7). In 2021, both the control and separated slurry treatments received comparable fertilizer N inputs (314.1 and 316.3 kg N ha^{-1}, respectively, without SPS). However, the separated slurry treatment led to higher proportions of fertilizer N recovered in aboveground biomass (28.8 % vs. 21.7 %) and soil (32.1 % vs. 25.6 %), as well as in belowground biomass (3.5 % vs. 1.6 %) ($p < 0.05$). Most notably, the slurry separation treatment reduced total gaseous N losses to 35.6 % of the fertilizer input compared to 51.1 % in the control treatment, representing a substantial decrease in emissions. By the end of the two-year period (2021–2022), the benefits of slurry separation became more pronounced. While total fertilizer N inputs differed between treatments (490.8 kg N ha^{-1}for control and 422.0 kg N ha^{-1} for the separated slurry treatment, without SPS), the separated slurry treatment consistently resulted in significantly higher N allocations to

Table 3
Root biomass; N storage; N content; and ^{15}N recovery. Sampling was conducted twice. In the table, 2021 represents the sampling results at 03.2022; while 2022 represents the sampling results at 03.2023. Lowercase a represents significant differences ($p < 0.05$) under different fertilization method.

	2021				2022			
	Root Biomass t/ha	Root N Storage kg/ha	Root N Content %	Root^{15}N Recovery %	Root Biomass t/ha	Root N Storage kg/ha	Root N Content %	Root^{15}N Recovery %
Control	5.6 ± 1.7	45.9 ± 12.4a	0.8 ± 0	1.6 ± 0.4a	5.9 ± 1.2	75.9 ± 9.2	1.3 ± 0.1	3.4 ± 0.1a
Slurry Separation	7.2 ± 2.1	64.9 ± 17.5b	0.9 ± 0.1	3.5 ± 1.1b	6.1 ± 1.6	75.1 ± 9.3	1.3 ± 0.4	5.5 ± 2.5b

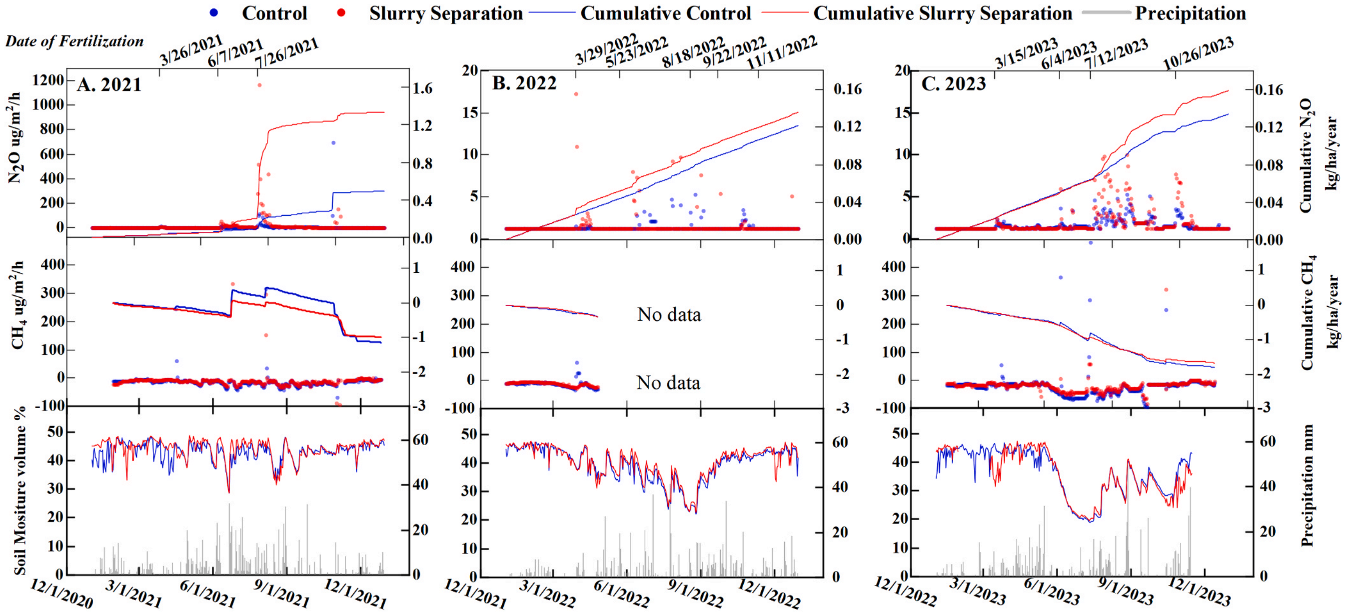

Fig. 5. GHG flux rates, cumulative emissions and soil moisture at 10 cm in 2021, 2022 and 2023 in columns A, B and C, respectively. While in 2021 and 2022, the first fertilization of the separated slurry treatment was the SPS, in 2023 it was the third fertilization.

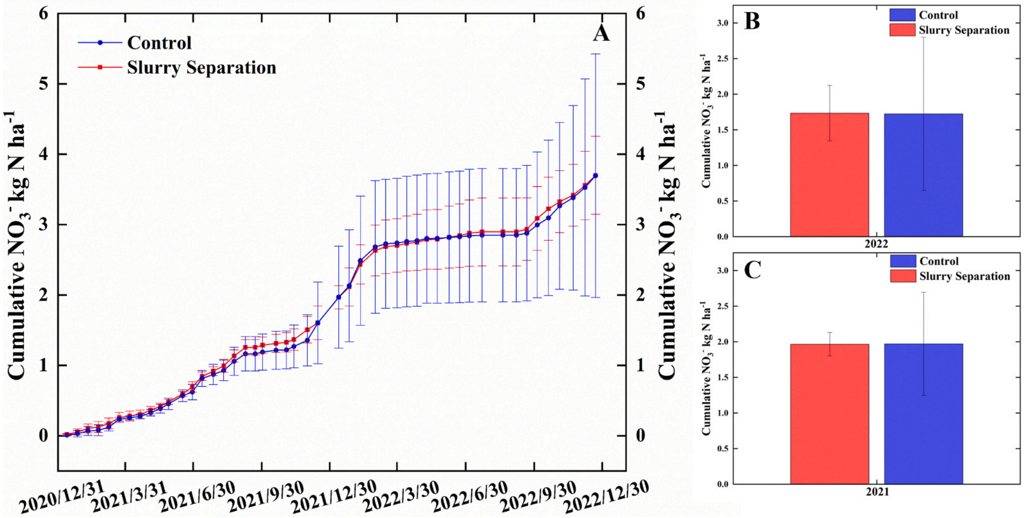

Fig. 6. Cumulative NO_3^- Leaching under Control and Separated Slurry Treatments from 2021 to 2022. A. Cumulative NO_3^- leaching was monitored over two years (2021–2022) across six lysimeters, including three control treatments (control 1–3) and three separated slurry treatments (slurry separation 1–3). B. Annual Cumulative NO_3^- leaching in 2021. B. Annual Cumulative NO_3^- leaching in 2022.

all recovery pools. ABG N recovery rose from 20.7 % in the control to 31.0 % in the slurry separation treatment, soil N recovery nearly doubled from 18.2 % to 27.7 %, and belowground biomass N recovery increased from 3.4 % to 5.5 % ($p < 0.05$). Concomitantly, total gaseous N losses were markedly lower with slurry separation (33.8 %) compared to the control (57.7 %), signifying a pronounced reduction in N emissions (Fig. 7).

3.9. *Total N balance*

In 2021, the fertilizer N inputs in control and slurry separation treatments amounted to 314.1 kg N ha^{-1}. The plant N export for the slurry separation treatment exceeded the control by 73.4 kg N ha^{-1}, and NO_3^- leaching was negligible (2.0 kg N ha^{-1}) (Fig. 6), but gaseous emissions were much smaller. Furthermore, the slurry separation treatment also received an additional 70.0 kg N ha^{-1} from SPS. Therefore, in the first year, the N balance for the control was negative (-27.5 kg N ha^{-1}), while it was positive for the slurry separation treatment (32.6 kg N ha^{-1}). At the end of the second year of the experiment, in March 2023, the calculated N balance showed that the control had a cumulative fertilizer N input of 490.8 kg N ha^{-1}, while the slurry separation treatment added 422.0 kg N ha^{-1} of fertilizer N and an additional 150.0 kg N ha^{-1} from solid organic N. The two-year cumulative plant N output for the slurry separation treatment was 93 kg N ha^{-1}a higher than for the control, and the gaseous N loss was 119 kg N ha^{-1} less. Over the two years, the N balance remained negative for the control treatment, whereas for the slurry separation treatment, the balance was positive at 84.8 kg N ha^{-1} (Fig. 8). Over the two years, in the control treatment, 0.53 kg N ha^{-1} was lost per kg of added fertilizer N, while this value with 0.25 N ha-1 was less than half of that when separated slurry was used (Fig. 8).

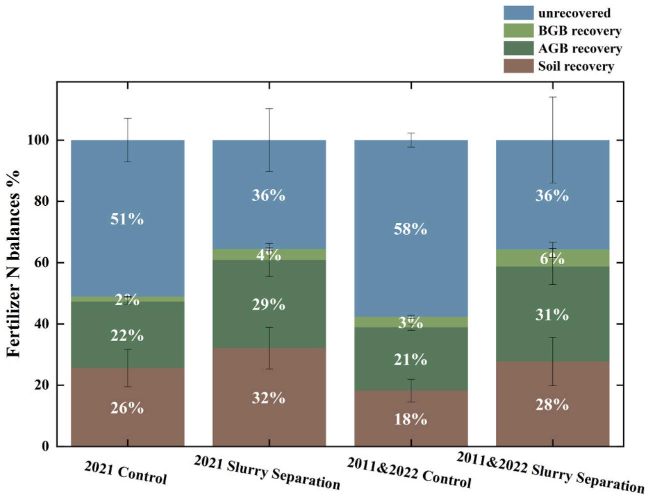

Fig. 7. Fertilizer N balances expressed in % of applied fertilizer for the first year of the experiment (2021) and the first plus second year of the experiment (2021&2022).

4. Discussion

4.1. Effects of slurry separation productivity, plant N uptake and export

During the two-year experiment, fertilization with separated slurry resulted in consistently higher plant biomass and N export by mowing compared to fertilization with regular slurry, although significant differences were not recorded at all sampling dates. This observation can mainly be attributed first to the characteristics of the LPS: over 70 % N was inorganic and dissolved in the liquid phase, allowing plants to absorb nutrients more directly and rapidly through the root system. In contrast, the proportion of inorganic N in the unseparated slurry was only 60 %. Furthermore, LPS had a lower C/N ratio because of its separation from SPS by centrifugation. The C/N ratio of unseparated slurry was twice that of the LPS. Previous research has demonstrated that the C/N ratio is considered to be a good indicator of the N mineralization potential, with a lower C/N ratio implying a higher N mineralization rate, which in turn increases NUE (Webb et al., 2013). Studies of Reijs et al. (2007) and Qian and Schoenau (2002) also pointed out that NUE is inversely related to the C/N ratio. This theory explains why the slurry separation treatment mediated higher yields (Qian et al., 2011; Reijs et al., 2007). Second, due to its higher water content and lower DM content, the LPS has reduced viscosity compared to unseparated slurry and SPS. This lower viscosity facilitates more uniform distribution and enhanced infiltration into soil micropores, covering a larger root-zone area thereby significantly improving nutrient accessibility and plant nutrient uptake efficiency. This was confirmed by significantly higher ^{15}N recovery in soil and roots in the slurry separation treatments. This role of LPS for plant N supply is also supported by the observation that after the first annual application of SPS, significant differences in plant biomass and N export by mowing were not observed between the slurry separation and control treatment. Consequently, after the application of LPS, plant biomass and N export by mowing were significantly higher in the slurry separation treatment than in the control, especially after the first LPS application, where the difference was most pronounced. The LPS contained more inorganic N than unseparated slurry, and this inorganic N could be quickly utilized by plants, while organic N needed to undergo mineralization before being absorbed by plants. This aligns with findings from corn studies, where LPS was shown to increase crop yields by providing readily available macro- and micronutrients (Evans et al., 1977; Sutton et al., 1986). Additionally, the DM yield in 2022 was lower than in 2021, primarily due to a decrease in precipitation of approximately 20 % compared to previous years. The insufficient precipitation limited plant growth and N absorption efficiency, which also impacted the overall performance of the production system. Soil moisture data, particularly from April to September, showed that in 2022, soil moisture levels were up to 10 % lower than in other years (Fig. 5). Lower soil moisture is generally associated with reduced mineralization rates (Paul et al., 2003). The high-water content in the LPS provided additional moisture to the soil, thereby improving soil moisture conditions, especially in the spring. At a depth of 10 cm, the soil moisture content of the slurry separation was 1 % higher than that of the control (Fig. 5). This more moist soil environment was favorable for N absorption by plants and also could have helped to enhance microbial activity in the soil, accelerating the decomposition of SOM into plant-available N, which is of particular importance for plant N nutrition in the investigated soils (Schlingmann et al., 2020). As a result, overall DM yield and N export were increased (Christie, 1987; Stevens et al., 1992; Fangueiro et al., 2015).

4.2. Fertilizer N flows and total N balance

With comparably small recovery in plant biomass, larger recovery in the soil, and large gaseous N losses, we also found the typical pattern of organic fertilizer N allocation as reported earlier for the investigated grassland systems (Schlingmann et al., 2020, Zistl-Schlingmann et al., 2020, Schreiber et al., 2023; Dannenmann et al., 2024). Apparently, plants largely rely on mineralized N rather than on recent fertilizer N, which bears the risk of SON mining. In such a system, fertilizer has the main function to fertilize the soil, i.e., to refill SON pools that tend to be depleted by large plant N exports by mowing, thereby avoiding N mining that can be related to SOC mining as well (Han et al., 2025, Garcia-Franco et al., 2024, Wang et al., 2021). In this context, reduced fertilizer N losses are key to close N balance gaps (Bowles et al., 2018; Tamagno et al., 2022).

In the present study, the fertilizer N allocation in the plant-soil system was generally improved by addition of separated slurry in all aspects, i.e. compared to the control it decreased environmental N losses, increased N stabilization in the soil and increased allocation to plant biomass. Fertilizer N partitioning to these pathways is generally influenced by a combination of factors such as fertilizer type and DM content, application timing, precipitation, soil moisture, and application practices (Cardenas et al., 2019). Even under favorable conditions, only up to 14 % of labeled fertilizer N were reported to be recovered in the plant biomass from slurry applied in fall (Dittert, Goerges, and Sattelmacher, 1998). However, NUE varies significantly, particularly in grasslands where organic fertilizers are applied. In previous ^{15}N experiments using conventional broadcast fertilization methods, plants only acquired 14–20 % of the N in the first year, which is similar to the results for the regular slurry control in this experiment (Zistl-Schlingmann et al., 2020; Schreiber et al., 2023; Han et al., 2025). In contrast, studies using acidification or injection methods for fertilization showed higher fertilizer N recovery in plants of around 30 % (Zistl-Schlingmann et al., 2020; Schreiber et al., 2023). In this study, the fertilizer N recovery rate in plant biomass exceeded 30 % when separated slurry was used. This comparison indicates that the use of separated slurry is among the good performing options to promote fertilizer N allocation to plant biomass (Christie, 1987; Stevens et al., 1992; Fangueiro et al., 2015). This improvement to be mainly induced by increased infiltration and mineral N content as discussed above.

Increased infiltration and, thus, distribution in the soil might also be key to explaining the higher fertilizer recovery in soil when separated slurry was used. The related mechanism particularly includes microbial immobilization, with subsequent stabilization of microbial necromass in SON (Zistl-Schlingmann et al., 2020, Ni et al.2021, Han et al., 2025). Furthermore, NH_4^+ in LPS can be strongly adsorbed by negatively charged colloids in the soil, effectively retaining NH_4^+-N in the soil and further reducing the risk of N loss via leaching (Fu et al., 2017). The addition of labile C via LPS further increases microbial N retention via

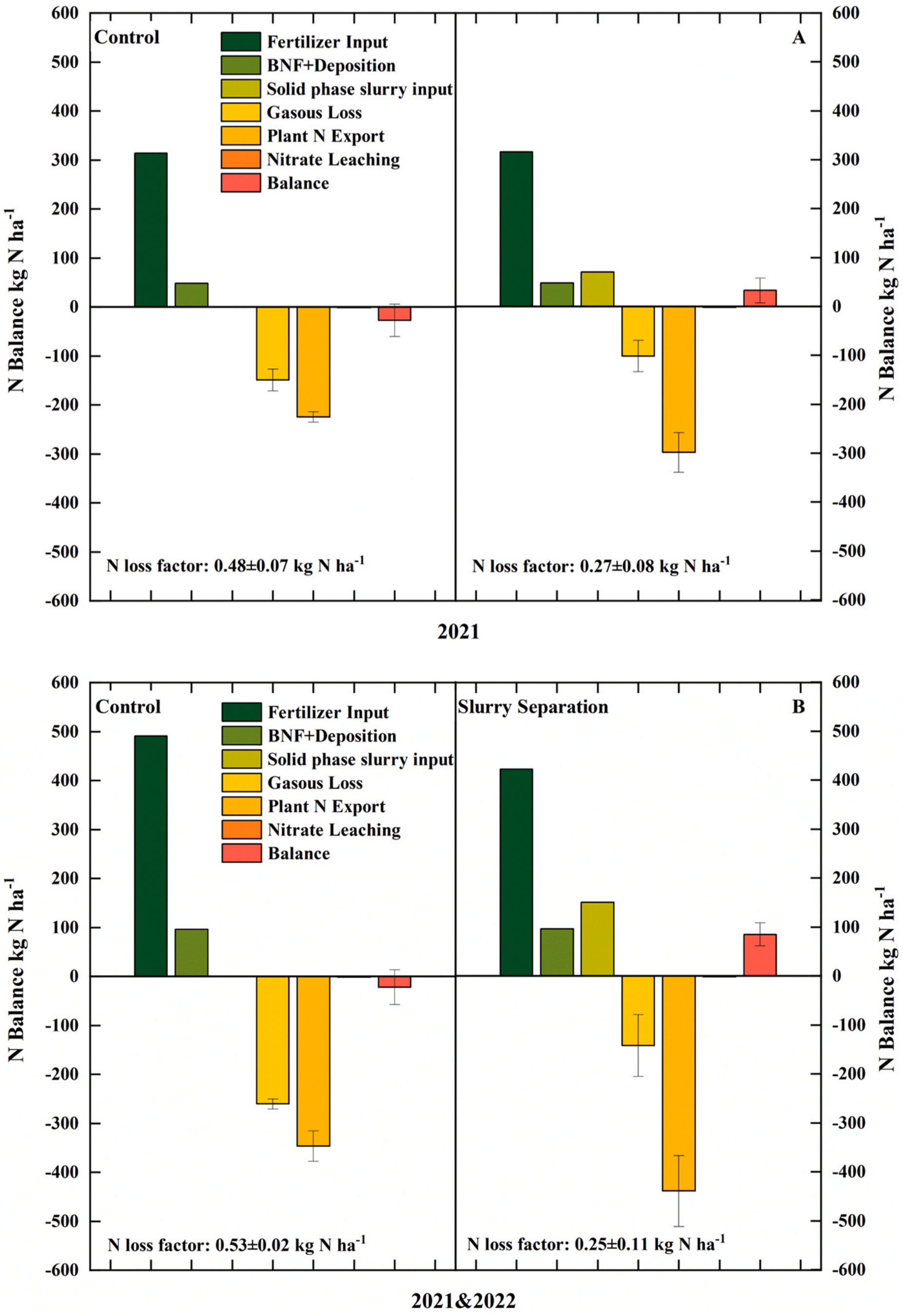

Fig. 8. Total N balances for the control and slurry separation treatments in 2021 (A. 1-year balance) and 2022 (B. 2-year balance). The 2021 N balance was calculated at the end of the first experimental year, while the 2022 N balance was determined after the conclusion of both the first plus second experimental years. The N balance includes inputs from fertilizer, biological N fixation (BNF), and deposition, as well as gaseous losses and plant N export (including AGB and BGB). For these considerations, the SPS N inputs were considered to be stable, i.e., not undergo N loss. N loss factor: Average gaseous and leaching N losses per kg of added fertilizer N.

heterotrophic microbial immobilization (Peacock et al., 2001), thereby also improving soil structure and physical properties (Celik, Ortas, and Kilic, 2004), factors that can collectively promote plant growth and enhance NUE. In our study, the use of separated slurry allowed additional SON formation compared to regular slurry of 34 kg N ha^{-1} in 2 years, which is larger than the 9–12 kg additional SON formation per year that were achieved in one year by slurry injection compared to broadcast application reported by Schreiber et al. (2023) for slurry injection in similar soils (Schreiber et al., 2023). Given the risk for N mining in these soils, this is a highly desirable effect, which supports soil functions including productivity in the long-term. The stabilized solid fraction (150 kg N in 2 years) might further add to considerable SOM formation (Diacono and Montemurro, 2011; Murphy, 2015).

The total N balance was slightly negative in the control treatment (-27.5 kg N ha^{-1} in 2021). This is generally consistent with earlier studies on slurry-fertilized grassland in the region, while earlier studies rather reporting larger N balance gaps, partly exceeding 100 kg N ha^{-1} (Schlingmann et al., 2020, Zistl-Schlingmann et al., 2020, Schreiber et al., 2023, Dannenmann et al., 2024). In contrast to earlier studies evaluating the mitigation potential of e.g. extensification or slurry acidification and injection, this is the first study in the pre-alpine region that showed that use of separated slurry can contribute to closing N balance gaps. Separated slurry achieved this despite enhanced plant N exports via a strong reduction of gaseous N losses (mainly NH_3 and N_2), and the stabilized solid phase N inputs. This is a remarkable result and emphasizes the potential of separated slurry to counteract the N and SOC mining in a warming climate, which was reported to enhance grassland SOM losses in humus-rich soils in the alpine region (Garcia-Franco et al., 2024; Schlingmann et al., 2020b). Still, limitations of this study such as partially deviating N application rates between treatments, estimating gaseous N losses by N mass balance approaches, unlabeled SPS, and the limitation to one soil type limit generalization of the findings. Thus, further studies should evaluate the potential of slurry separation on both soil nitrogen and carbon balances. For this, we recommend full (including organic C and N) labelling of used slurry with ^{15}N and ^{13}C. This could be achieved e.g. based feeding of ^{15}N labelled C4 plant fodder to produce slurry for application to C3 plant-affected soil. Also, direct measurements of dominating gaseous N losses such as NH_3 and N_2 are desirable, but difficult to conduct and also highly uncertain.

4.3. GHG fluxes, NO_3^- losses and microbiome composition

The strongly enhanced N_2O emissions in 2021 during wet-warm summer conditions and after high N additions were the only undesired effect observed due to application of LPS. The fact that the related emissions were observed within a few days illustrates the huge inherent temporal dynamic of N_2O emissions (Butterbach-Bahl et al., 2013), which require automated measurements to be sufficiently captured (Wolf et al., 2010). It also needs to be considered that despite the emission peaks appear very pronounced in 2021 compared to 2022 and 2023 – these emissions still are comparably low. Given N application rates of > 300 kg N, even the strongly enhanced N_2O emissions of the separated slurry treatment in 2021 of 1.3 kg N ha^{-1} yr^{-1} reveal a relatively small N_2O emission factor of 0.3 % for separated slurry and 0.1 % for the control treatment, with negligible background emissions. This is at the lower end of N_2O emission factors for grassland in Germany and elsewhere (Mathivanan et al., 2021; O'neill et al., 2020; Jerray et al., 2024). Negligible background emissions are is clearly illustrated by the low annual N_2O emission in 2022 and 2023, when the amount of N applied was lower and soils were drier. These results generally confirm an earlier study, which also found negligible N_2O emissions in such grassland soils in the study region (Unteregelsbacher et al., 2013). These low emissions can be explained by the N_2O consumption in the terminal step of denitrification in the studied calcareous soils caused by high pH values, which favor N_2O reductase activity (Chen et al., 2015), thereby producing high N_2 emissions while consuming virtually all produced N_2O produced by denitrification (Zistl-Schlingmann et al., 2019). Also, large microbial immobilization and plant uptake of NH_4^+ and NO_3^- might have contributed to low N_2O emissions on top of the importance of high N_2O reductase activity (Dannenmann et al., 2024). Still, the summer fertilization event in 2021 showed that N_2O reductase activity might be still inhibited even in these soils under conditions of extremely high mineral N application with LPS while high gross N mineralization and nitrification already produce large amounts of NO_3^- (Wang et al., 2016; Schlingmann et al., 2020b). This is likely related to large NO_3^- availability which is then increasingly preferred as electron acceptor over N_2O, leading to an increasing share in incomplete denitrification and associated increases in N_2O emissions (Butterbach-Bahl et al., 2013). Again, it should be stressed that this only occurred once in 2021 under conditions of large N application with LPS, high temperatures and high soil moisture. This provides important information on how to avoid such N_2O pulse emissions due to improved management timing. We therefore recommend applying in wet, warm summers the SPS instead of LPS, which might have additional positive effects in spring on plant N uptake instead as outlined above, while N mineralization is still limited by temperatures. Indeed, in 2023 with SPS application in summer, no N_2O pulses were observed but they were likely also prevented by the lower N application rates and particularly the drier soil conditions.

Generally, the molecular data on the nitrifier and denitrifier microbiome, based on gene abundance, did not reflect the high N_2O emissions in the year 2021 under LPS fertilization. Capturing such events might require mRNA-based activity measurements (Chen et al., 2015). The application of separated slurry did not affect the abundance of nitrifiers (AOA and AOB) compared to the regular slurry indicating that the initial steps of soil mineral N turnover, leading to NO_3^- production, remained relatively stable after two years of application. The high ratios between nitrifiers and denitrifies, which may be more pronounced at longer term applications by the separated slurry treatment, suggest a great emphasis on nitrification in this system (Khalil et al., 2004), which is in line with year-round measurements of gross nitrification rates in adjacent sites (Wang et al., 2016). In this context, the low N_2O emissions are particularly remarkable and also confirm the strong role of N_2O reductase in limiting N2O emissions.

Throughout the study period, the grassland ecosystem consistently showed a minor net uptake (negative flux) of CH_4 in both the slurry separation and control treatments. The CH_4 uptake gradually increased over the increasingly dry three-year study period, with values in 2021 of −1.15 and −0.10 kg CH_4 ha^{-1} yr^{-1} for the control and slurry separation treatments, respectively, further increasing to −1.80 and −1.67 kg CH_4 ha^{-1} yr^{-1} by 2023 for the control and slurry separation treatments, respectively. Given that soil-atmosphere exchange of CH_4 is mainly regulated by diffusive properties in the investigated soils, the decreasing soil moisture 2022 and 2023 compared to 2021 facilitates increased CH_4 and O_2 availability to methanotrophic microbes (Unteregelsbacher et al., 2013), thereby explaining the increasing the net CH_4 sink strength. We did not find large effects of separated slurry, only a minor increase in net CH_4 uptake in 2021. This result is somewhat surprising, since the large NH_4^+ addition via LPS should rather inhibit CH_4 oxidation due to the homology of the enzymes methane monooxygenase and ammonia monooxygenase (De Visscher and Cleemput, 2003). Still, CH_4 fluxes are relatively small and of negligible importance for the greenhouse gas balance in this study.

5. Conclusions

This three-year study demonstrated that fertilization with centrifuge-separated slurry with starch and clay additives enhances grassland productivity and NUE compared to conventional unseparated slurry application due to improved infiltration and N supply of the LPS. At the same time, environmental N losses – except for N_2O emissions - were more than halved, and formation of SON was promoted, which can strongly contribute to closing typical grassland N balance gaps, without the use of additional mineral fertilizers. To optimize timing and to avoid increased N_2O emissions, we suggest applying the SPS instead of LPS in warm, wet periods of the growing season. In contrast, LPS should be used for first fertilizer application of the season. Given the beneficial effects for both agronomic and environmental soil functions, centrifuge-based slurry separation is a holistic management scenario, also considering the stability of the SPS with low storage N losses.

CRediT authorship contribution statement

Heinz Rennenberg: Writing – review & editing, Supervision, Conceptualization. **Jincheng Han:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Benjamin Wolf:** Writing – review & editing, Supervision, Methodology, Formal analysis, Data curation. **Michael Dannenmann:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Rainer Gasche:** Writing – review & editing, Data curation, Conceptualization. **Noelia Garcia-Franco:** Writing – review & editing, Formal analysis, Data curation.

Steffen Schweizer: Writing – review & editing, Investigation, Formal analysis, Data curation. **Ralf Kiese:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Martin Wiesmeier:** Writing – review & editing, Supervision, Funding acquisition, Formal analysis, Data curation. **Diana Andrade:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Ulrike Ostler:** Writing – review & editing, Formal analysis, Data curation. **Marcus Schlingmann:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Michael Schloter:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dannenmann, Michael reports equipment, drugs, or supplies (specifically: the organic fertilizers used in the experiment) was provided by HKF Clean Tech AG. If there are other authors, they 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. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2026.110275.

Data availability

Data will be made available on request.

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