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High importance of organic fertilizer nitrogen applied to temperate grassland for plant nitrogen uptake in the years following fertilization

Jincheng Han [a], Marcus Schlingmann [a,b], Rainer Gasche [a], Noelia Garcia-Franco [c], Martin Wiesmeier [c,d], Ralf Kiese [a], Ulrike Ostler [a], Heinz Rennenberg [e], Michael Dannenmann [a,*]

[a] *Karlsruhe Institute of Technology, Institute for Meteorology and Climate Research, Atmospheric Environmental Research (IMK-IFU), Kreuzeckbahnstrasse 19, Garmisch-Partenkirchen, Germany*
[b] *Landwirtschaftliches Zentrum Baden-Württemberg (LAZBW), Atzenberger Weg 99, Aulendorf, Germany*
[c] *TUM School of Life Sciences Weihenstephan, Chair of Soil Science, Technical University of Munich, Freising, Germany*
[d] *Bavarian State Research Center for Agriculture, Freising, Germany*
[e] *Center of Molecular Ecophysiology (CMEP), College of Resources and Environment, Southwest University, No. 2, Tiansheng Road, Beibei District, Chongqing 400715, PR China*

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ABSTRACT

We applied ^{15}N labelled cattle slurry over one year to large grassland lysimeters in Southern Germany to study its importance for plant nitrogen (N) nutrition not only in the year of application, but also in the following four years, and for both current and future climatic conditions. In the year of ^{15}N fertilizer application, the recovery of ^{15}N in harvested aboveground plant biomass was as low as 16 %, with several fold larger ^{15}N recovery in soil organic nitrogen (SON). In the first year after its application, the labelled ^{15}N was of equal importance for plant N nutrition compared to the year of application, as illustrated by a plant ^{15}N recovery of 9–14 %. The recovery of ^{15}N fertilizer in mowed plant biomass remained relatively stable and only gradually declined in the following years, ultimately resulting in a cumulative ^{15}N recovery rate of 33–37 % in mowed biomass after five years. Total ^{15}N recovery in plant and soil after five years was 67.6 ± 3.8 % for the climate change treatment and 75.6 ± 10.8 % for the climate control treatment, suggesting increased long-term fertilizer N losses under warming. Overall, this study reveals a minimal contribution of recent fertilizer N to plant nutrition, but a surprisingly quick recycling via SON and re-allocation to plants. The findings support a concept that fertilizing such grasslands is largely a fertilization of soils rather than a direct fertilization of plants, with a fast turnover of fertilizer-derived SON promoting the risk of soil N mining.

1. Introduction

The cycling of the macronutrient nitrogen (N) is vital for plant growth and other soil functions of grasslands (Elser et al., 2007). However, organic fertilizers applied to grasslands are often not efficiently utilized, resulting in N losses via nitrous oxide (N_2O), dinitrogen (N_2) and ammonia (NH_3) emissions as well as N leaching, thereby lowering the apparent retention of fertilizer N in the soil-plant system (Lalor et al., 2011; Misselbrook et al., 2002; Zistl-Schlingmann et al., 2020). The N losses from organic fertilization in grasslands including their undesired environmental impacts such as radiative forcing, impaired air quality and human health effects, decline in biodiversity as well as water contamination and eutrophication are increasingly well understood (Merbold et al., 2014; Porter et al., 2013; Shao et al., 2018; Swift et al., 1998). To mitigate the environmental impact of N fertilizers, especially NH_3 emissions and nitrate (NO_3^-) contamination of groundwater, Germany has implemented the EU's 2016 NEC Directive in its national fertilizer regulations. This includes setting a maximum limit for the application of organic fertilizers at 170 kg of N per hectare per year in intensive management. Still, the long-term fate of this organic fertilizer N in the grassland plant-soil systems remains unclear (Dai et al., 2023; Dourado-Neto et al., 2010; Yan et al., 2020). This is a major problem as recent research showed negative N balances in montane temperate grassland soils (Garcia-Franco et al., 2024; Schlingmann,

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* Corresponding author.
E-mail address: michael.dannenmann@kit.edu (M. Dannenmann).

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2020) which is caused by high N losses, high productivity, and a low importance of recent fertilizer for plant N nutrition, with plant N nutrition by up to 90 % or more being based on mineralized SON (Schreiber et al., 2023; Zistl-Schlingmann et al., 2020). Therefore, knowledge of multi-year fate of organic fertilizers in soil and temporal dynamics of their re-allocation to plants is of decisive importance to implement grassland management systems that prevent N mining. This is of particular importance in a changing climate, as warming in temperate grassland is increasing N mineralization (C. Wang et al., 2016).

Fertilizer N that is not lost via hydrological or gaseous pathways is either exported by aboveground biomass harvest or retained in the system in the short term in microorganisms and roots, and in the long term particularly in SON (Dourado-Neto et al., 2010; Yan et al., 2020). Therefore, high fertilizer N input can lead to N accumulation in the soil under conditions of sufficient SOC availability and moderate N losses, thereby increasing soil fertility (Dessureault-Rompré et al., 2013; Epstein et al., 2001; Sebilo et al., 2013). Most of the research on the long-term fate of N in soil-plant systems has primarily focused on cropland systems and on mineral N fertilizers (Buthelezi and Buthelezi-Dube, 2022; Li et al., 2022; Sebilo et al., 2013; Zhu et al., 2023). In a 30-year (1982–2012) experiment involving sugar beet and winter wheat in France, the recovery rate of ^{15}N by plants in the year of ^{15}N fertilizer application was 23–34 %. Over the 30 years of the experiment, plants cumulatively recovered nearly 65 % of the ^{15}N. The recovery rate of ^{15}N in soil initially was 32–37 %, and after 30 years, 12–15 % of the ^{15}N remained in the soil. This indicates a very slow recycling of SON until re-allocation to plant uptake. This pattern of low plant recovery and high soil recovery was also observed in grassland studies with organic fertilizers (Sebilo et al., 2013). In a short-term arid grasslands study in Northern China, it was found that in the year of organic fertilizer application, the N recovery rate by plants in grassland systems was only 3.8–11.7 % (Jiao et al., 2018). Between 50.4 % and 84.4 % of the labeled ^{15}N was retained in the top soil layer (Jiao et al., 2018). However, the long-term fate of N from organic fertilizers in grassland systems remains largely unknown and is particularly desirable to know for intensive management regimes with liquid slurry fertilizer, where biological N_2 fixation via legumes is suppressed.

Due to high mean annual precipitation and low mean annual temperature, mountainous grassland soils exhibit relatively high primary productivity and carbon inputs, coupled with relatively slow organic matter decomposition, making them significant hotspots for both SOC and SON storage regionally and globally (Leifeld and Menichetti, 2018; Garcia-Franco et al., 2024). However, climate change threatens these large SOC and SON stores and associated key soil functions. The warming climate in temperate and montane grasslands can extend the growing season, elevate photosynthesis and plant productivity, and enhance overall productivity (Rustad et al., 2001; Wu et al., 2011;, Wang et al., 2019). Besides the effects on grassland productivity, climate change can also change plant physiological processes and nutrient utilization rates (Jentsch et al., 2011). Several studies have highlighted that warming might increase N mineralization in grasslands, potentially elevating N availability (Emmett et al., 2004; Z. Wang et al., 2016). However, climate change will also lead to more frequent extreme weather events. Schwarzak et al. (2015) projected that central-eastern Germany would continue to experience increased summer drought events until 2100. The reduction in precipitation or alterations in precipitation patterns due to climate change could limit productivity, with large-scale droughts decreasing primary productivity by 30–49 % (Gilgen and Buchmann, 2009). This limitation is especially pronounced in drought-sensitive grassland types (Xu et al., 2021). In contrast, in wet mountainous regions, warming could increase SOM mineralization and productivity even under a decline in precipitation, thereby decreasing SOC and SON stocks (Garcia-Franco et al., 2024; Schlingmann, 2020; Schreiber et al., 2023; C. Wang et al., 2016; N. Wang et al., 2021). A decline in available water may thus either promote N mineralization or nitrification due to increased soil oxygen availability or impair soil microbial activity due to moisture stress depending on precipitation levels (Schmidt et al., 2004). Research by Gilgen and Buchmann (2009) revealed that regions with lower annual precipitation (975 mm) are more susceptible to summer drought effects, reducing above-ground net primary productivity, whereas more humid subalpine regions (1534 mm) are less negatively impacted by droughts. Given this decisive but variable effect, it is essential to better understand the dynamics influencing fertilizer N fluxes, stores, and turnover processes in grassland ecosystems and their potential future shifts under climate change conditions (Gilgen and Buchmann, 2009).

Therefore, the goal of this study was to assess the multi-year fate of fertilizer N in the plant-soil system in the largest grassland belt of Germany, the pre-alpine region, as affected by climate change. Building upon the ^{15}N fertilizer tracing experimental setup of the 1-year study of Zistl-Schlingmann et al. (2020), we continued to analyze ^{15}N excess recovery in four following years, while the newly added fertilizer was not ^{15}N-enriched. Based on the anticipated high stability of SON, we expected that the importance of "old" fertilizer N for recent plant nutrition would be minimal compared to the importance in the year of the fertilizer application. Specifically, we hypothesized that the recovery of ^{15}N in plant biomass would be small but still measurable and would decline in the years following the introduction of the ^{15}N -labeled fertilizer, given that the average turnover time of SON derived from the fertilizer upon re-mineralization and allocation to plant N uptake would likely be in the order of many decades rather than years. Furthermore, we expected that climate warming would accelerate the SON turnover rate via increased microbial activity due to higher temperatures, thus enhancing the recycling of old fertilizer ^{15}N from SON and its allocation to plants.

2. Materials and methods

2.1. Study sites and experimental setup

We used the experimental setup of the intensively managed treatment of the Schlingmann et al. (2020) study, i.e., six large intact grassland lysimeters excavated at the Graswang site (11.03° E, 47.57° N) in the TERENO pre-alpine observatory at an altitude of 865 m in 2011 (Pütz et al., 2016). The weighed lysimeters are equipped with soil temperature and moisture sensors at five depths (Kiese et al., 2018; Schlingmann et al., 2020). Three of the lysimeters remained at Graswang as a control, and the other three lysimeters were translocated to the Fendt site (600 m) to simulate climate change expected in the following decades within a space-for-time approach (Pütz et al., 2016; Kiese et al., 2018). Based on historical records (2011–2018), the mean annual precipitation (MAP) at Fendt and Graswang is 961 mm and 1284 mm, respectively, and the mean annual temperatures (MAT) are 9.2 °C and 7.1 °C, respectively. The Fendt site was 2.1°K warmer and received 323 mm less precipitation per year compared to the Graswang site (Wang et al., 2021). Thus, the lysimeters at Graswang are the high elevation climate control treatment (HE), while lysimeters translocated to the low elevation site Fendt within a space for time approach represent the climate change treatment (LE). The selected sites have a flat topography and represent typical soils in the area, which were formed from calcareous and dolomitic parent materials. The investigated soil is a Haplic Cambisol (IUSS Working Group WRB, 2015). Since the establishment of the lysimeters in 2012, both Fendt and Graswang lysimeters have been intensively managed in accordance with local farming practices, i.e., underwent 4–5 fertilization and subsequent harvest cycles per year. In line with the German Fertilizer Ordinance, the maximum organic N input for intensive management is capped at 170 kg/ha per year, with each single cattle slurry fertilization contributing approximately 30–40 kg N/ha. The cattle slurry used for the experiment was obtained from a local farmer at the Fendt site and analyzed for N and nutrient content by a commercial laboratory (Raiffeisen Laborservice, Ormont, Germany).

2.2. 15N tracing approach

^{15}N-labelled slurry was applied during the first four fertilization events of 2017, while un-labelled slurry was used in the following fertilization events (2018–2021, Fig. 1). The fate of N in the plant-soil system was traced using slurry amended with 99 % atom% $^{15}NH_4^+$ and ^{15}N- urea (Zistl-Schlingmann et al., 2020). The slurry had an average N content of 4.2 % total N, with 53 % of the N present as NH_4^+-N and 47 % as organic N, including urea. Therefore, the ^{15}N labelling covers the majority of slurry N, excluding polymeric organic N. The addition of the ^{15}N label resulted in an average relative increase of 3.5 % in the N content of the slurry. Before fertilization, the ^{15}N tracer was thoroughly mixed and the plastic barrel was agitated to ensure even distribution of the tracer in the slurry. The excess ^{15}N applied per fertilization event in the 1 m^2 lysimeter was 253 mg, resulting in a total ^{15}N excess amount of 1012 mg applied to the lysimeters in 2017. Further details are provided by Schlingmann et al. (2020). In the subsequent four years (2018–2021), slurry with ^{15}N at natural abundance was used. Subsequently, we tracked the ^{15}N applied in 2017 through all harvested plant biomass from 2018 to 2021. In December 2021, final soil and root samplings were conducted to determine the recovery rates of ^{15}N in the soil and roots to set up a 5-years fertilizer ^{15}N balance.

2.3. Sampling, sample preparation and analysis

The harvesting of biomass on the 1 m^2 lysimeter surfaces followed local farmers practice, i.e., we cut biomass 5 cm above the ground using scissors. Also, the frequency and timing of harvesting followed local farmers practice (Fig. 1). The harvested biomass was placed in paper bags and transported to IMK-IFU's lab for further processing. The biomass was turned every two days and allowed to dry for a week before being transferred to an oven set at 60 °C until constant weight. The dried biomass was weighed, then cut into small pieces using scissors, homogenized, and a representative subsample was ground into homogeneous powder for 3 minutes at 30 rpm/s using a ball mill (Retsch, MM301, Germany). The soil sampling was conducted in November 2021 using a motor driller (Cobra TTe, Atlas Copco, Germany) with three replicated drillings at each lysimeter. The entire soil column, measuring 1 m in depth and 4.7 cm inner diameter, was extracted from the lysimeters and immediately transported to IMK-IFU's Lab for further processing. Sampling depths were 0–5 cm; 5–10 cm; 10–20 cm and 20–30 cm. The soil samples were sieved by using a 2 mm mesh, dried at 60 °C until a constant weight was achieved, and ground using a ball mill (Retsch, MM301, Germany). For sampling of roots from a defined volume, we used separately sampled cores of 100 cm^3 volume for 0–5 cm depth, thereby covering the main rooting depth (Wang et al., 2016). Roots were handpicked and washed with distilled water. The roots were immersed in a capacious container or sink filled with water and swirled gently to eliminate any residual soil. Finally, the roots were dried and pulverized as described above. Aliquots of biomass (2 mg), root (4 mg), and soil samples (2–8 mg) were weighed into 5x9mm tin capsules (IVA Analysentechnik, Meerbusch, Germany). The N concentration and ^{15}N enrichment were analyzed using an EA-IRMS system at the IMK-IFU in Garmisch-Partenkirchen, with an elemental analyzer (Flash 1112 EA, Thermo Scientific, Waltham, Massachusetts, USA) coupled to an isotope ratio mass spectrometer (Delta Plus XP, Thermo Scientific, Waltham, Massachusetts, USA) via a ConFlo III interface (Thermo Scientific, Waltham, Massachusetts, USA) similar to the system described by Guo (Guo et al., 2013). Mineral N in soil solution was originally determined in soil water samples collected from bottles with suction cups attached (Fu et al., 2017). We collected water samples from 140 cm collection depth every 2 weeks. From these samples, we filtered 50 ml subsamples using a 0.45 µm hydrophilic cellulose acetate membrane (Sartorius Stedim Biotech GmbH, Göttingen, Germany) and a syringe. The filtered subsamples were poured into Falcon tubes and stored frozen until further analysis (Yankelzon et al., 2024).

2.4. ^{15}N isotope calculations

Excess ^{15}N amount in samples was calculated using the following Eq. (1):

$$^{15}Nexcess = \frac{APE}{100} * Npool \tag{1}$$

Where N_{pool} is the amount of N in mg in the biomass, depth-specific soil and roots N_{pool} per lysimeter or m^2, and APE (atomic percent excess) is ^{15}N excess enrichment (atom%^{15}N measured minus the natural abundance atom%^{15}N) of the respective N_{pool}. ^{15}N natural abundance is 0.3663 %. The recovered ^{15}N excess amount was divided by the cumulative ^{15}N excess addition applied with fertilizer in 2017 (1012 mg ^{15}N per lysimeter) in order to calculate the ^{15}N recovery rate in %.

2.5. 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 (Harris et al., 2020; Hunter, 2007). Before proceeding with the analyses, the normal distribution of the data was verified using the Kolmogorov-Smirnov test, and the homogeneity of variances was assessed using the Levene test. To evaluate the significant differences in biomass, roots, soil, and 15 N recovery under different climatic conditions, one-way analyses of variance (ANOVA) were conducted. With different climate conditions as the factor, differences among climate conditions were identified at a significance level of 0.05 using the Tukey test. When the data did not follow a normal distribution, the non-parametric Kruskal-Walli's test was used, followed by pairwise comparisons between groups at a 0.05 probability

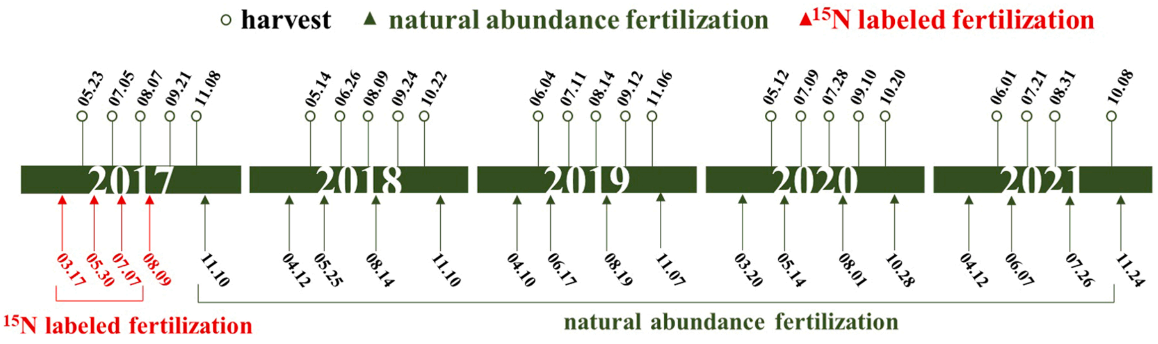

Fig. 1. Harvest and fertilization schedule. Only the first 4 fertilizations in 2017 were ^{15}N labelled, the following fertilizations were not enriched with ^{15}N.

level.

3. Results

3.1. Meteorological conditions

During the 5-years experimental period, the space for time translocation of the lysimeters to simulate climate change resulted in increased soil and air temperatures and decreased precipitation and soil moisture (Fig. 2). The 5-year average annual air temperature was 6.9 °C at HE. In comparison, the LE site had an average of 8.9 °C, confirming an average temperature difference of approximately 2.0 °C between the two sites. During the GS (growing season, from April to September), the average air temperature was 12.8 °C at HE and 14.3 °C at LE, which is a 1.5 °C difference. The average annual soil temperature at a depth of 10 cm was 9.0 °C and 10.9 °C for HE and LE, respectively, i.e., a difference of 1.9 °C. Considering GS, the soil temperature at 10 cm depth for the HE experimental site was 14.3 °C, and for the LE site it was higher by 1.8 °C (16.1 °C). The 5-year average soil moisture at 10 cm depth was 47.1 vol% for HE and 43.7 vol% for LE, revealing a mean difference of 3.4 vol%. The 5-year MAP for the HE site amounted to 1415 mm, while it was 1003 mm for the LE site. Precipitation at both sites predominantly occurred between April and September, with an annual difference of 225 mm between the two sites during the growing season. The year 2018 was clearly warmer and drier than the other years, and both 2018 and 2019 showed the most pronounced decline in soil moisture during the growing season in the LE site compared to the HE site (Fig. 2).

3.2. Plant biomass yields and N Content

From 2017–2021, the annual plant biomass yield at HE and LE was relatively stable around ca 10 t ha^{-1} yr^{-1} (Fig. 3). We found a trend of higher annual biomass yield at LE than HE in all years except for 2018, but this was not significant. In the drought year 2018, there was significantly lower plant biomass harvest at LE (8.1 t ha^{-1}) compared to the control treatment at the HE site (10.5 t ha^{-1}). Considering the cumulative biomass export over the entire period, this yield difference at LE was almost outbalanced in the following years (Fig. 3). Typically, the highest yields were observed during the first harvest of each year and declined with later harvests. Compared with plant biomass, the related data on plant N export through mowing showed more pronounced differences between HE and LE. The five-year cumulative N export at LE (956 kg N ha^{-1}) was significantly lower than that of HE (1061 kg N ha^{-1}) ($p < 0.05$). Annual plant N export in most cases was larger than annual fertilizer N addition (Table 1). We found the lowest plant N export due to the climate change treatment (LE) in the drought affected years 2018 (69 kg N ha^{-1} less at LE than at HE ($p < 0.05$), and in 2019 (46 kg N ha^{-1} less $p = 0.12$) (Fig. 3). Similarly, the N concentration in harvested biomass at LE (2.1 % N) was significantly lower ($p < 0.005$) compared to HE (2.3 % N) (Fig. 3).

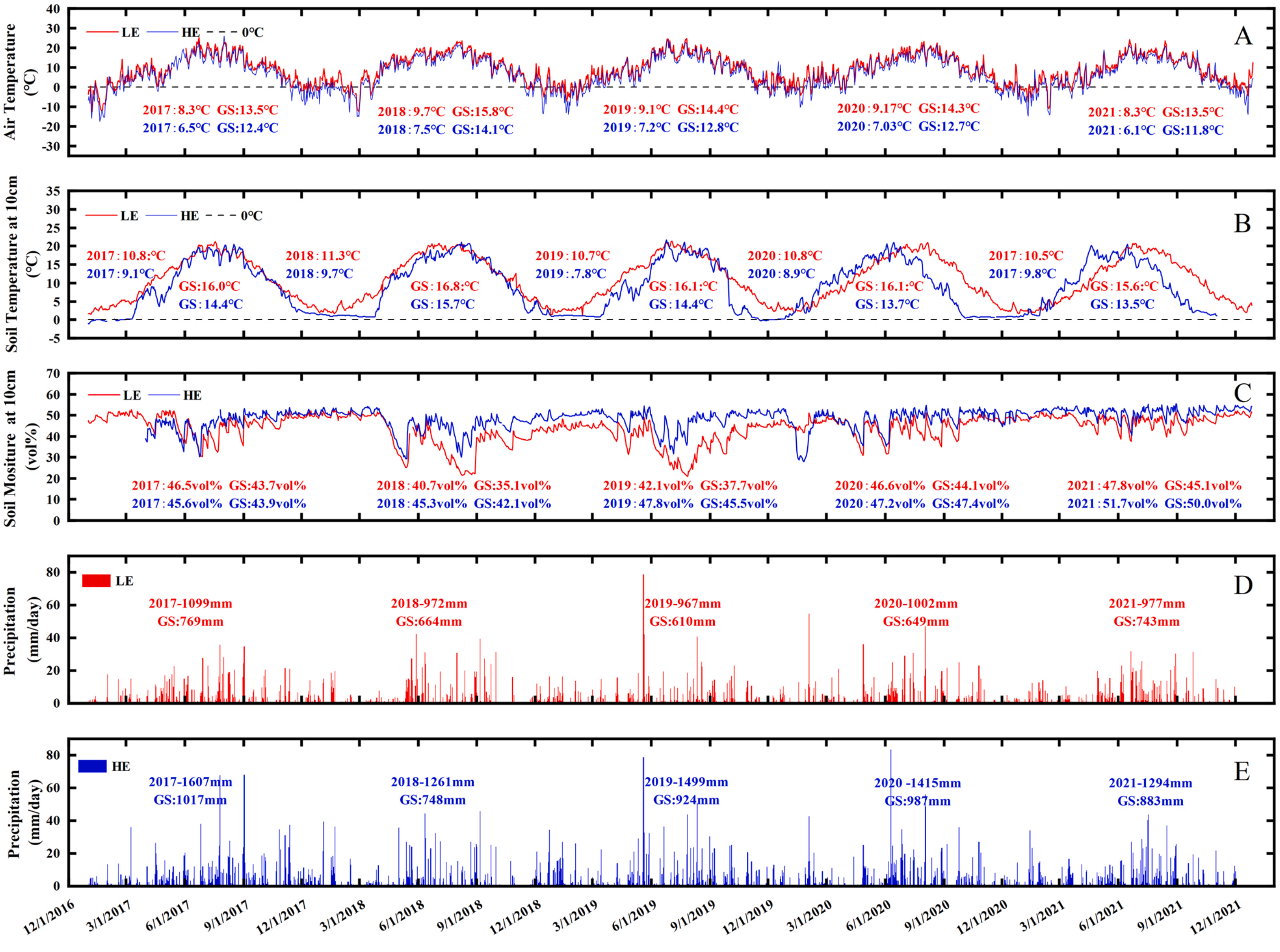

Fig. 2. Meteorological conditions of HE (control site, blue) and LE (climate change site, red) from 2017 to 2021. A. Air temperature B. Soil temperature measured in the lysimeters at 10 cm depth; C. Volumetric soil moisture measured in the lysimeters at 10 cm depth; D. Climate change site precipitation E. Control site precipitation. The graph also numbers of annual mean values and mean values for the period April to September ("GS"). HE (high elevation site, as climate control); LE (low elevation site, as climate change).

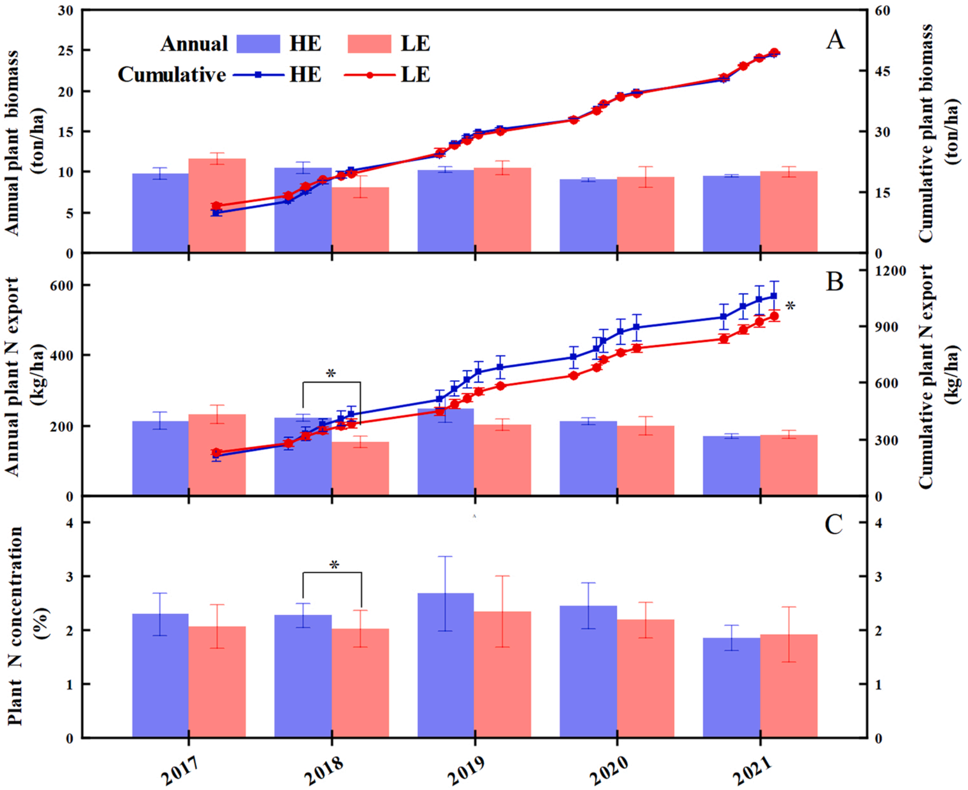

Fig. 3. A. Annual plant biomass production (from 4 to 5 harvests) and cumulative productivity from 2017 to 2021. B. Plant annual N export and cumulative plant N export from 2017 to 2021. C. Annual average of plant N concentrations. HE (high elevation site, as climate control); LE (low elevation site, as climate change).

Table 1
Annual fertilization, annual plant N export, and importance of fertilizer applied in 2017 for plant N uptake. HE (high elevation site, climate control treatment); LE (low elevation site, climate change treatment). Different indices (a, b) indicate statistically significant differences between the HE and LE treatments.

	HE (Climate Control)					LE (Climate Change)				
	2017 (^{15}N)	2018	2019	2020	2021	2017 (^{15}N)	2018	2019	2020	2021
Fertilizer Application kg N ha^{-1}	170	170	170	170	170	170	170	170	170	170
Plant N Export kg N ha^{-1}	217.6 ± 32.9	222.0 ± 9.4 a	246.0 ± 37.3	211.0 ± 10.3	169.0 ± 6.7	194.1 ± 13.8	153.0 ± 15.3 b	200.9 ± 16.4	199.0 ± 26.6	173.3 ± 11.1
Fertilizer N from 2017 in %	15.7 ± 3.4	13.7 ± 1.5 a	3.9 ± 0.2	2.4 ± 0.3	1.2 ± 0.08 a	16.5 ± 0.8	9.8 ± 0.5 b	3.6 ± 0.5	2.4 ± 0.1	1.6 ± 0.1 b
Fertilizer N from 2017 fertilization kg N ha^{-1}	26.7 ± 5.7	23.4 ± 2.6 a	6.7 ± 0.3	4.1 ± 0.6	2.1 ± 0.1 a	28.1 ± 1.3	16.7 ± 0.8 b	6.2 ± 0.8	4.1 ± 0.2	2.8 ± 0.2 b

3.3. ^{15}N excess recovery in AGB, BGB and soil

A clearly detectable amount of fertilizer ^{15}N excess applied in 2017 was recovered in every single plant biomass sample harvested during the years 2018–2021. Hence, cumulative ^{15}N recovery was constantly increasing over the experimental period (Fig. 4). Compared with the ^{15}N fertilizer recovery in plant biomass in the year of application (2017, ca. 15–16 %), the recovery in the subsequent year (2018, ca 9–14 %) was similar, despite no further ^{15}N labelled fertilizer was added. In the following years, annual ^{15}N recovery in harvested plant biomass decreased further from 4 % in 2019 to 1 % in 2021. By the end of 2021, the cumulative ^{15}N recovery in harvest plant biomass was 33 % and 41 % at LE and HE, i.e. significantly lower ($p < 0.05$) at LE. This means that far more than twice as much of fertilizer N was exported with plant biomass in the four years after its application compared to the year of application (Table 1). Although the ^{15}N recovery rates in mowed plant biomass in 2017 showed minimal variation between HE and LE, by the end of 2021, the five-year cumulative ^{15}N recovery rate for the HE (37.0 %) was significantly higher than that of the LE (34.0 %) (Fig. 4). This disparity was largely caused in the dry year 2018, when the annual ^{15}N recovery rate at the HE was exceeding that of the LE by 4 % ($p < 0.05$).

To complete experiment in December 2021 with a full fertilizer N balance, the recovery rates of ^{15}N were measured in the soil at four different depths (0–5 cm, 5–10 cm, 10–20 cm, and 20–30 cm) and in roots in 0–5 cm depth. The total N stocks in 0–10 cm depth amounted to 4.2 ± 1.0 and 4.2 ± 0.7 t N ha^{-1} at LE and HE, respectively. Within the 0–30 cm soil profile average recovery rate of soil ^{15}N was 38.5 % at HE, while it was 33.2 % at LE ($p = 0.4833$) (Fig. 5). At the depths of 0–5 cm and 5–10 cm, no significant differences in the ^{15}N recovery rates in soil were found between HE and LE. However, at 10–20 and 20–30 cm depth, HE soil exhibited ^{15}N recovery rates that were significantly higher than those of LE soil by 5.6 % and 0.9 %, respectively ($p < 0.05$). Notably, ^{15}N recovery in soil strongly declined with depth and was negligible for the mass balance in the last depth (Fig. 5). The ^{15}N recovery in belowground biomass was of minor importance with values of 0.1 ± 0.06 % for HE and 0.3 ± 0.2 % for LE, but this difference between HE and LE was statistically significant ($p < 0.005$).

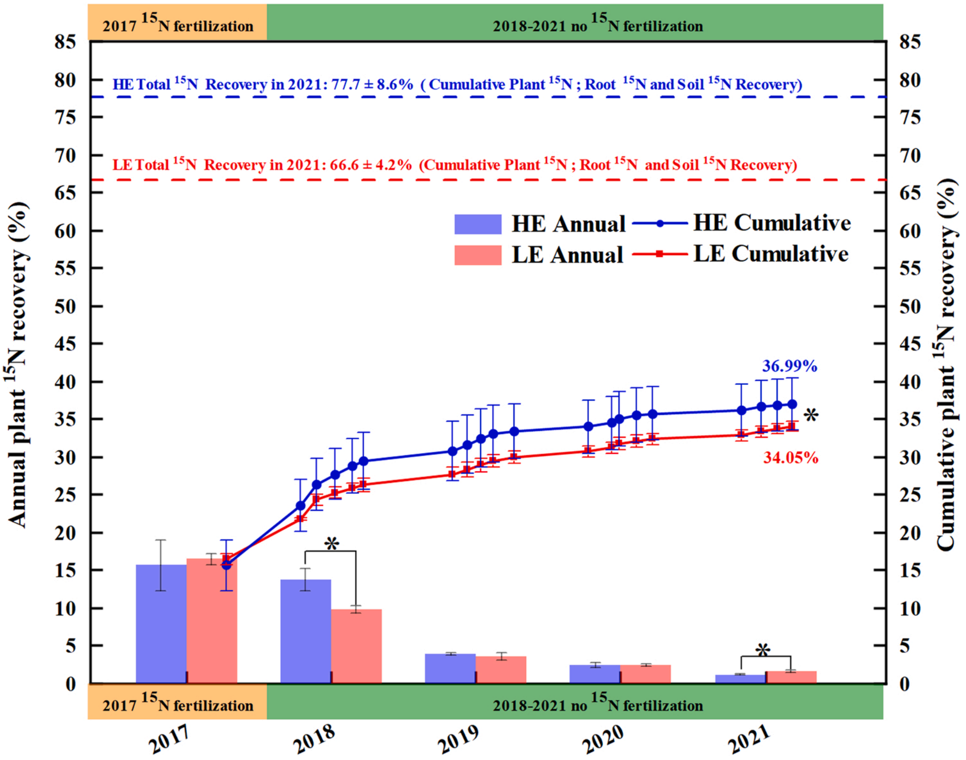

Fig. 4. Annual and cumulative recovery of ^{15}N fertilizer excess in harvested plant biomass in the years 2017–2021. Note that ^{15}N labeled fertilizer was only applied in 2017, while fertilizer applied 2018–2021 was not ^{15}N enriched. HE (high elevation site, as climate control); LE (low elevation site, as climate change).

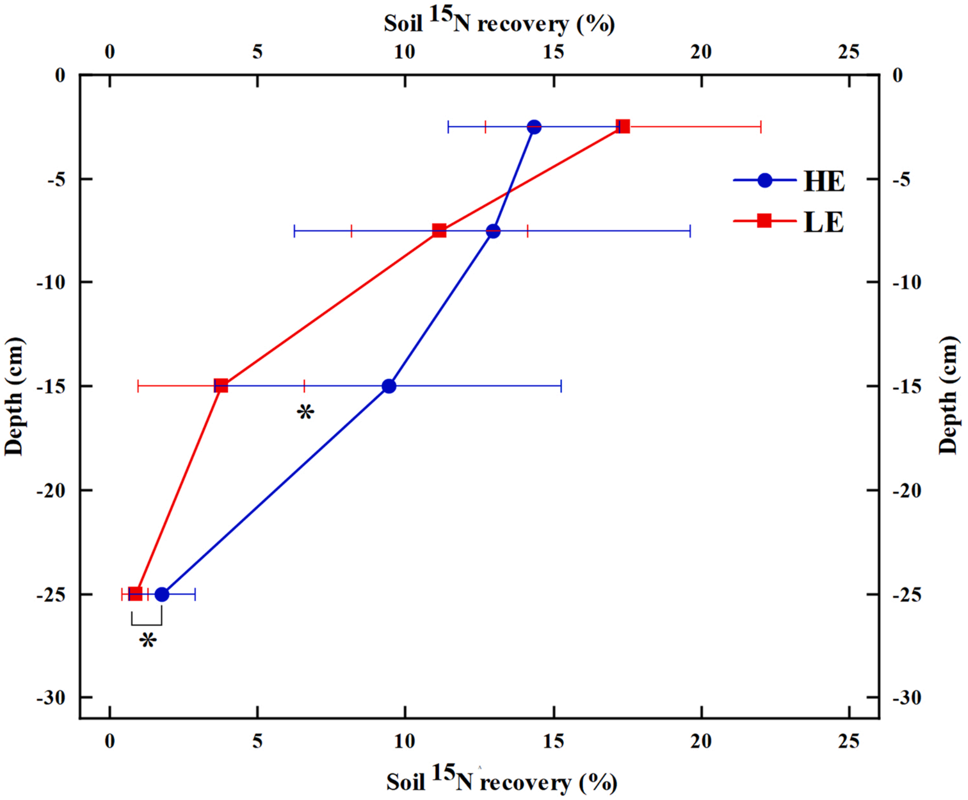

Fig. 5. Recovery of excess 15 N at the end of the experiment in 2021 in the soil at 0–5 cm; 5–10 cm; 10–20 cm ($p < 0.05$) and 20–30 cm ($p < 0.05$) HE (high elevation site, as climate control); LE (low elevation site, as climate change).

3.4. Total ^{15}N recovery and 5-year fertilizer N balance

Summing up individual components, the total recovery of fertilizer ^{15}N applied in 2017 was 75.6 ± 10.8 % (HE) and 67.6 ± 3.8 % (LE), with about half contribution by harvested plant biomass N and soil recovery (Table 2). A total of 170 kg/ha N was applied in 2017, of which 128.6 kg N/ha (HE) and 114.9 kg N/ha (LE) were recovered. So, the unrecovered fertilizer ^{15}N five years after its application at HE was 24.4 ± 10.8 % and LE was 32.4 ± 3.8 %. Specifically, the biomass at HE showed a 3 % higher ^{15}N recovery rate ($p < 0.05$), while the 10–20 cm soil layer exhibited a 5.6 % higher ^{15}N recovery rate compared to LE ($p < 0.05$). Although the 20–30 cm soil layer and the BGB at HE displayed a significantly higher ^{15}N recovery rate compare with LE, the absolute difference in values was relatively small. For the 0–10 cm soil layer, the ^{15}N recovery rate remained consistent at 27 % for both the HE and LE, showing negligible differences.

4. Discussion

4.1. Grassland fertilizer N utilization and long-term fate

The 5-year organic fertilizer ^{15}N balance presented here allows for several insights into grassland N cycling under simulated climate change. First, the higher ^{15}N recovery in the climate control treatment suggests that warming led to increased fertilizer N losses in the years after its application, which cannot be explained by increased plant ^{15}N export. Hence, this attributed to increased microbial mineralization, nitrification and denitrification in the climate change treatment as a consequence of increased temperatures and DOC availability (Wang et al., 2016), with unreactive N_2 rather than the greenhouse gas N_2O being the main N loss compound (Zistl-Schlingmann et al., 2019). Second, our study clearly showed that cattle slurry fertilizer N was of minor importance for grassland plant N nutrition in the year of its application, but a several fold larger contribution to plant N uptake in the years following the application. Fertilizer N use efficiency by plants and other fertilizer N fates including losses are largely influenced by factors such as fertilizer type and timing, precipitation and soil moisture, as well as management practices (Calderón et al., 2005; Chadwick et al., 2000; Fangueiro et al., 2008; Van Kessel and Reeves, 2002). In contrast to grasslands, abundant related information is available for cropping systems. A meta-analysis of studies involving the use of ^{15}N-labeled mineral fertilizers in agricultural systems revealed that crops such as maize, rice, and cereals derive only approximately 41 %, 32 %, and 37 % of the total N uptake, respectively, from N fertilizers applied in the year of fertilizer application (Gardner and Drinkwater, 2009; Yan et al., 2020). Moreover, crops exhibit relatively lower utilization rates of organic N fertilizers, estimated at 27 %, yet the N recovery efficiency of these organic fertilizers in subsequent years (approximately 10 %) surpasses that of mineral fertilizers (Yan et al., 2020; Zhu et al., 2023). A 30-year ^{15}N study conducted in an agricultural system in France has demonstrated that isotopically labeled N applied 30 years ago continues to be recycled and utilized. Specifically, 12–15 % of the ^{15}N remains in soil organic matter (SOM) pools, indicating a prolonged recovery period for a significant portion of the fertilizer N bound to SOM (Sebilo et al., 2013). Previous studies on grasslands have shown that SON is very stable with three times slower turnover and higher residence time than found in intensive agricultural systems (Gerzabek et al., 2004; Jansson and Persson, 1982; Ladd et al., 1983).

Table 2

Total ^{15}N recovery at the end of the experiment and flows of the 2017-year fertilizer by end of 2021. HE (climate control); LE (climate change). N leaching losses were negligible. Different indices indicate significant differences between HE and LE.

Fertilizer flow applied in 2017 to N pools by 2021	HE (^{15}N %)	LE (^{15}N %)	HE (fertilizer N flow in kg/ha)	LE (fertilizer N flow in kg/ha)
AGB (cumulative)	37.0 ± 3.4 % a	34.0 ± 0.7 % b	62.9 ± 5.8A	57.9 ± 1.2 B
BGB	0.1 ± 0.0 % a	0.3 ± 0.1 % b	0.2 ± 0.0 A	0.6 ± 0.1 B
Total Soil Pools (0–30 cm)	38.5 ± 10.9 %	33.1 ± 5.0 %	65.5 ± 18.5	56.4 ± 8.4
0–5 cm Soil Pools	14.4 ± 2.9 %	17.4 ± 4.6 %	24.4 ± 4.9	29.5 ± 7.9
5–10 cm Soil Pools	13.0 ± 6.7 %	11.2 ± 3.0 %	22.0 ± 11.4	19.0 ± 5.1
10–20 cm Soil Pools	9.4 ± 5.8 % a	3.8 ± 2.8 % b	16.0 ± 9.9A	6.4 ± 4.8 B
20–30 cm Soil Pools	1.8 ± 1.1 % a	0.9 ± 0.4 % b	3.0 ± 1.9A	1.5 ± 0.7 B
Losses	24.4 ± 10.8 %	32.4 ± 3.8 %	41.4 ± 18.3	55.1 ± 6.4
Total N recovery	75.6 ± 10.8 %	67.6 ± 3.8 %	128.6 ± 18.3	114.9 ± 6.4

This contradicts the most striking findings of this study, i.e., the high importance of organic fertilizer N in the years after its application for plant N nutrition, which was several folds larger than its importance in the year of application. In this study of grassland systems, using traditional slurry fertilizer broadcast application methods resulted in a ^{15}N recovery rate of only 15–16 % in the first year. However, experiments conducted under the same grassland and climatic conditions using acidified slurry or slurry injection methods showed higher ^{15}N recovery rates, with approximately 30 % recovery in the year of fertilization (Schreiber et al., 2023). The relatively lower fertilizer recovery rates observed in this study can be attributed to intense competition for N among plants and microorganisms in high-altitude grasslands. A significant proportion of ^{15}N may be immobilized by microbial biomass (Gerzabek et al., 2004; Jaeger et al., 1999), particularly in soils with high SOC content, which promotes heterotrophic microbial activity and N immobilization (Booth et al., 2005; Elrys et al., 2022). Despite the absence of added ^{15}N-labeled fertilizers, the cumulative ^{15}N recovery rates in harvested biomass reached 34–41 % from the second to the fifth year (2018–2021). Compared to the year of application, both HE and LE treatments exhibited similar trends, with the annual ^{15}N recovery rates declining from 9 % to 14 % in 2018 to 4 % in 2019 and dropping to 1 % by the end of the experiment in 2021. These findings suggest a surprisingly rapid cycling of N within SOM until remobilization and plant uptake with a residence time of fertilizer N in SON of years rather than decades. More specifically, in our study we observed that ^{15}N excess in plant biomass increased from end of 2017 to end of 2021 by 18 % (LE) and 22 % (HE) of applied ^{15}N excess (Fig. 4). Total ^{15}N recovery in 2021 and cumulative plant ^{15}N recovery end of 2017 reveal a maximum initial soil ^{15}N recovery of 50 % (LE) and 62 %. This suggests that at least 35 % (both for HE and LE) of applied fertilizer ^{15}N excess cycled through SON until re-provision to plants within 4 years. From this, a rough lower estimate of the mean turnover rate of fertilizer-derived SON of about 11 years can be calculated, however this is not considering different SON pools with variable stability. The turnover of fertilizer-derived SON is governed by the stability of the respective SON pools, and by mineralization rates, with the latter mainly depending on DOC availability (Wang et al., 2016). Available DOC might origin both from depolymerization of SOC and from rhizodeposition, so that plant productivity exerts feedback on N mineralization.

The limited contribution of fertilizer N to plant nutrition in the year of application, and the predominance of mineralized SON in the plant uptake of these grasslands compared to direct contributions of fertilizer N, underscore the concept that fertilization in these grasslands largely fulfills the function to refuel SON rather than directly benefiting the plants, thus supplementing the plant harvest N outputs fueled by mineralization of SON stocks. In this sense, the gaseous and hydrological N fertilizer losses do not only have environmental consequences such as air pollution, radiative forcing, and eutrophication, but also prevent new SON formation, thereby promoting N mining. The SON-mineralization based N uptake of grassland plants has recently been shown in several studies in montane grasslands (Dannenmann et al., 2024; Schlingmann et al., 2020a). From a plant ecological perspective, it has advantages as N mineralization is generally high in these SOM rich soils (Booth et al., 2005; Schlingmann et al., 2020; C. Wang et al., 2016), so that even if plants access considerably less of mineralized N compared to microbial immobilization, this is sufficient to meet a high demand. Furthermore, from a spatial perspective, N mineralized from SON might be much better available to roots, also due to rhizospheric priming (Kuzyakov and Xu, 2013). Finally, a high abundance of arbuscular mycorrhizal fungi in such grasslands (Anrade-Linares et al., 2023) might allow plants to access depolymerized N before ammonification in form of monomeric organic N based on symbiosis with mycorrhizal fungi (Kuzyakov and Xu, 2013; Leifheit et al., 2024).

The soil ^{15}N recovery after application of labelled slurry in the studied soil type is almost exclusively attributable to unextractable, polymeric organic N (Schlingmann et al., 2020; Dannenmann et al., 2024). Given that grassland plants can only use dissolved monomeric organic N or mineral N, rapid re-use of OM-N by plants within few years through depolymerization and/or ammonification is suggested by this study. However, it needs to be considered that the study of Schlingmann et al. (2020) did not consider root biomass, i.e., the total fertilizer ^{15}N recovery of approximately 50 % at the end of the year 2017 (Zistl-Schlingmann et al., 2020) does not consider root ^{15}N and might be higher. This could theoretically also explain the higher total ^{15}N recovery (67–75 %) in 2021 after five years compared to 2017 after one year of ^{15}N labelling. However, a parallel study with similar soil and a similar experimental setting, fertilization and timing reported root ^{15}N recovery at the end of the season under intensive management of only 1–2 % (Schlingmann et al., 2020). Furthermore, it needs to be considered that measurements of total ^{15}N recovery in 2021 show much less uncertainty than those in 2017 because they include a much larger share of plant ^{15}N measurements (sampling of the entire lysimeter area) which are highly spatially representative compared to soil measurements (3 drillings per lysimeter), which represent the largest uncertainty in such ^{15}N mass balance studies (Yankelzon et al., 2024). Therefore, the majority of the plant ^{15}N uptake in 2018–2021 is originating from SON with a short N residence time.

Generally, our study confirms a series of recent work that plant N nutrition in OM-rich grasslands is largely based on N mineralization, rather than on using recently added slurry-N. The large rates of N mineralization in the investigated soil (Wang et al., 2016) are likely promoted by a narrow C:N ratio between 9 and 10 in the 0–15 cm topsoil (Garcia-Franco et al., 2024), which indicate high decomposability of organic matter and high N yields during organic matter breakdown (Knicker, 2011). In that regard, the fertilizer function is not to directly fertilize plants, but to refuel the SON that is mineralized, thereby supporting plant N uptake in following years. In our study this function was partly prevented by large N losses, so that – together with large plant N exports – there was a N deficit up to 86 kg N ha^{-1} in 2017 (Schlingmann et al., 2020). The residence time of fertilizer N in SON will then largely determine the vulnerability of the system to N losses, with such N losses being accompanied by C losses as well (Wang et al., 2021). Generally, the fast re-cycling of fertilizer N through SON and its subsequent uptake and export by plants might explain the strong decline of SON and SOC stocks observed in OM rich grassland soil under slurry fertilization (Garcia-Franco et al., 2024). Especially in cases where the high productivity of plants and the continuous N export by plants exceed the total N input, a more negative N balance will result. However, the present work has the limitation that the flow of fertilizer N through SON pools of different stability is not directly followed – a task we recommend for future studies. In this sense, it is also essential to know whether traditional farmyard manure management not only reduces N losses but also results in more stable SON and SOC (Köninger et al., 2021; Rodríguez-Espinosa et al., 2023). Furthermore, the long-term N balances of extensification, i.e., reduced slurry N addition are unclear. We therefore recommend that future studies should also focus on different N application rates and application of different types of organic fertilizer.

4.2. Impact of climate change on grassland productivity and N export by mowing

Understanding climate change effects on grassland productivity and N uptake is crucial since harvest N exports are the largest component of the grassland N balance, and climate warming is particularly strong in the pre-alpine regions. The response of productivity and annual N export to experimentally simulated climate change was highly variable in this study, ranging from a climate change induced increase in the wet year 2017 to a decrease in the very dry year 2018 and consecutive 2019 and no effect in the years 2020–2021. Generally, it is thought that global warming extends the growing season of grasslands, enhances photosynthesis plant productivity, and thus increases the overall productivity (Rustad et al., 2001; H. Wang et al., 2020). A meta-analysis of climate change experiments suggests that climate change results in 19 % increase in plant productivity and 50 % in root biomass (Rustad et al., 2001). However, despite the climate change treatment LE experienced a longer vegetation period (temperatures above 5 °C) by ca. 30 days each year compared to the control treatment HE, both treatments maintained a relatively consistent grassland biomass yield of on average 9.6 ton/ha, with no significant differences over a 5-year period. In other studies conducted in the alpine and pre-alpine areas, climate change had no effect on biomass, whether intensively managed (14–19 tons/ha) or extensively managed (8–9 tons/ha) (Schlingmann et al., 2020). Additionally, a long-term study over 30 years on the Tibetan Plateau's alpine grassland showed no change in biomass yield (3.7ton/ha) even with an average annual temperature increase of 1.2 °C (Liu et al., 2018). This implies that climate change does not systematically alter above-ground grassland net primary production, potentially due to the combination of different climate change factors such as increasing temperature with growing season length and decreasing moisture. Notably, in the particularly dry year 2018, the annual yield of the LE was lower than HE, which appeared to be related to a spring drought with > 40 mm reduced precipitation in April compared to other years, which lead to the lowest growing season precipitation values and soil moisture values of LE observed in the monitoring period of this study, with lower soil moisture at LE persisting until 2019 (Fig. 3). Also, the timing of this drought within the year might have been decisive as it occurred before the first grassland harvest, which contributes most to annual productivity and N export. Additionally, in 2018, temperatures at both sites were the highest in 5 years, with an increase of 0.8–1.9 degrees compared to other years, leading to increased evapotranspiration and dryer soil conditions (Fig. 3). Other studies also indicate that grassland productivity is highly dependent on soil moisture conditions (Cantarel et al., 2013). Warming experiments in perennial grasslands in Central France showed an increase in spring biomass following warming (Bloor et al., 2010). This suggests that rising temperatures positively affect above-ground plant growth, with the benefits outweighing potential adverse outcomes from increased evaporation and soil moisture fluctuations in the short term. However, over time, as plants cannot rapidly regenerate and replenish reserves, the biomass yield gradually declines due to climate change (Arft et al., 1999). Soil moisture appears to positively impact root systems, while environmental fluctuations might disrupt the balance between above and below-ground production, potentially leading plants to reallocate biomass among different organs to optimize growth (Bloom et al., 1985; Chapin et al., 1987; Hui and Jackson, 2006). In the final sampling period of our experiment, we noted that root density in HE soil was 1.6 times lower than in LE soil, consistent with previous findings (Rustad et al., 2001). Due to drier conditions at LE, plants enhanced their root growth, enabling the entire plants to absorb more water, ensuring stable primary production in the ecosystem under climate change conditions.

Compared to the unchanged biomass productivity over a five-year period, our study revealed that climate change significantly influenced plant N content. At the HE site, biomass N content was significantly higher (0.2 %) than at LE site, which is also suggesting higher fodder quality. Consequently, over a period of five years, the cumulative N export in grasslands at HE exceeded that at LE by 105 kg N ha^{-1}. With 69 kg N ha^{-1}, the majority of the reduced plant N export was related to the spring drought period at LE in 2018, which might be a combined consequence of plant physiological limitations and reduced N mineralization during this drought period. Additionally, HE generally experienced lower winter soil temperatures and more frequent freeze-thaw cycles during the course of our study (Fig. 2), which physically disrupts soil aggregates, roots, and microorganisms, thereby releasing soil nutrients for grassland plant utilization (Budge et al., 2011; Garcia-Pausas et al., 2017; Guan et al., 2018). Wang et al. (2016) confirmed increased gross N mineralization in winter under colder soil temperatures for the soil of our study, however in the monitored time span 2011–2012 colder temperatures were observed in the climate change treatment at LE due to missing snow cover, while a thick snowpack prevented soil freezing and freeze-thaw events at the HE site. In particularly dry years such as in 2018, the reduced soil moisture content at LE could have led to reduced N mineralization (Paul et al., 2003), which can reduce grassland N export (Wang et al., 2016; Giraud et al., 2021). However, using the same soil and lysimeters as this study in the years 2012–2014, Fu et al. (2017) observed increased N export by mowing in the climate change treatment, despite annual precipitation was similar in these years compared to years of this study (Fu et al., 2017). Given the particularly strong decline in SOC and N content in these soils in the climate change treatment (Garcia-Franco et al., 2024), a change to lower N export in the climate change treatment could also be a consequence of reduced SON stocks limiting N mineralization and thus, plant N supply. Furthermore, with the changing fertilizer ordinance in Germany, N fertilization rates of the lysimeters were reduced from 250 to 300 kg N ha^{-1} $year^{-1}$ to 170 kg N ha^{-1} $year^{-1}$ in 2016. This could also have contributed to both reduced plant N supply and SON stocks, thereby outbalancing the positive effect of warming and prolonged growing seasons on plant N acquisition.

5. Conclusion

The high importance of cattle slurry fertilizer N for plant N nutrition in the years following fertilizer application on the one hand increases the plant N use efficiency, which is over multiple years apparently larger than the very low ca. 10 % suggested by previous single year studies. On the other hand, these findings suggest a surprisingly short residence time of fertilizer-derived N in SON until re-mineralization and provision to plant N uptake of ca a decade only. Given the dominant role of plant N export for the negative total ecosystem N balances, the rapid re-provision of slurry-N to plant export can mechanistically explain the quick decline of SON observed in the studied soils after changing from traditional farmyard manure fertilization to liquid slurry fertilization. In order to protect SON and likely as well SOC – stocks, grassland management should ensure low fertilizer N losses as well as long-term stabilization of fertilizer N in SOM, and reduced plant N export. When liquid cattle slurry is used as organic fertilizer, surface-close application such as injection or acidification reduce N losses. Combined with reduced number of cuts, this can result in less negative N balances in intensively used pre-alpine grassland under the auspices of a changing climate.

CRediT authorship contribution statement

Jincheng Han: Writing – review & editing, Writing – original draft, Visualization, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marcus Schlingmann:** Methodology, Investigation, Conceptualization. **Martin Wiesmeier:** Writing – review & editing. **Ralf Kiese:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Rainer Gasche:** Software, Project administration. **Noelia Garcia-Franco:** Writing – review & editing. **Michael Dannenmann:** Writing – original draft, Supervision, Project administration, Funding acquisition, Data curation, Conceptualization. **Ulrike Ostler:** Methodology, Data curation. **Heinz Rennenberg:** Writing – original draft, Supervision, Conceptualization.

Declaration of Competing Interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2025.109653.

Data availability

Data will be made available on request.

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