



Impacts of slurry acidification and injection on fertilizer nitrogen fates in grassland

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Abstract Low nitrogen (N) use efficiency of broadcast slurry application leads to nutrient losses, air and water pollution, greenhouse gas emissions and—in particular in a warming climate—to soil N mining. Here we test the alternative slurry acidification and injection techniques for their mitigation potential compared to broadcast spreading in montane grassland. We determined (1) the fate of ^{15}N labelled slurry in the plant-soil-microbe system and soil-atmosphere exchange of greenhouse gases over one fertilization/harvest cycle and (2) assessed the longer-term contribution of fertilizer ^{15}N to soil organic N formation by the end of the growing season. The isotope tracing approach was combined with a space for time

climate change experiment. Simulated climate change increased productivity, ecosystem respiration, and net methane uptake irrespective of management, but the generally low N_2O fluxes remained unchanged. Compared to the broadcast spreading, slurry acidification showed lowest N losses, thus increased productivity and fertilizer N use efficiency (38% ^{15}N recovery in plant aboveground plant biomass). In contrast, slurry injection showed highest total fertilizer N losses, but increased fertilization-induced soil organic N formation by 9–12 kg N ha $^{-1}$ season $^{-1}$. Slurry management effects on N_2O and CH_4 fluxes remained negligible. In sum, our study shows that the tested alternative slurry application techniques can increase N use efficiency and/or promote soil organic N formation from applied fertilizer to a remarkable extent. However, this is still not sufficient to prevent soil N mining mostly resulting from large plant N exports that even exceed total fertilizer N inputs.

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Introduction

Liquid cattle slurry has become an important fertilizer in grasslands across Europe, thereby often replacing former farmyard manure (Capriel 2013). Broadcast application has been the common application

technique for slurry, but involves a range of environmental issues resulting from the low N use efficiency and high N losses induced by this fertilization technique (Thompson et al. 1990; Misselbrook et al. 1996, 2002; Uusi-Kämpä & Mattila 2010; Lalor et al. 2011; Zistl-Schlingmann et al. 2019, 2020). These issues include high gaseous losses in form of ammonia (NH_3) and greenhouse gases (CH_4 , N_2O) with their undesired impacts on air quality, human health and global warming. Also nitrate leaching and associated groundwater pollution is a serious issue that has been linked to liquid cattle slurry management (WWAP 2013, 2015; Ryden et al. 1984; Cuttle and Scholefield 1995; Ryan & Fanning 1996; Haas et al. 2001; Vellinga et al. 2001; Maris et al. 2021). Recent studies in the C- and N-rich soils of the pre-alpine grassland belt of Southern Germany indicate negative N balances under slurry broadcast application that are a result of high productivity fueled by high N mineralization of soil organic matter (SOM) and consequently sufficient nutrient availability also between fertilization events. Low slurry N use efficiency can further aggravate the imbalance (Zistl-Schlingmann et al. 2020; Wang et al. 2016). In this context, high fertilizer N losses and thus, low fertilizer use efficiencies are not only linked to NH_3 emissions but also to usually not considered high dinitrogen (N_2) emissions (Zistl-Schlingmann et al. 2019). The negative N balances in these grasslands have been reported to range between 32–239 kg N ha⁻¹ yr⁻¹ depending on the management (intensive > extensive) and extent of experimentally simulated warming (Schlingmann et al. 2020). The N balance of such soils is dominated by high plant productivity and associated N exports in the course of mowing, with plant N demand largely being not met by recent fertilizer rates but by N mineralization from organic matter (Schlingmann et al. 2020; Berauer et al. 2019; Wang et al. 2016; Hoekstra et al. 2010; Schröder et al. 2005; Sørensen 2004). In this context, climate warming has been shown to stimulate soil N mineralization and increase plant N export and thus, negative soil N balances. Due to the tightly linked C and N cycles, this might also negatively affect soil organic carbon (SOC) stocks. This has been confirmed in a recent study (Wang et al. 2021a, b), which showed that already under current climate conditions, the investigated pre-alpine grassland site was a source of C (positive net ecosystem C balance of 1.7–1.8 t ha⁻¹ yr⁻¹) that further increased

under climate warming to 2.3–2.9 t ha⁻¹ yr⁻¹. Such losses of soil total N and SOC do not only turn slurry-managed grassland ecosystems from a greenhouse gas sink to a source but on the long-term also suggest losses of soil organic matter related soil functions such as productivity, nutrient retention, filter function, erosion and flood control (Wilson et al. 2012; Gibon 2005).

The key to avoid such negative impacts of slurry management is to reduce slurry N losses and thereby to increase fertilizer N use efficiency of plants and to stimulate soil organic matter formation (Jensen et al. 2000; Sørensen 2004; Hoekstra et al. 2010; Bierer et al. 2017; Zistl-Schlingmann et al. 2019, 2020; Schlingmann et al. 2020; Wang et al. 2021a, b). To reduce the environmental impacts of N fertilization, above all the release of ammonia, the national fertilizer ordinance of Germany implements the EU's 2016 NEC-Directive by 2025. Besides an upper threshold of organic fertilizer application of 170 kg N ha⁻¹ yr⁻¹, farmers will—with only few exceptions—be legally asked to use alternative liquid slurry application techniques which directly apply manure close to the soil surface by bandspreading instead of broadcast spreading, or even injecting slurry into soil. In a review about impacts of manure application methods on emissions of ammonia Webb et al. (2010) showed that NH_3 emissions on grassland could be reduced by trailing hose and trailing shoe application of slurry on average by 35% and 65% compared to broadcast slurry application techniques. Such approaches require expensive and heavy machinery which are difficult to apply in grassland regions where farm and field sizes are small and slopes are common. In contrast, slurry acidification is considered to be a more easily applicable approach to avoid NH_3 emissions by creating conditions that minimize the concentrations of NH_3 relative to NH_4^+ by decreasing slurry pH via adding of acids or other acidifying substances such as whey (Fangueiro et al. 2015a, b). The lowest pH values tested range from 4.0 to 4.5 resulted in ammonia (NH_3) emission less than 1% compared to non-acidified slurry (Stevens et al. 1989; Hartung & Phillips 1994). Fangueiro et al. (2015a, b) reported in a review a reduction of NH_3 emissions from acidified cattle slurry in the range of 15–80%, depending on the final pH of the slurry and the type of the amendment. Though decreased pH can generally increase soil N_2O emissions through

inhibiting the N_2O reductase and thus the reduction of N_2O to N_2 (Butterbach-Bahl et al. 2013), a recent meta-analysis study of Emmerling et al. (2020) showed for slurry acidification a reduction of NH_3 emissions by 69% without pollution swapping towards increased greenhouse gas emissions like nitrous oxide (−21%), methane (−86%) and carbon dioxide (−15%). Nonetheless, some studies indicated a slight stimulation of N_2O emissions due to acidification (Malique et al. 2021). In this context it needs to be considered that depending on the parent material the soil pH buffering capacity might prevent slurry acidification effects on soil denitrifiers in the short-term so that increased N_2O emissions could be rather a long-term consequence.

Compared to acidifying, slurry injection on average shows slightly lower (−61%) reduction of NH_3 but clearly increases N_2O emissions by 196% as reported in a recent meta-analysis (Emmerling et al. 2020). However, single studies on slurry injection reported widely varying results. In a pot experiment with a Cambic Arenosol, Fangueiro et al. (2017) showed insignificant reduction of NH_3 emissions and unchanged N_2O emissions by soil injection compared to surface application of cattle slurry. Seidel et al. (2017) found NH_3 emissions to be reduced by 31–61% in perennial grassland depending on distances between double disc injectors, while significantly higher N_2O losses were only observed in one of the monitored years. A field study in permanent grassland by Maris et al. (2021) reported an increase of N_2O emissions by slurry injection by 32% compared to broadcast application and a decrease of NH_3 volatilization only in autumn under relatively dry soil conditions and showed that differences in weather and soil conditions can lead to variations in total N losses of up to 146%. In this context, increased N_2O emissions are caused by a stimulation of the coupled soil microbial N-mineralization and nitrification/denitrification processes (Fangueiro et al. 2015a, b). Consequently, the injection depths and patterns are important for the reduction of N_2O and NH_3 emissions and might explain the often-opposing findings on rates of pollution swapping from NH_3 to N_2O .

Besides gaseous N losses, slurry injection and acidification slurry may also affect plant N availability and uptake. The response of crops to slurry injection can widely vary from decreasing, no changes, to increasing plant N uptake and yield (Webb et al.

2010). For example, Seidel et al. (2017) found no effects of open slot injection on dry matter yield at perennial grassland sites in southern Denmark (sandy soil) and Northern Germany (marsh, clay soil). In contrast, in the same study acidified slurry (pH 6.0) resulted in higher N uptake among slurry treatments at least for one of the study years at one study site. A review of acidification of animal slurry (Fangueiro et al. 2015a, b) reported also on significant increases in yields of crops like winter wheat, spring barley, maize. Fangueiro (2017) referred to significant higher oat dry yields if fields were fertilized with incorporated acidified (pH 5.5) cattle slurry, compared to surface slurry application. In a pot experiment with loamy topsoil, ryegrass and maize showed an increase of 40% and 20%, respectively, using acidified (pH 6.0) pig slurry (Loide et al. 2020).

While an increasing number of published studies is dealing with trade-offs of slurry treatment and application techniques, thereby considering greenhouse gas fluxes, nitrate leaching (Maris et al. 2021; Cameira et al. 2019; Park et al. 2018; Kayser et al. 2015; Powell et al. 2011) and productivity (Baral et al. 2021; Regueiro et al. 2020; Fangueiro et al. 2017; Huijsmans et al. 2016), very little is known on fertilizer N partitioning in the plant-soil-system and on full N balances of conventional slurry broadcast spreading compared to injection and acidification. This particularly applies for montane grasslands of the alpine region in Europe and still prevents a holistic assessment how these refined slurry application techniques affect the actual plant N uptake and fertilizer N stabilization in the soil, which is key to preserve soil fertility on the long term.

Therefore, the main objectives of the present study are (1) to assess the effects of refined slurry techniques (slurry injection and slurry acidification compared to traditional broadcast spreading) on the short-term fate of ^{15}N labelled fertilizer N in the plant-soil-microbe system after a fertilization/harvest cycle in a montane grassland; (2) to quantify related impacts on soil-atmosphere exchange of the greenhouse gases CH_4 , N_2O and ecosystem respiration; and (3) to assess the longer-term potential of these alternative slurry techniques for SON formation compared to broadcast slurry spreading after several fertilization cycles in the growing season. In the pre-alpine target region, climate change is particularly pronounced with warming twice as fast compared to

global average (Smiatek et al. 2009; Wagner et al. 2013; Pepin et al. 2015). In order to assess the suitability of the investigated management options in a warming climate, we used a mesocosm-based space for time translocation climate change experiment. We hypothesized that both slurry injection and slurry acidification would lead to decreased total N losses and increased plant N use efficiency, but increase N₂O emission. Furthermore, we expected that warming would increase productivity and thus plant N demand so that the alternative management would be particularly effective in the climate change treatment. Furthermore, we hypothesized that slurry injection would stimulate the formation of fertilizer derived, stable soil organic N, thereby counteracting the risk of soil N mining.

Materials and methods

Study sites and experimental design

The investigated grassland sites Graswang (47°57' N, 11°03' E) and Fendt (47.83° N, 11.06° E) are located in the alpine and pre-alpine region in southern Germany (DE-Fen, DE-Gwg; Kiese et al. 2018; for a map see <https://access.onlinelibrary.wiley.com/doi/full/10.2136/vzj2018.03.0060>). Within a mesocosm translocation approach, the elevation gradient from Graswang (860 m a.s.l.) to Fendt (600 m a.s.l.) is used to simulate climate warming of ~2 K (mean annual temperature increases from 6.5 to 8.6 °C) and reduced mean annual precipitation by ~300 mm (from 1398 to 1033 mm) (space for time approach). The soil in Graswang is a Haplic Cambisol derived from alluvial gravel with 9% SOC and 0.8% total N (TN) in the 0–200 mm topsoil and a pH of approximately 7 (Unteregelsbacher et al. 2013). The vegetation is dominated by the perennial herbs *Plantago lanceolata* L., *Trifolium repens* L. and *Prunella vulgaris* L., and the perennial grass *Festuca rubra* L. (Zistl-Schlingmann et al. 2019).

To study climate change and management effects, 60 intact plant-soil mesocosms (diameter 300 mm, height 400 mm) were taken at the high elevation site Graswang in August 2016. Sampling was based on a geostatistical pre-exploration of the site in order to gain representative mesocosms (Unteregelsbacher et al. 2013). While half of the mesocosms were

translocated to the lower elevation site Fendt for simulating climate change (for the Graswang mesocosms), the other half were operated as control at the high elevation Graswang sampling site.

In the experimental period from April 10th to September 23rd, 2019, the average air temperature in Graswang was 13.2 °C with a total precipitation of 882 mm. On the climate change site Fendt, the average air temperature was 1.5 °C higher and precipitation was reduced by 227 mm. Hence, the experimental period was well representative for long-term climatic differences between the sites, thereby representing predicted future regional climate conditions, i.e. warmer and drier summer months (Smiatek et al. 2009; Gobiet et al. 2014; Warscher et al. 2019; Rajczak et al. 2013).

From 2016 to 2018, all grassland mesocosms were intensively managed, i.e. fertilized through broadcast slurry application and mowed four to five times each year, according to local farmer's practice. For the present study conducted in 2019, the 30 mesocosms at each site were split in three different treatments: (1) traditional slurry (TS) surface spreading as in the years before; (2) slurry injection (SI) in soil within slits of 100 mm distance and 50 mm depth; (3) slurry application as in (1) but using acidified slurry (AS). Total fertilization rate of any treatment was 102 kg N ha⁻¹ split in applications on April 10, June 17 and August 19, thereby using ¹⁵N labelled slurry in order to trace the fate of fertilizer N. A scheme of management and mesocosm harvests in the study year 2019 is provided in Fig. S1.

Production and use of ¹⁵N labeled cattle slurry

We produced ¹⁵NH₄⁺ and ¹⁵N-urea labelled cattle slurry as described in detail by Schlingmann et al. (2020). The liquid fresh cattle slurry was supplied by the local farmer at the Fendt field site (organic family farm) and analyzed by a commercial laboratory (Raiffeisen-Laborservice, Ormont, Germany) for N compounds. Due to organic farming, the slurry has rather low N content of an average 1.75 kg N t⁻¹ fresh weight, consisting of 55% NH₄⁺-N and 45% organic N including urea. For labeling the mesocosms with ¹⁵N by different slurry application techniques, 4 l of liquid fresh slurry were needed for the 30 mesocosms at each site and each application date. For the intended ¹⁵N enrichment of 5 atom%, we added

1.33 g of enriched ammonium sulfate and 0.6 g of urea (both 99 atom% ^{15}N) in equal N amounts to the liquid cattle slurry immediately prior to fertilization at the field sites (Schlingmann et al. 2020). Each mesocosm (area of 0.07 m^2) was fertilized with an amount of 130 ml manure equal to $18.5\text{ m}^3\text{ ha}^{-1}$ following farmers practice. This resulted in an addition of $18.7\text{ mg }^{15}\text{N}$ in excess of natural abundance per mesocosm and fertilization event. Addition of ^{15}N label marginally increased the N content of slurry by 7.3%. The plant-soil systems were fertilized with either broadcast application of acidified slurry, slurry that was injected into the soil, or traditional broadcast application of the slurry. To lower the pH from ~ 7.5 to ~ 5.5 , hydrochloric acid (25%) was added to the ^{15}N labeled slurry for the acidified treatment. For the injection treatment, two parallel approximately 50 mm deep slurry slits were made with a custom-made tool that imitates injection machinery, followed by filling of the slits with ^{15}N labelled slurry.

Sampling and sample preparation

On June 3, 2019 (after ~ 7 weeks after first addition of ^{15}N labelled slurry) and September 23, 2019 (after three times addition of ^{15}N labelled slurry) nine mesocosms from each site, three per treatment, were dug out and brought to the laboratories of KIT-IMK-IFU in Garmisch-Partenkirchen for the harvest of soil and plant biomass. In a first step, the mesocosm was opened by cutting the plastic cylinder vertically. Subsequently, the aboveground biomass was removed by cutting the turf and the soil column was separated into three layers with a saw (depths 0–100 mm, 100–200 mm and 200–400 mm) and weighed. Two stainless steel soil sampling cores were taken from each layer and the respective volume ($2 * 10,000\text{ mm}^3$) analyzed for below ground biomass (BGB). The roots were hand-picked, washed with tap water, dried, weighed and BGB for the whole mesocosm layer was obtained by scaling from the sampling volume to the total layer volume. The remaining soil of any layer was homogenized by hand in a bucket for at least 10 min. Representative soil samples were taken (Schlingmann et al. 2020) to analyze gravimetric soil water content and N concentrations as well as ^{15}N enrichment in the following pools: total soil N, ammonium, nitrate, dissolved organic N, microbial biomass N.

Analysis of N concentrations and ^{15}N enrichment in plant and soil N pools

Sample preparation and analyses followed procedures described earlier in detail by Guo et al. (2013) and by Schlingmann et al. (2020) for similar soil. Fig. S2 provides a schematic visualization of the subsequent steps of sample processing and analysis. The above and belowground biomass samples as well as soil samples were dried for 3 days at $55\text{ }^\circ\text{C}$ until constant weight. Samples were ground to a fine powder with a ball mill (Retsch Schwingmühle MM2, Haan, Germany), weighed in tin capsules and stored in a desiccator with silica gel. To calculate TN concentrations and the $^{15}\text{N}/^{14}\text{N}$ isotope ratio (At.% ^{15}N), the samples were analyzed via an elemental analyzer (Flash EA, Thermo Scientific, Waltham, MA, USA) coupled to an isotope ratio mass spectrometer (Delta PlusXP, Thermo Scientific, Waltham, MA, USA) (Guo et al. 2013).

For analysis of extractable N pools, 80 g of fresh soil were extracted immediately after soil homogenization (ratio 1:2) with 0.5 M K_2SO_4 solution to determine NH_4^+ and NO_3^- , MBN and DON concentrations as well as the pool-specific ^{15}N enrichment. For the determination of MBN, 40 g of fresh soil were fumigated with ethanol-free chloroform and extracted the following day. The soil extracts were directly frozen until further processing. In order to determine the soil NH_4^+ and NO_3^- concentrations, subsamples from soil extracts were analyzed by a commercial laboratory (Raiffeisen-Laborservice, Ormont, Germany). The ^{15}N enrichment in NH_4^+ , NO_3^- and DON was analyzed by sequential diffusion on acid filter traps as described by Guo et al. (2013). Microbial biomass N and ^{15}N was determined from the respective differences in total ^{15}N in fumigated and unfumigated extracts (Guo et al. 2013) without applying a correction factor for extraction efficiency. Soil extracts were also analyzed for DOC and TN concentrations (Multi N/C 3100, Analytik Jena, Germany) according to Dannemann et al. (2016). All isotopic analyses of ^{15}N enrichment in dissolved N compounds of soil extracts were conducted as described in Wang et al. (2016).

Calculation of ^{15}N recovery

Excess ^{15}N amount, $m^{15}\text{N}_{\text{pool}}$ [mg], in all investigated pools was calculated using the following equation, according to Schlingmann et al. (2020).

$$m^{15}\text{N}_{\text{pool}} = m\text{N}_{\text{pool}} * \left(\frac{^{15}\text{N}_{\text{pool}} - 0.3663}{100} \right)$$

$m\text{N}_{\text{pool}}$ is the amount of ^{14}N and ^{15}N [mg N] in the plant or depth-specific soil N pool. $^{15}\text{N}_{\text{pool}}$ is the enrichment (atom% ^{15}N) of the respective N pool. 0.3663 [%] is used as the natural abundance of ^{15}N ; 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 $m^{15}\text{N}_{\text{pool}}$ in the analyzed pools by the ^{15}N addition through slurry fertilization (see section Production of labeled slurry) at the sampling time revealed the ^{15}N excess recovery, which was expressed as a percentage.

To calculate soil organic N formation during the season from N applied during the three fertilization events, we multiplied ^{15}N recovery (%) in unextractable soil N, i.e., the difference between SON and the sum of NH_4^+ , NO_3^- , MBN and DON, as measured in September with the cumulative fertilizer N addition of all three application events (kg N ha^{-1}).

CO_2 , N_2O and CH_4 emissions

Soil-atmosphere exchange of CO_2 , N_2O and CH_4 were measured during the first fertilization/harvest cycle by use of manual static chambers and GC analysis (8610 C; SRI Instruments, Torrence, USA) of sampled headspace concentrations ($N=4$) as described in detail by Schlingmann (2020). Measurements started the day following slurry application (April 11th) and ended in June 28th before the second fertilization. A total of 17 measurements (app. twice per week) were averaged and temporarily scaled to the total investigation period.

Basic weather and soil environmental parameters

Air temperature and precipitation was monitored by on site weather stations at both sites (Kiese et al. 2018). Soil temperature and volumetric soil moisture was continuously recorded by probes (Decagon)

in the mesocosms (Schlingmann et al. 2020). Soil pH values (0.01 M CaCl_2) were measured using a combined electrode as described by Dannenmann et al. (2018) for samples of the June harvest. Gravimetric water content of soil was determined at any sampling time based on drying of ca 300 g of soil at 105 °C until constant weight. Bulk density was calculated for each soil layer (0–100 mm, 100–200 mm, 200–400 mm) based on the volume and soil dry mass.

Statistical analysis

In this study, the mesocosms were used as statistical replicates ($N=3$). A (2-way) ANOVA and a rm-ANOVA (to test the cumulative greenhouse gas fluxes) was used to test the effect of climate change by translocation, management and sampling time. The non-parametric Wilcoxon signed-rank test was used to evaluate the effect of the management and climate change treatments. All statistical data analyses were performed with R-3.6.3 (PBC, Boston, MA). For graphical display Microsoft Office, Excel (2019) was used.

Results

Basic soil parameters

Soil bulk density was not influenced by climate and management treatments (data not shown). Total soil N concentration did also not differ across treatments (Table 1), indicating that mesocosms were comparable before treatments were established. Soil pH values varied between 6.8 and 7.4 and did not differ across treatments (data not shown), indicating that acidified slurry is not yet affecting the soil pH on short time-scales due to the soil carbonate buffer system (Malique et al. 2021). Gravimetric water content (Table 1) was significantly reduced in the climate change treatment. Slurry injection increased the soil water content in 0–100 mm depth compared to the other slurry application treatments.

CO_2 , N_2O and CH_4 emissions

Soil-atmosphere greenhouse gas fluxes were highly variable in space and time (Fig. S3). Translocation to lower elevation increased ecosystem respiration

Table 1 Soil total N concentrations and gravimetric soil water content \pm SD for the June harvest in different depths of the mesocosms depending on treatments

Treatment/ Soil depth [mm]	Soil total N concentrations [g N kg ⁻¹ dry soil]		Gravimetric water content [%]					
			Broadcast		Injected		Acidified	
	Control	Injected	Graswang	Fendt	Graswang	Fendt	Graswang	Fendt
0–100	10.58 \pm 3.09	10.55 \pm 1.61	74.8 Aa \pm 9.1	52.3 Ba \pm 6.1	84.5 Ab \pm 7.7	59.0 Bb \pm 4.0	78.5 Aa \pm 5.3	55.0 Ba \pm 3.8
100–200	5.85 \pm 0.90	5.77 \pm 1.01	65.8 Aa \pm 4.6	55.1 B \pm 2.7	69.5 A \pm 7.0	55.1 B \pm 3.0	64.1 A \pm 1.2	57.0 B \pm 2.9
200–400	1.41 \pm 0.38	1.31 \pm 0.32	32.9 \pm 4.0	33.2 \pm 3.7	32.9 \pm 2.0	28.8 \pm 3.0	34.9 \pm 5.2	32.2 \pm 2.8

Soil total N concentrations were neither affected by management nor by climate change treatments. Climate change persistently reduced soil water content in 0–200 mm depth irrespective of management treatment (capitalized indices A, B). Only for the soil depth 0–100 mm, slurry injection increased gravimetric soil water content compared to other slurry application, both in control and climate change treatments (indices a, b)

under all slurry treatments (Table 2). With regard to management, both acidification and injection increased ecosystem respiration compared to the control slurry treatment, irrespective of the climate treatment. Nitrous oxide emissions were low, variable and over the entire monitoring period neither significantly affected by climate change nor by slurry treatments (Table 2). Nonetheless, slurry acidification increased N₂O emissions during the first days after application at the climate change site Fendt, but not at the control site Graswang (Fig. S3). Methane fluxes mostly remained negative indicating soil CH₄ uptake, and climate change increased the net CH₄ sink of the soil. In contrast, slurry treatments were not consistently affecting the soil-atmosphere exchange of CH₄.

Extractable soil N Pools

Microbial biomass N concentrations exceeded soil DON concentrations by roughly one order of magnitude and soil NH₄⁺ and NO₃⁻ concentrations by roughly two orders of magnitude, respectively (Table S1). Soil microbial biomass N concentration was more than twice as large in September compared to June. The climate and management treatments occasionally affected extractable soil N concentrations during the two harvest events, however these effects were inconsistent across treatments and sampling dates.

Plant parameters

Simulated climate change generally increased aboveground biomass in the June harvest irrespective of management (Table 3). Across all management treatments, mesocosms incubated at the climate change site had a 32% larger productivity (i.e., an increase by 3.1 t ha⁻¹) compared to mesocosms at the Graswang site. However, climate change significantly decreased the plant N concentrations so that total mowed N export was not significantly different between the two climate treatments. The Slurry application approach had more variable effects. Compared to slurry injection, slurry acidification resulted in higher AGB and N export. Generally, the plant N export with the highly productive first cut was more than twice as

Table 2 Cumulative greenhouse gas fluxes for the 79 days of monitoring (11.04. – 28.06.2019) for CO₂-C, N₂O-N and CH₄-C ± SE

Graswang	Reco [CO ₂ -C t ha ⁻¹] 11.04.— 28.06.2019	N ₂ O-N [kg ha ⁻¹] 11.04.— 28.06.2019	CH ₄ -C [kg ha ⁻¹] 11.04.—28.06.2019
Control	2.16 ± 0.54 <i>Aa</i>	0.12 ± 0.12	0.07 ± 0.43 <i>Ac</i>
Injected	2.60 ± 0.45 <i>Ab</i>	0.05 ± 0.03	-0.30 ± 0.10 <i>Aa</i>
Acidified	2.39 ± 0.27 <i>Ab</i>	0.04 ± 0.03	-0.31 ± 0.09 <i>Ab</i>
Fendt			
Control	2.70 ± 0.39 <i>Ba</i>	0.04 ± 0.05	-0.37 ± 0.11 <i>Ba</i>
Injected	3.39 ± 0.38 <i>Bb</i>	0.07 ± 0.03	-0.41 ± 0.09 <i>Ba</i>
Acidified	3.11 ± 0.48 <i>Bb</i>	0.08 ± 0.04	-0.30 ± 0.16 <i>Ba</i>
p (climate)	< 0.001	0.757	< 0.001
p (management)	< 0.001	0.711	< 0.001
p (climate* management)	0.676	< 0.001	< 0.001

Significant treatment effects are highlighted as bold p values

Data are based on 17 manual chamber measurements for the climate treatments Graswang and Fendt on the different management treatments “control”, “injected” and “acidified”. Capital letters indicate significant ($p < 0.05$) differences between climate—and lower-case letters for the management treatments. Reco: ecosystem respiration

large as the slurry N input of 42 kg N ha⁻¹. Slurry injection decreased AGB compared to other slurry techniques for control mesocosms but not for climate change mesocosms. Acidified slurry in contrast, did not change productivity for Graswang mesocosms but increased productivity for Fendt mesocosms. Belowground biomass and root to shoot ratio were not significantly affected by management or by climate. Slurry acidification resulted in highest plant N exports both at the control and climate change sites.

Short-term. ¹⁵N fertilizer partitioning in the plant-soil-system (June harvest)

Seven weeks after the first ¹⁵N fertilizer application, about half of applied ¹⁵N excess was recovered in plant biomass (AGB + BGB) (range 42.0 to 47.5%), while about a quarter was recovered in total soil N (range 23.0–28.9%) or remained unrecovered (range 19.8 to 27.4%). Since ¹⁵N tracer recovery in these compartments was not affected by different climate conditions we pooled the data from Graswang and Fendt sites. Following this approach, Fig. 1 shows that the AGB of acidified slurry showed

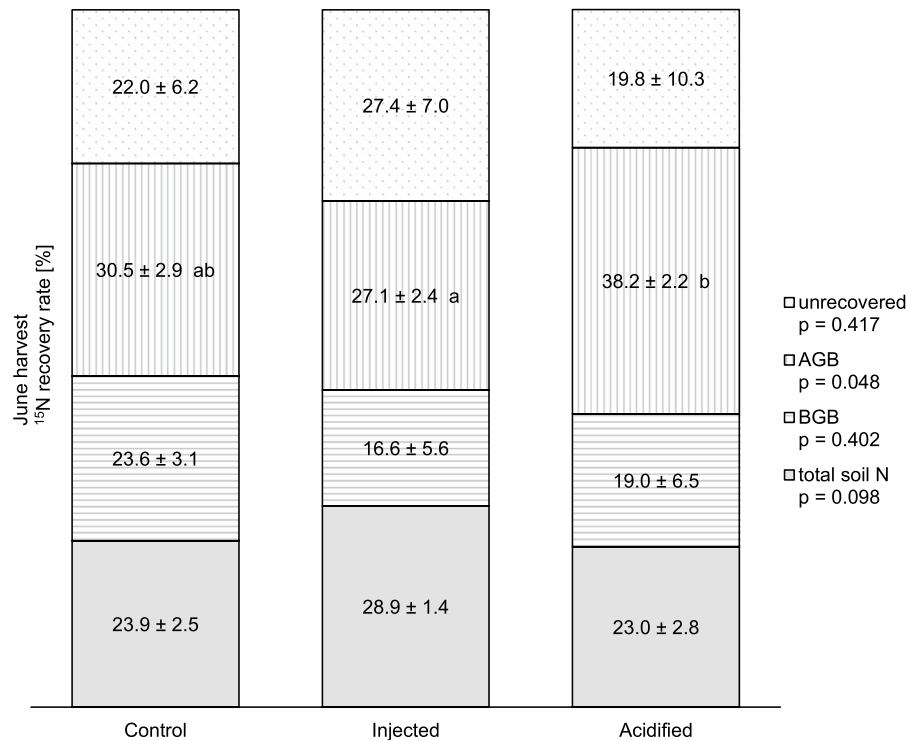
Table 3 Plant parameters measured during the June harvest with standard deviations

Site	Treatment	AGB [t ha ⁻¹]	AGB-N [%]	N-export ± SD [kg N ha ⁻¹]	Below ground biomass [t ha ⁻¹]	Root/shoot ratio
Graswang	Control	7.2 ± 0.5 <i>Aab</i>	1.46 ± 0.02 <i>A</i>	90.9 ± 17.6	13.3 ± 0.6	1.9
	Injected	5.7 ± 0.2 <i>Aa</i>	1.48 ± 0.10 <i>A</i>	83.4 ± 10.5	11.4 ± 3.0	2.0
	Acidified	6.7 ± 0.1 <i>Ab</i>	1.44 ± 0.01 <i>A</i>	95.1 ± 1.9	19.4 ± 3.3	2.9
Fendt	Control	8.5 ± 0.4 <i>Bab</i>	1.08 ± 0.21 <i>B</i>	103.1 ± 11.0	22.4 ± 1.2	2.6
	Injected	9.0 ± 0.9 <i>Ba</i>	1.12 ± 0.02 <i>B</i>	99.5 ± 17.5	19.1 ± 5.5	2.1
	Acidified	11.3 ± 0.9 <i>Bb</i>	1.10 ± 0.10 <i>B</i>	121.9 ± 14.5	16.4 ± 3.3	1.5
p (climate)		< 0.001	< 0.001	0.128	0.193	0.658
P (management)		0.021	0.798	0.117	0.902	0.802
P (climate* management)		0.088	0.960	0.065	0.250	0.193

Statistically significant treatment effects are highlighted as bold p values

Capital letters indicate significant ($p < 0.05$) differences between climate—and lower-case letters for the management treatments

Fig. 1 Recovery rates of ^{15}N from labelled slurry [%] \pm SE of the June harvest for total soil nitrogen, above- and belowground plant biomass (AGB, BGB), and unrecovered addition (environmental losses) for the different management treatments “control” = broadcast application, “injected” and “acidified”. Statistically significant differences are indicated by different indices



a significantly higher ^{15}N recovery compared to the AGB from the slurry injection treatment ($p=0.03$). Vice versa, slurry injection resulted in significant higher ^{15}N recovery in the total soil compared to the control treatment ($p=0.06$). Most of the tracer was recovered in the topsoil (0–100 mm), with a persistent pattern inject > acid > broadcast spreading observed for both sampling dates and irrespective of climate treatment (Fig. 2).

In soil sampled in June, NH_4^+ , NO_3^- and DON together contributed 2.9% and MBN 8.7% to total soil ^{15}N recovery (data not shown), i.e., about 90% of the fertilizer N recovered in soil was present in unextractable SON.

Long-term ^{15}N recovery at the end of the season and SON formation from fertilizer

At the end of the growing season, extractable mineral N and DON together contributed little to ^{15}N recovery from fertilizer in total soil N (range 1.1–3.1%), while microbial biomass contributed 4.6–15.5% and unextractable N harbored the majority of ^{15}N

recovered in soil (83.1–92.2%, Table 4). Most of the soil ^{15}N recovery still was observed in the topsoil and was highest for injected slurry, lower for acidified slurry and lowest for the broadcast slurry control treatment ($p=0.008$) (Fig. 2). Also, for the entire soil columns of 400 mm, highest ^{15}N recovery rates in soil TN were found for the slurry injection treatment, while lowest ^{15}N recovery rates in soil were found in the broadcast slurry (control) treatment (Table 4). These management effects on ^{15}N recovery were not affected by different climate conditions (Table 4).

Using ^{15}N recovery in unextractable soil N to calculate SON formation during the growing season from applied fertilizer revealed rates of 21.8 to 37.2 kg N ha $^{-1}$ (Table 4). These SON formation rates significantly differed across management treatments ($p=0.01$). Specifically, SON formation from injected slurry was on average 9.2 and 12.2 kg N ha $^{-1}$ larger than SON formation from acidified slurry or slurry from broadcast application, respectively. These effects remained only marginally affected by climate with a slight tendency towards lower SON formation from fertilizer under the imposed climate change treatment.

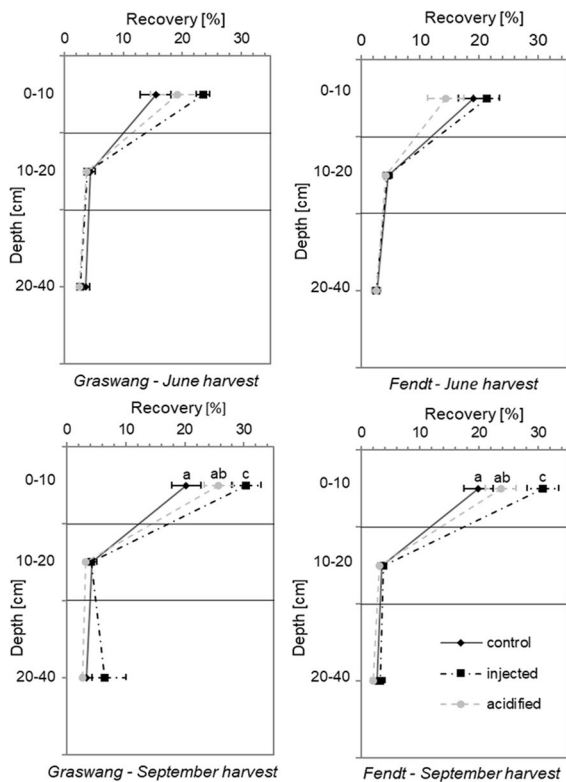


Fig. 2 ^{15}N tracer recovery from labelled slurry in total soil N [%] \pm SE for the June and September harvest events in Graswang (ambient climate) and Fendt (climate change) for the different management treatments “control” = broadcast application, “injected” and “acidified”. Statistically significant differences between management treatments are indicated by different indices

Table 4 ^{15}N Recovery rates [%] \pm SE in soil total N, the contribution of unextractable N to this recovery rate, and soil organic N formation from fertilizer as obtained from the harvest at the end of the growing season after 3 ^{15}N labelled fertilizer additions

Treatment	Graswang			Fendt		
	% recovery in soil TN (0–400 mm)	Thereof in unextractable N	SON-formation (kg N ha ⁻¹)	% recovery in soil TN (0–400 mm)	Thereof in unextractable N	SON-formation (kg N ha ⁻¹)
Control	27.6 \pm 2.6 a	90.1 \pm 2.8 a	25.1 \pm 3.3 a	26.1 \pm 2.3 a	83.1 \pm 2.0 a	21.8 \pm 2.5 a
Injected	40.9 \pm 4.2 b	92.1 \pm 4.1 b	37.2 \pm 5.5 b	37.8 \pm 2.0 b	89.4 \pm 2.2 b	34.1 \pm 2.7 b
Acidified	31.3 \pm 2.1 a	86.0 \pm 2.0 ab	27.1 \pm 2.4 ab	28.9 \pm 2.9 a	89.5 \pm 2.9 ab	25.8 \pm 3.7 ab
p (climate)	0.409	0.125	0.392			
p (management)	0.008	0.050	0.012			
p (climate* management)	0.973	0.016	0.953			

Significant treatment effects are highlighted as bold p values

Data are from mesocosms incubated at Graswang (ambient climate) and Fendt (climate change) and the different slurry management treatments “control = slurry broadcast application”, “injected” and “acidified”. Treatments were not affected by climate change simulation. Statistically significant differences for management treatments are indicated by small letters

Discussion

Short-term fate of fertilizer N in the plant-soil-microbe system and greenhouse gas emissions as affected by different slurry techniques

The generally high plant ^{15}N recovery in this study of ca 30% (AGB) exceeded that of an earlier study using the same soils and sites solely under broadcast fertilization technique (Zistl-Schlingmann et al. 2020) by a factor of 2–3, indicating large interannual variability of fertilizer N use efficiency by plants. Corresponding to our findings of AGB ^{15}N recovery rates of 31% of the broadcast slurry control treatment, Jensen et al. (2000) also found recovery rates of 32% in AGB after two months of slurry application using the broadcast technique on a loamy sand soil. Differences in fertilizer N recovery and losses across years and studies might be largely related to different weather conditions. Likely precipitation, wind speed and air temperatures at given dates of slurry application and thereafter, affects fertilizer incorporation into soil and N losses through NH_3 volatilization, denitrification and N leaching (Schröder et al. 2005; Hoekstra et al. 2011; Lalor et al. 2011; Fu et al. 2017; Wang et al. 2016; Misselbrook et al. 1996; Häni et al. 2016; Seidel et al. 2017). Compared to the study of Zistl-Schlingmann et al. (2020) weather conditions for our first fertilizing event were quite different, with lower maximum wind speed (max. 1.3 vs. 2.7 ms⁻¹) and temperatures (max. 11 °C vs. 19 °C) and higher

amount of precipitation (2.7–5.4 mm vs. 0–1.7 mm), which are in favor for higher fertilizer N recovery.

Particularly high fertilizer N use efficiency by plants, productivity and N uptake/export were observed after fertilization with acidified slurry. Clearly this appears to be related to reduced NH_3 and other gaseous losses like N_2 in the acid slurry treatment (Emmerling et al. 2020), which is confirmed in our study by lowest values for unrecovered ^{15}N associated with acidified slurry, too. Such a reduction of gaseous N losses under acidified slurry is caused by the lower share of NH_3 in favor of NH_4^+ under the slurry pH reduction to 5.5 realized in our study. Seidel et al. (2017) reported that NH_3 losses were significantly reduced compared to broadcast slurry application technique by 31–61% by slurry injection and 42–79% by slurry acidification respectively. For an in-situ experiment in montane grassland and supporting laboratory incubations, Mar et al. (2021) identified a reduced NH_3 volatilization by approximately 50% by application of acidified cattle slurry. In a pot experiment over 67 days with different cattle slurry application techniques it was shown by Fangueiro et al. (2017), that an even stronger decrease of N losses can be achieved by surface application of acidified slurry followed by soil incorporation. Moreover, they constrain 81% less NH_3 emissions from acidified slurry compared to non-acidified ones and report much smaller reduction rates of NH_3 emissions from slurry injection. Hence, our results on lowest unrecovered N and highest plant N use efficiency and productivity under acidified slurry are likely mainly due to reduced NH_3 emissions, while effects on the terminal denitrification product N_2 remain uncertain.

Reduced NH_3 losses result in a higher amount of plant available N and likely also in an increase of P availability for plants (Fangueiro et al. 2015a, b, 2017; Cocolo et al. 2016). Since the solubility of manure inorganic P is pH dependent, acidification increases the amount of dissolved P in manure (Pedersen et al. 2016; Fangueiro et al. 2015a, b). Higher mobility of N, P and other nutrients favor the nutrient uptake of plants and promote plant growth (Agren et al. 2012; Wieder et al. 2015). Regarding the overall N balance and considering the high productivity, the relative importance of recent fertilizer N for plant N nutrition is still low, even despite increased N availability in the acidification treatment. This is particularly evident

in view of the enormous total plant N export of 95.1 and 121.9 kg N ha⁻¹ on the “current climate site” Graswang and the “climate change site” Fendt in the course of the season’s first harvest, which are highly exceeding slurry N application of 42 kg N ha⁻¹. Slurry recovery in plant suggests thus that 80.7–83.1% of N demand was not met from recent slurry but from decomposition soil organic matter N. Thus, N derived from organic fertilizers acts mainly retarded via cycling through the SON pool. It has been argued that the small direct utilization from organic fertilizers in the year of application is because of the slow-release characteristics of organically bound N and the medium- and long-term N immobilization in soils (Whitmore and Schröder 1996; Jensen et al. 2000; Sørensen and Amato 2002). The short-term (i.e. year of application) N availability of organic fertilizers depends largely on two factors: urea and mineral N and N content of larger organic substances (Gutser et al. 2005). Given that ammonium and urea typically account for much more than half of total slurry N, we attribute the low direct utilization of slurry N by plants rather to high heterotrophic microbial immobilization and subsequent N stabilization in organo-mineral associations or soil aggregates (Bimüller et al. 2014; Bierer et al. 2017; Jensen et al. 2000) than to slow release of N from slurry. This is supported by the observations that microbial biomass ^{15}N is dominating ^{15}N recovery in extractable soil N and even more that ^{15}N recovery in unextractable soil is either equaling (this study) or exceeding ^{15}N recovery in plant by several fold (earlier studies by Schlingmann et al. 2020 and Zistl-Schlingmann et al. 2020). High microbial immobilization is favored by high SOC content of the studied soil and also might contribute to low N_2O emission. The latter is typical for the high pH soils of this study which promotes the terminal step of denitrification, i.e., the reduction of N_2O to N, which has been shown both by direct $\text{N}_2\text{O}/\text{N}_2$ flux measurements and molecular analyses of the denitrifying microbiome (Wu et al. 2020; Chen et al. 2015; Zistl-Schlingmann et al. 2019). In the context of high microbial competition for N, injecting slurry resulted in higher soil ^{15}N recovery, likely because it promoted microbial access to fertilizer N with subsequent stabilization of microbial necromass in soil (Angst et al. 2021; Wang et al. 2021a, b; Ma

et al. 2022; Zhang et al. 2019; Thiele-Bruhn et al. 2012).

From a purely agronomic perspective on the short-term slurry injection has the side effect of lower yields compared to surface application. The reduction in yield may have been caused by sward damage from the injector tool, particularly at the first cut (Rees et al. 1993; Smith et al. 1995; Misselbrook et al. 1996; Rohde et al. 2006). On the longer term, such effects can be compensated by the larger amount of $\text{NH}_4\text{-N}$ left due to reduced N volatilization (Misselbrook et al. 1996; Rahmann et al. 2001; Mattila et al. 2003). Thus, injection of slurry can have a relatively greater effect on plant N concentration than on dry matter (van der Meer et al. 1987; Mattila et al. 2003) due to reduced NH_3 volatilization and by introducing manure-N to the soil closer to the roots. In our study, both root damage and intense microbial competition for N might have led to slightly lower yields under slurry injection compared to surface application of acidified slurry (Seidel et al. 2017; Sørensen 2004). In this context, the positive effect of slurry injection on CO_2 emissions could reflect increased root turnover and microbial respiration as a consequence of the physical soil disturbance.

In this study across all slurry treatments 19.8–27.4% of applied fertilizer ^{15}N remained unrecovered after the first harvest event. These losses were highest for the injected and lowest for the acidified slurry management technique. The environmental losses correspond roughly to figures presented by Jensen et al. (2000) of 23% unrecovered $^{15}\text{NH}_4\text{-N}$ cattle slurry for the experimental time of 7 month on a loamy sand soil before sowing in September of a winter wheat crop. However, a study from Schlingmann et al. (2020) for the same grassland soils and study sites determined much higher (50.5 to 57.1%) unrecovered ^{15}N rates through environmental losses after broadcast slurry application. Both Schlingmann et al. (2020) and Fu et al. (2017) in a three-year dataset reported similarly low leaching rates of 1 to 4.8 $\text{kg N ha}^{-1} \text{ yr}^{-1}$ for the grassland soils studied here. Therefore, we assume, that the majority of losses in this study also occurred in gaseous forms, i.e., NH_3 and N_2 . According to Zistl-Schlingmann et al. (2019) for the same grassland sites up to 40–50% of the applied manure was lost via N-gas emissions dominated by N_2 with up to 65% of all N losses. It needs to be noted however that this was measured

in dark incubations, i.e., neglecting plant uptake. Under mass balance considerations N_2O emissions of this study (0.04–0.12 kg ha^{-1} for a cumulative period of 79 days) are of minor importance and are also not affected by slurry management. Low N_2O emission levels and no significant difference for across five different application techniques were also found by Seidel et al. (2017), who applied cattle slurry on a perennial grassland. Though our N_2O flux data need to be carefully interpreted due to their limited temporal resolution, they confirm a recent study using better-constrained N_2O flux data (Malique et al. 2021) that shows a certain potential of pollution swapping to N_2O emission only in the days following application of acidified slurry to the investigated grassland.

Soil organic N formation from slurry fertilizer

In view of the high importance of SON for plant nutrition and the risk of N mining in the soils of this study, the additional formation of SON from fertilizer N by slurry injection of about 10 kg N ha^{-1} N from organic fertilizer N (Table 4) appears a highly desirable measure to reduce N mining, though negative N balances typically are several fold to one order of magnitude larger (Schlingmann et al. 2020; Zistl-Schlingmann et al. 2020). As confirmed by our data, SON formation is likely promoted by slurry injection due to increased inorganic N availability caused by assimilating heterotrophic soil microbes after injection, followed by stabilization of microbial necromass in polymeric organic matter compounds (Bierer et al. 2017; Jensen et al. 2000; Sørensen 2004; Hoekstra et al. 2011). Our soil depth-specific data clearly show that this process is most important in the densely rooted uppermost topsoil, generally characterized by highest microbial activity (Wang et al. 2016).

The increase in inorganic N availability (calculated as $\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$) induced by injection for some climate treatments and sampling dates in this study is in line with to earlier work. Bierer et al. (2017) calculated increased total inorganic N ($\text{NH}_3\text{-N} + \text{NO}_3^-\text{-N} + \text{NH}_4^+\text{-N}$) through slurry incorporation by 31% in a clay loam and 108% in a sandy loam relative to the control treatment. Kulesza et al. (2014) reported an increase of inorganic N by 71% and 105% for loam and sandy loam respectively after poultry litter injection.

Besides N, heterotrophic microbial immobilization requires easily available C. Such C supply might not only originate from root-derived C but also be readily oxidizable slurry-C, both increasing heterotrophic biological activity and microbial immobilization with subsequent remineralization of necromass or stabilization of N in organic matter (Sørensen 2004; Jensen et al. 2000). In this context, the initially higher respiration rates after addition of acidified slurry or slurry injection (Fig. S3) compared to broadcast application could reflect higher slurry-derived C availability for microbes under the alternative slurry application techniques. Nitrogen immobilization after slurry application occurred mainly within the first two weeks after slurry application (Kirchmann & Lundvall 1993; Jensen et al. 2000; Sørensen 2004). Hence, the relatively low ^{15}N recovery in microbial biomass and much higher recovery in unextractable soil N shows stabilization of microbial necromass in SOM, e.g., in organo-mineral associations.

The residence time of immobilized slurry N in SOM is largely unknown but might depend both on the chemical form and its physical protection e.g., in soil aggregates. Applying unlabeled slurry in the following seasons while tracing ^{15}N release from SOM into plant biomass would be needed to further assess the importance of stabilized fertilizer N for plant N nutrition in the following years.

Conclusion

In sum, we show that slurry injection did not show the same positive effects of slurry acidification on reducing N losses, increasing yield and plant N uptake, but is superior in increasing SON formation from organic fertilizer. Since plant nutrition is largely based on SON mineralization and high plant N export are the decisive factor for soil N mining, the positive effect of slurry injection on SON formation from fertilizer appears particularly desirable. However, we also show that the additional refueling of SON stocks promoted by slurry injection is likely not sufficient to avoid the much larger extent of current soil N mining, which remains driven by enormous plant N harvest. A combination of slurry acidification and slurry injection might further promote SON formation from organic fertilizer but increases time demand, work

safety requirements and costs for farmers. Still further measures such as reducing plant N harvest e.g., by reduced number of cuts, higher cutting levels and spring and/or autumn farmyard manure application, might be needed to reduce soil N mining to a significant extent in the pre-alpine grassland soils.

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Declarations

Competing interests The authors declare no competing interests.

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