Long-term changes of $\delta^{15}N$ natural abundance in 72 temperate grassland soils are small and indicate management rather than climate and biodiversity

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Abstract

The bulk soil $\delta^{15}N$ signature in grassland soils is fingerprinting nitrogen inputs, and losses, both shaped by land management, climate, and biodiversity. $\delta^{15}N$ has been explored as an indicator of these factors in multiple studies with mixed results regarding its primary drivers. This study quantified changes in soil $\delta^{15}N$ natural abundance for 72 German grassland sites after 12 years (2011 vs. 2023) and linked these changes to detailed records of land-use intensity, biodiversity, and climate. Land-use intensity was calculated from management activities including mowing, grazing and fertilisation. In the top 10 cm of the soil, $\delta^{15}N$ changes between 2011 and 2023 ranged from -0.86 to +2.49‰ (equating to an average increase of +0.35‰ per decade). Over two-thirds of our study sites showed a long-term increase in $\delta^{15}N$. These sites with rising $\delta^{15}N$ were associated with high land-use intensity, particularly high rates of fertilisation and grazing. Our findings indicate that management with organic fertiliser application and grazing are the dominant drivers of increases in $\delta^{15}N$. A biodiversity-induced decrease in $\delta^{15}N$ due to a tightening of the N cycle and reduction of N losses was not observed. The slow decadal-scale shifts show that single sampling of soil $\delta^{15}N$ without known starting values is only informative for long-term processes spanning > 50 years, and does not allow for insights regarding the impacts of more recent management practices. Thus, our findings emphasize the long-term character of bulk soil $\delta^{15}N$ as an indicator of management in temperate grasslands, particularly when repeated sampling is not possible.

Keywords: ^{15}N natural abundance, soil, grasslands, fertilisation, grazing, nitrogen cycling, stable isotopes

1. Introduction

Grasslands make up one-third of Germany's agricultural area and are primarily used for the production of biomass which supplies fodder for livestock and contributes to energy generation (Destatis, 2022; Rösch et al., 2009). In addition to agricultural and cultural benefits, grasslands provide a range of vital ecosystem services including long-term carbon (C) sequestration, nutrient cycling, water filtration and retention, erosion control, habitat provision and biodiversity conservation (Bengtsson et al., 2019). To protect these valuable ecosystems and the ecosystem services they provide, it is crucial to understand the interactions of land-use, plant biodiversity, and soil properties in shaping soil biogeochemistry, particularly the nitrogen (N) cycle.

In the past three decades, there have been major advances in using the N isotopic signature in agroecosystems as an indicator for the magnitude of N fluxes and for tracing individual pathways within the N cycle (Chalk et al., 2019). The isotopic signature refers to the concentration of the heavier ^{15}N compared to the lighter and much more common ^{14}N. The concentration of ^{15}N in the soil is determined by processes of N turnover and losses which fractionate, which means the lighter ^{14}N isotope is preferentially used leaving more of the heavier ^{15}N in the soil (Denk et al., 2017; Mudge et al., 2013; Stevenson et al., 2010; Wang et al., 2021). The N isotopic signature is usually given as the ratio between ^{15}N and ^{14}N expressed as $\delta\ ^{15}N$ given in ‰ (Watzka et al., 2006).

The soil $\delta\ ^{15}N$ signature is not only a result of turnover processes and losses (particularly gaseous losses) but also of the $\delta\ ^{15}N$ signature of the systems N inputs (Dawson et al., 2002; Gerschlauer et al., 2019). Biological nitrogen fixation (BNF) and mineral fertilisers have a $\delta^{15}N$ signature near 0‰ reflecting their origin from atmospheric N_2, whereas organic fertilisers such as manure or slurry show much higher signatures (Michener and Lajtha, 2008; Watzka et al., 2006). Fractionation processes in soil are relatively well known and the isotopic signature of different N inputs (such as BNF or various fertilisers) is too (or can be quantified). Therefore soil $\delta^{15}N$ has recently been tested for its potential to serve for the independent validation of complex process-oriented biogeochemical ecosystem models (Denk et al., 2019).

Since N input, turnover and losses are influenced by or interrelated with land management, climate and vascular plant biodiversity, the bulk soil $\delta^{15}N$ signature has also been investigated as an indicator of the history of those factors in multiple studies (Kleinebecker et al., 2014; Michener and Lajtha, 2008; Watzka et al., 2006). Grassland management mainly consists of mowing, grazing, and fertilisation all of which impact soil organic carbon (SOC), soil total nitrogen (TN) and the plant community composition (Gilmullina et al., 2020; Jia et al., 2022; Yang et al., 2023; Zhang et al., 2023). Multiple studies have shown that an increased N input e.g. through intensified management leads to higher gaseous N emissions in the form of nitrous oxide (N_20) and nitric oxide (N0), ammonia volatilization (NH_4+) and nitrate (NO_3-) leaching into the groundwater (Dittert et al., 1998; Estavillo et al., 1997). With losses fractionating against ^{15}N, their increase leads to an accumulation of ^{15}N in the soil. Studies using $\delta^{15}N$ as ab indicator agree on the processes enriching or depleting $\delta^{15}N$ in the bulk soil total N pool, although the results on the main driver determining the soil $\delta^{15}N$ signature vary widely. Some identified the influence of plant species richness on soil N turnover as the key driver of $\delta^{15}N$ values (Kleinebecker et al., 2014), while others found land management or abiotic factors such as climate to be more influential (Brenner et al., 2001; de Vries et al., 2012).

The diverging results regarding the drivers of the soil $\delta^{15}N$ signature in grasslands might originate from two key problems in retrospective analysis which lead to large uncertainties and unknowns, and thus also potentially to misinterpretation. Firstly, $\delta^{15}N$ has typically been measured only once and then used as an indicator of management, climate or biodiversity history of an undefined duration and with undefined initial soil $\delta^{15}N$ values. Secondly, there are large differences in the uncertainties of these potential drivers of $\delta^{15}N$ in such studies. Management history is mostly unknown or only known for restricted periods of the recent past, climate may be more reliably known for longer time scales, while biodiversity is usually measured at the time of sampling and may have been influenced by management at unknown timescales. These differences in uncertainty might bias the attribution of explanatory power to $\delta^{15}N$ drivers.

Therefore, the goal of this study was to quantify actual changes of grassland bulk soil $\delta^{15}N$ at decadal timescales based on repeated sampling in order to provide urgently needed knowledge on the potentials and constraints of $\delta^{15}N$ as an indicator. We quantified changes in soil $\delta^{15}N$ in 72 different sites under grassland management over 12 years (2011-2023) and linked these changes to precisely quantified management, biodiversity and climate history in this time span. With this approach, we wanted to (1) quantify the magnitude and direction of soil $\delta^{15}N$ changes in grassland sites, and (2) identify the main drivers in order to constrain the information that is contained in $\delta^{15}N$ changes, thereby supporting future studies using $\delta^{15}N$ as an indicator. We hypothesized that (1) $\delta^{15}N$ is generally higher at increasing land use intensity; (2) given the notion that $\delta^{15}N$ is an indicator of land management in the recent past, we expect $\delta^{15}N$ increases over time to be higher with higher land use intensity; (3) $\delta^{15}N$ changes are a mixed signal of increases due to management, and decreases at lower management and at higher biodiversity, which tightens the N cycle and thus reduces N losses.

2. Methods

2.1. Study design

We studied 72 grassland sites spread across three distinct regions in Germany the Schorfheide-Chorin (SCH) in the north-east (centroid latitude 53.0071, longitude 13.7695, 3-140 m above sea level (asl)), the Hainich-Dün (HAI) area in the centre (centr. lat. 51.158, long. 10.4762, 285-550 m asl) and the Schwäbische Alb (ALB) in the south-west (centr. lat. 48.4374, long. 9.38938, 460-850 m asl). The sites span a geographic gradient of approximately 550 km north to south and 300 km east to west. A decline in temperature and an increase in altitude and precipitation can be observed between areas from north to south. The areas belong to the Biodiversity Exploratories project infrastructure priority program of the German Science Foundation where biodiversity of different trophic groups, land-use parameters, and climate have been recorded for a total of 150 grassland sites since 2006 (Fischer et al., 2010; Hänsel et al., 2024; Hinderling and Keller, 2023; Ostrowski et al., 2020). In 2011, data on bulk soil TN concentrations and $\delta^{15}N$ natural abundance values for the top 10 cm of the soil were collected and stored in the Biodiversity Exploratories database (BExIS) covering all 72 sites under study (Klaus et al., 2012; Schöning and Trumbore, 2012). We then re-sampled these sites in 2023 following the same protocols. Sampling took place within the same 50 x 50 m sites of 2011.

The management records include detailed annual data on the land-use components of mowing, grazing, and fertilisation, including precise amounts of N fertiliser for every single management event during this time span (Ostrowski et al., 2020; Vogt et al., 2019).

From this data, an overall land-use intensity index (LUI) is calculated for each site and each year based on the parameters of mowing, grazing, and fertilisation. For each site p, the overall land-use intensity LUI_p is defined as:

$$LUI_p = \sqrt{\frac{G_p}{G_{BE}} + \frac{M_p}{M_{BE}} + \frac{F_p}{F_{BE}}}$$

Where G_p represents grazing intensity, measured by the density of livestock (livestock units per day of grazing $ha^{-1}\ yr^{-1}$), M_p is the frequency of mowing events per year, and F_p is the fertilisation level (kg N $ha^{-1}\ yr^{-1}$) on site p for a given year. The terms $G_{BE,}$ M_{BE} and F_{BE} are the respective means across all 150 grassland sites of the Biodiversity Exploratories for that year. Due to this standardization by ratios, the LUI is dimensionless (Blüthgen et al., 2012). Since soil samples in 2011 and 2023 were taken in early May the LUI for each site was calculated for each year from 2011 until 2022 and then averaged across the years (Ostrowski et al., 2020). For plant species richness (PSR) we also used the mean value of the annual records between 2011 and 2023 (Hinderling and Keller, 2023).

2.2. Fieldwork and laboratory analysis

To allow for a second time point of $\delta^{15}N$ bulk soil data 12 years after the sampling in 2011 (for methods see Kleinebecker et al., 2014), we sampled soil in 25 different grassland sites in each grassland region in early May 2023. SCH is the only area where 13 grassland sites were located on organic soils (peat soils) which were analysed separately due to a lack of comparability to mineral soils. Results from 3 mineral soil sites had to be disregarded for this analysis due to faulty measurements.

The sites were chosen to represent a full spectrum of the land-use types and intensities within each region. On each site, four soil samples down to a maximum of 100 cm depth were taken at the endpoints of a cross-shaped 22 m transect using a gasoline-powered percussion hammer (Cobra TT) equipped with a 45 mm diameter soil column cylinder (Eijkelkamp, Netherlands). The sample cores were separated into 10 cm depth increments. The samples from each depth on every site were homogenized and sieved with a 4 mm sieve to remove roots and rocks. The soil was dried at 60°C, ground with a pebble ball mill (Retsch MM400 Mixer Mill, Retsch, Haan, Germany) and analysed for total nitrogen (TN) and soil organic carbon (SOC) concentrations, as well as $\delta^{15}N$ using an isotope ratio mass spectrometer (Delta Plus, Finnigan MAT, Bremen, Germany) coupled to an elemental analyser (NA 1110; Carlo Erba, Milan, Italy), following standard procedures (Wittmer et al., 2010). The $\delta^{15}N$ concentration was calculated using the equation below (Mariotti, 1983).

$$\delta^{15}N\ ‰ = \left(\frac{R(^{15}N/^{14}N)_{sample}}{R(^{15}N/^{14}N)_{standard}} - 1 \right) x\ 1000$$

Each sample was measured against a working standard N_2 gas calibrated against secondary isotope standards (IAEA-N1, -N2 and –N3; accuracy of calibration <0.1 ‰). Each sample was measured only once. The precision of measurement runs was estimated via a solid laboratory standard (SLS; a finely ground wheat flour) run after every tenth samples to account for possible instrument drift. Long-term precision of SLSs over the measurement period was 0.17 ‰.

For this analysis, we focus on the top 10cm of soil as data from 2011 was collected only to this depth. Detailed data on soil properties were recorded for 2011 (see Baumann et al., 2022) but not for 2023. All data recorded in 2023 has also been made available in the BExIS database (Kepp and Schwärzler, 2025).

2.3. Data analysis

We applied linear and linear mixed effects models to analyse how the difference in $\delta^{15}N$ between 2011 and 2023 ($\Delta\delta^{15}N$) was influenced by LUI for fertilisation (LUI F), LUI grazing (LUI G), plant species richness (PSR), the difference in soil organic carbon (SOC) and TN concentrations and C to N ratio (ΔC%, ΔN% and ΔC/N) between 2011 and 2023, mean annual precipitation (MAP) and mean annual air temperature at 2 m above the ground (MAT) for each site within that timeframe.

LUI for mowing (LUI M) was not used in these models as we found a very strong correlation between LUI M and LUI F ($|r| > 0.73$). We decided to remove LUI M and keep LUI F in the models based on the higher impact of LUI F on the $\delta^{15}N$ signature through predominant organic fertiliser use and the fact that LUI M also had a relatively strong correlation with LUI G ($|r| > 0.51$). The correlation between LUI F and LUI M is explained by linked fertilisation-mowing cycles particularly in intensively managed grassland. A strong correlation was also found between MAP and MAT ($|r| > 0.64$). We decided to drop MAT from the model as MAP had a higher variance (measured via the coefficient of variation (cv) = 0.308 for MAP, cv = 0.076 for MAT) among all sites and might therefore offer more insight than MAT.

First, a linear mixed-effects model was used, with the study region included as a random effect to account for spatial clustering. However, when comparing a model with and without the random effect the corrected Akaike information criterion (AICc) revealed that the random effect decreased the model fit (AICc = 127.9 with the random effect vs. 99.5 without). This suggests that there is minimal variability attributable to differences between study areas in this analysis and the random effect was therefore dropped.

Measures of ΔC% and ΔN% also showed a very strong correlation ($|r| > 0.67$) and a Variance Inflation Factor (VIF) with values above the commonly used threshold of 5 (for ΔC% VIF = 9.7, for ΔN% VIF = 5.5)

indicating high multicollinearity. Consequently, ΔC% having the highest VIF value was removed from the model. However, because the roles N and C play in the N cycle are quite different and we were interested in both we went through the rest of the model selection process twice, once with ΔN% and once with ΔC%. This confirmed the similarity in their effects but showed no major impact on any other variables and their effects. The ΔC/N was always near 0 which is attributable to the synchronous changes in N% and C% over this time period. Therefore, ΔC/N would only offer minimal explanatory power and was subsequently removed from the model. The AICc was used to compare the performances of both final models (one with ΔN% and one with ΔC% instead) and revealed that the model with ΔN% had higher explanatory power(AICc 94.6 for ΔN%, AICc 99.7 for ΔC%). We tested all pairwise interactions between the remaining predictors and ranked them according to the difference in the AICc. Out of all models with an δAICc < 2 we chose the most parsimonious model to avoid overfitting and ensure interpretability of the model. The final model selected was the following linear model: $\Delta\delta^{15}N$ ~ LUI F + LUI G + ΔN% + MAP + PSR + LUI G *MAP + LUI G * PSR. Model diagnostics confirmed the validity of the assumptions, with residuals approximately normally distributed and homoscedastic. Interactions between variables were analysed and visualized using the Johnson-Neyman technique (Johnson and Neyman, 1936).

We also divided our data into two groups one with sites that showed an overall increase in $\delta^{15}N$ between 2011 and 2023 and sites that showed an overall decrease. The differences in those groups with regards to their average LUI, LUI F, LUI M, and LUI G, PSR between 2011 and 2023 as well as C %, N% and C/N in 2011 and 2023, and ΔC%, ΔN% and ΔC/N were analysed.

Additionally, we grouped all sites into three management categories, one where land-use is dominated by fertilisation (LUI F > LUI G), one where it is dominated by grazing (LUI G > LUI F) and one where land-use is mainly extensive (LUI G and LUI F both < 1) and determined the average $\delta^{15}N$ value for each group for each exploratory for 2011 and 2023. SCH was the only region with no fertilisation-dominated management.

All statistical analyses were performed in RStudio (R Version 4.4.2) using the packages dplyr, MuMIn, lme4, ggplot2, car and effects. Multicollinearity diagnostics were conducted using the car package, while model selection relied on the MuMIn package. Graphs were generated with the packages ggplot2 and interactions.

3. Results

Out of our 72 sites 59 were located on mineral soils and 13 on organic soils. For the 59 mineral soil sites soil $\delta^{15}N$ in 2011 ranged between 0.92 ‰ and 6.89 ‰ and between 0.93 ‰ and 6.80 ‰ in 2023. The $\Delta\delta^{15}N$ changes from 2011 to 2023 ranged between -0.86 and +2.49 ‰ $\delta^{15}N$ with an average of +0.42 ‰ $\delta^{15}N$ (SE = 0.09). Based on this result the average change of $\Delta\delta^{15}N$ per decade in mineral soils was +0.35 ‰ $\delta^{15}N$. For the organic soil sites, $\delta^{15}N$ in 2011 ranged between 2.62 ‰ and 4.83 ‰ and between 3.37 ‰ and 5.54 ‰ in 2023. The change of $\Delta\delta^{15}N$ ranged between -0.10 and +1.02 ‰ $\delta^{15}N$ with an average of +0.65 ‰ $\delta^{15}N$ (SE = 0.09).

For the mineral soil sites, the differences in $\delta^{15}N$ changes from 2011 to 2023 were significant and positive in all mainly grazed and mainly fertilized sites (Figure 1). For extensive sites both an increase and a decrease in $\delta^{15}N$ from 2011 to 2023 were observed but differences were not significant in any of the study regions (Figure 1). The organic soil sites were all located in the SCH study area and showed a significant difference between bulk soil $\delta^{15}N$ in 2011 and in 2023 under both extensive and grazing dominated management (Figure 2, Table 2). For both mineral and organic soil sites average annual fertilization rates in kg N per hectare (kg N ha^{-1}) and grazing intensities in livestock units (LU) and days per hectare (LU*d ha^{-1}) between 2011 and 2023 for each management category are given in Table 1.

*Table 1: Range and mean values of fertilisation rates (in kg N ha-1 yr-1) and grazing (in livestock unit days (LU*d) ha-1 yr-1) between 2011 and 2023 for all 59 mineral soil sites and 13 organic soil sites grouped by management category.*

Management	Fertilisation range (kg N ha^{-1} yr^{-1})	Fertilisation mean (kg N ha^{-1} yr^{-1})	Grazing range (LU*d ha^{-1} yr^{-1})	Grazing mean (LU*d ha^{-1} yr^{-1})
		Mineral soils		
Extensive	0.00 - 19.27	5.42	14.02 - 125.87	51.01
Mainly grazed	0.00 - 30.73	12.22	164.23 - 809.04	387.13
Mainly fertilised	26.13 - 183.67	94.13	6.37 - 144.15	42.45
		Organic soils		
Extensive	0.00 - 100.00	7.97	0.00 - 224.55	33.29
Mainly grazed	0.00 - 100.00	5.13	0.00 - 747.82	324.30

Across all three regions, extensively managed mineral soil sites showed the lowest $\delta^{15}N$ signature and smallest difference in $\delta^{15}N$ between 2011 and 2023 (Table 2). In the SCH region, only 3 sites were extensively managed and their average $\delta^{15}N$ signature decreased over time. For grazing-dominated management the $\delta^{15}N$ signature is higher than for extensive management and so is the $\Delta\delta^{15}N$. For the ALB and HAI regions, the most enriched $\delta^{15}N$ values are found in fertilisation-dominated management (4.34 ‰ in 2011 and 5.04 ‰ 2023). These sites also show the largest changes in $\delta^{15}N$.

The same grouping was applied to the 13 remaining organic soil sites which were all located in the SCH region and therefore did not include fertilization dominated sites. Similar to the SCH values on mineral soils the average $\delta^{15}N$ signature in organic soils in 2011 was 3.49 ‰ (SE ±0.22) for the 8 extensively managed sites and 4.05 ‰ (SE ±0.47) for the 5 grazed sites. In 2023, extensively managed sites had a mean $\delta^{15}N$ signature 4.13 ‰ (SE ±0.24) which is higher than that observed in the mineral soil sites. The grazed sites on the other hand had a $\delta^{15}N$ signature of 4.72 ‰ (SE ±0.47) which is lower than that of the mineral soil sites.

Table 2: Mean values of $\delta^{15}N$ in 2011 and 2023 and their difference with the standard error (SE) for 59 mineral soil sites and 13 organic soil sites grouped by management categories and study areas. Sample size of each group is also given.

Region	Management	Mean $\delta^{15}N$‰ 2011 (SE)	Mean $\delta^{15}N$‰ 2023 (SE)	Mean $\Delta\delta^{15}N$‰ (SE)	Sample size
			Mineral soils		
ALB	Extensive	3.11 (± 0.27)	3.17 (± 0.28)	0.06 (± 0.08)	12
ALB	Mainly grazed	3.39 (± 0.67)	3.48 (± 0.70)	0.09 (± 0.06)	5
ALB	Mainly fertilised	4.34 (± 0.51)	5.00 (± 0.42)	0.63 (±0.14)	7
HAI	Extensive	2.80 (± 0.37)	3.27 (± 0.40)	0.48 (± 0.20)	6
HAI	Mainly grazed	3.50 (± 0.65)	3.76 (± 0.87)	0.26 (± 0.34)	4
HAI	Mainly fertilised	3.65 (± 0.18)	4.25 (± 0.26)	0.60 (± 0.20)	15
SCH	Extensive	3.63 (± 0.82)	3.42 (± 0.76)	-0.21 (± 0.20)	3
SCH	Mainly grazed	4.06 (± 0.29)	5.04 (± 0.57)	0.98 (± 0.42)	7
			Organic soils		
SCH	Extensive	3.49 (± 0.22)	4.13 (± 0.24)	0.63 (± 0.08)	8
SCH	Mainly grazed	4.06 (± 0.47)	4.72 (± 0.37)	0.67 (± 0.20)	5

In 40 of the 59 mineral soil sites, we found an overall increase of $\delta^{15}N$ from 2011 to 2023 and in 19 sites we found a decrease (Table 3). For organic soils only 1 out of 13 sites showed a decrease in $\delta^{15}N$. In combination with the inherent differences between organic and mineral soils and the fact that all our organic soil sites are located in the SCH region, this led us to exclude organic soil sites from further analysis.

Table 3: Mean values and their standard error (SE) of variables for all mineral soil sites and for those sites grouped by increase or decrease in $\delta^{15}N$ from 2011 to 2023 and the p value of the difference between these groups.

Variable	***Mean all sites (SE)***	***Mean group increase $\delta^{15}N$ (SE)***	***Mean group decrease $\delta^{15}N$ (SE)***	***P value difference between groups***
Sample size	*59*	*40*	*19*	
$\Delta\delta^{15}N$‰	*0.42 (± 0.09)*	*0.72 (± 0.10)*	*-0.21 (± 0.05)*	*>0.0001*
$\delta^{15}N$‰ 2011	*3.55 (± 0.14)*	*3.51 (± 0.15)*	*3.67 (± 0.30)*	*0.722*
$\delta^{15}N$‰ 2023	*4.01(± 0.17)*	*4.19 (± 0.20)*	*3.44 (±0.31)*	***0.037****
PSR[1]	*33.42 (± 1.41)*	*32.19 (± 1.63)*	*36.01 (± 2.7)*	*0.205*
LUI	*1.71 (± 0.08)*	*1.87 (± 0.10)*	*1.36 (± 0.10)*	***0.00069******
LUI F	*1.24 (± 0.23)*	*1.66 (± 0.31)*	*0.36 (± 0.12)*	***0.0068****
LUI M	*0.98 (± 0.11)*	*1.1 (± 0.13)*	*0.73 (± 0.17)*	*0.117*
LUI G	*1.09 (± 0.19)*	*1.16 (± 0.26)*	*0.94 (± 0.25)*	*0.5989*
C% 2011	*5.21 (± 0.29)*	*5.56 (± 0.34)*	*4.48 (± 0.52)*	*0.0909*
C% 2023	*4.91 (± 0.21)*	*5.08 (± 0.24)*	*4.56 (± 0.43)*	*0.295*
Δ C%	*-0.29 (± 0.12)*	*-0.47 (± 0.15)*	*0.08 (± 0.15)*	***0.0109****
N% 2011	*0.5 (± 0.03)*	*0.53 (± 0.03)*	*0.42 (± 0.05)*	*0.0694*
N% 2023	*0.5 (± 0.03)*	*0.51 (± 0.03)*	*0.45 (± 0.05)*	*0.311*
Δ N%	*0.00 (± 0.01)*	*-0.02 (± 0.01)*	*0.03 (± 0.01)*	***0.00129****
C/N 2011	*10.35 (± 0.09)*	*10.27 (± 0.09)*	*10.54 (± 0.19)*	*0.1396*
C/N 2023	*10.19 (± 0.14)*	*10.08 (± 0.18)*	*10.42 (± 0.19)*	*0.267*
Δ C/N	*-0.17 (± 0.12)*	*-0.19 (± 0.15)*	*-0.12 (± 0.19)*	*0.769*

**significant, **highly significant, ***very highly significant*

[1]plant species richness

Compared to those with a decrease in $\delta^{15}N$ the sites with an increase showed higher LUI (p= < 0.001), particularly with regards to fertilization (LUI F) (p= 0.0068) (Figure 3, Table 3). In contrast, the difference in PSR between groups with increasing and decreasing $\delta^{15}N$ was not statistically significant (p= 0.205) though the mean PSR of sites with a $\delta^{15}N$ increase was lower than that of those with a decrease (Table 3).

Soils with increasing $\delta^{15}N$ had slightly higher SOC and TN concentrations compared to those with decreasing $\delta^{15}N$ (Table 3). However, soils with increasing $\delta^{15}N$ showed decreasing SOC and TN concentrations while decreasing soil $\delta^{15}N$ was related to unchanged or slightly increasing SOC and TN concentrations over time (Figure 4, Table 3). This indicates more of an increase in C% and N% in the

soil in those sites with a decrease in soil $\delta^{15}N$ over time. The observation that ΔC% and ΔN% show the same trend is also reflected in the similarity in the ΔC/N of both the $\delta^{15}N$ increase and decrease groups.

3.1. Linear model

Model fitting revealed that LUI F had a significant positive effect on $\Delta\delta^{15}N$ (Estimate = 0.132, SE = 0.045, t = 2.896, p = 0.00555). The opposite could be observed for ΔN% (Estimate = -4.470, SE = 1.116, t = -4.005, p = 0.000202) (Figure 5). Furthermore, LUI G showed a significant effect on $\Delta\delta^{15}N$ (Estimate = 1.725, SE = 0.315, t = 5.486, p < 0.0001). While MAP and PSR did not have a direct significant effect on $\Delta\delta^{15}N$, they were found to interact with LUI G. Specifically, at lower values of MAP, LUI G has a more positive effect on $\Delta\delta^{15}N$, but at higher MAP values, this effect decreases (Estimate = -0.00066, SE = 0.00025, t = -2.591, p = 0.0124). The Johnson-Neyman analysis revealed that this effect is significant for all observed values of MAP, and within the observed MAP range, the direction of LUI G's effect on $\Delta\delta^{15}N$ does not change (Figure 6).

A similar interaction was found between PSR and LUI G (Estimate = -0.036, SE = 0.008, t = -4.565, p < 0.0001). For PSR below 38, we found a positive relationship between LUI G and $\Delta\delta^{15}N$ which increased with decreasing PSR. Conversely, at PSR above 63, the model found a negative effect of LUI G on $\Delta\delta^{15}N$. However, this applies only to one site and is therefore not considered a robust finding. For PSR values between 38 and 63, the interaction was not deemed significant according to the Johnson-Neyman interval (Figure 7).

Our selected model ($\Delta\delta^{15}N$ ~ LUI F + LUI G + ΔN% + MAP + PSR + LUI G *MAP + LUI G * PSR) explained 55.2% of the variation in $\Delta\delta^{15}N$ (multiple R^2 = 0.552, adjusted R^2 = 0.490). Plotting the predicted $\Delta\delta^{15}N$ values against the actual measured data confirmed the good fit of the model and revealed no apparent differences in model fit between the three sampling regions (Figure S1). Overall, these findings highlight the significant role of grazing and fertilisation in shaping nitrogen isotope dynamics over time.

4. Discussion

4.1. Speed and direction of $\delta^{15}N$ changes over time

A main result of our long-term and large-scale study of temperate grassland soils was that, while both increase and decrease of $\delta^{15}N$ were observed, there was an average increase of +0.42 ‰ from 2011 to 2023. For extensively managed sites the observed 12-year changes were very small, hardly changing the initial $\delta^{15}N$ values in 2011. Intensively grazed sites as they are found in SCH and intensively fertilized sites in HAI and ALB show higher $\delta^{15}N$ starting values but also much larger increases in $\delta^{15}N$. This shows that N inputs decreasing $\delta^{15}N$ (such as BNF) are dominated by N inputs and processes increasing $\delta^{15}N$, particularly in grazed and fertilised sites. Other studies examining the impact of land-use on $\delta^{15}N$ have yielded widely varying results with regard to direction, speed and magnitude of changes in $\delta^{15}N$ (Chalk et al., 2019; Marriott et al., 1997; Mudge et al., 2013; Sieve et al., 2021; Stevenson et al., 2010; Watzka et al., 2006). This variation highlights the complexity of N inputs and turnover effects on $\delta^{15}N$ in different ecosystems and the necessity of long-term studies at a multitude of sites to capture meaningful trends.

Contrasting our results, Marriott et al. (1997) demonstrated a decrease from 6.1 ‰ to 5.1 ‰ $\delta^{15}N$ over one year under grazing and mineral fertilization and no such change in their ungrazed and unfertilized controls. However, fertilisation and grazing had only stopped on those unmanaged sites 3 years prior to their first sampling. The use of mineral fertilizers and a potentially altered plant species composition favouring inputs via biological nitrogen fixation may explain these rather unexpected changes.

Our findings reveal that, particularly for extensively managed and grazed sites (apart from SCH region, where grazing is more intensive), long term observations are required to capture slow long-term trends. Here, changes in $\delta^{15}N$ were so small that they fell well within the range of variability of $\delta^{15}N$ among similarly managed sites in 2011. For a meaningful analysis of natural abundance $\delta^{15}N$ in such sites long-term treatment and ideally also historic $\delta^{15}N$ values of the same sites for comparison are needed.

The overall magnitude of changes in $\delta^{15}N$ we found is in line with Mudge et al (2013) who compared grazed pasture soils over a 50-year period and observed changes of about 1 ‰ in a fertilisation trial and 1.4 ‰ over 45 years in an irrigation trial. Interestingly, in the irrigation trial, $\delta^{15}N$ remained stable for the first 10-15 years before beginning to diverge, illustrating the slow nature of nitrogen isotopic changes, particularly in response to irrigation compared to fertilisation.

Watzka et al. (2006) studied permanent grasslands aged 8-50 years under different fertiliser and cutting regimes. They only sampled once but management regimes had been the same since the grasslands were established. The $\delta^{15}N$ of both vegetation and soils closely mirrored management differences, with higher values observed under increased fertilisation.

In line with this, Sieve et al. (2021) did find short-term trends of $\delta^{15}N$ changes when examining three years of data following a management change. They observed significant differences between treatments differing in fertilisation. However, within each treatment, changes over the short period were minimal, emphasizing the need for long-term studies to discern broader and more fine-scale trends.

A somewhat faster increase than what we observed of 2.1 ‰ in the top 10 cm of soil over two decades was found by (Jones and Dalal, 2017) when investigating soil nitrogen dynamics after the transformation of an area of native forest to croplands. Neither the forest nor the later cropland was ever fertilised so the increase in $\delta^{15}N$ which went along with a decrease in total N reflects the removal of N (particularly the preferentially plant assimilated ^{14}N) through cropping. This goes to show that more drastic changes in land-use also reflect faster changes in the natural abundance of soil $\delta^{15}N$. Even more drastic land-use changes were investigated by (Stevenson et al., 2010) who found that $\delta^{15}N$ values increased with land-use intensity in a single-time sampling: from +2.1‰ under native forest, to +2.8‰ under plantation forestry, +3.8‰ in pasture under drystock, +5.4‰ in pasture under dairy management, and +6.2‰ under intensive cropping. Similarly, Qiu et al., (2015) observed an increase of approximately 1.5‰ in $\delta^{15}N$ over 27 years following grassland conversion to cropland or shrubland. Longer-term studies, such as that of Liao et al. (2006) assessing 150 years of grassland invasion by woody plants, found no significant differences in $\delta^{15}N$ between age groups, only between grassland and woodland. In line with our findings, this suggests that non-management-related changes are detectable primarily over very long periods or under drastic shifts in vegetation structure. Whereas, changes related to management, particularly fertilisation and intensive grazing are faster.

These findings underscore the value of long-term data collection, particularly with comparative historical records. The slow nature of $\delta^{15}N$ changes in extensively managed systems necessitates multi-decade studies to discern patterns beyond the variability observed among treatments. In our study, for such sites, the 12-year shifts in $\delta^{15}N$ were much smaller than the initial variation observed in 2011. This highlights that single-time bulk soil $\delta^{15}N$ sampling in extensive systems reflects cumulative drivers over several decades. With regards to management $\delta^{15}N$ changes are faster and can capture more recent changes in management. Nonetheless, if the areas studied are not exclusively intensively managed, observations over multiple years or ideally decades and comparative data are necessary to use $\delta^{15}N$ as a robust indicator of management or climate-driven nitrogen dynamics.

4.2. What is driving $\delta^{15}N$ changes in temperate grassland?

Our comprehensive study based on well-reported quantitative management and climate data over 12 years found that management effects are the main drivers of the $\delta^{15}N$ signature of grassland top soils, which confirms some earlier studies conducted at different scales (Apostolakis et al., 2022; Sieve et al., 2021; Stevenson et al., 2010). In contrast, in other studies based on single sampling also other parameters such as PSR effects on N retention and losses seemed to dominate over management (Kleinebecker et al., 2014). However, it has also become evident that the separate aspects of management namely mowing, fertilisation, and grazing affect this signature to different extents.

4.2.1. Organic fertilisation

We found that fertilisation has a strong positive effect on $\delta^{15}N$ which is in line with the findings of other studies (Mudge et al., 2013; Schimel and Bennett, 2004; Watzka et al., 2006). Overall fertilisation on

our sites ranged between 0 and 180 kg N ha^{-1} yr^{-1}. Mainly organic fertiliser was used only occasionally supplemented by mineral fertiliser.

Organic fertiliser consists largely of animal excreta which generally show a $\delta^{15}N$ signature between 19 and 20 ‰ (Bedard-Haughn et al., 2003). This proportionately larger addition of ^{15}N compared to ^{14}N necessarily impacts the $\delta^{15}N$ signature of the system due to the significant amounts of N added annually through fertilisation. Additionally, organic fertilisers are particularly prone to N losses via volatilization which further enriches ^{15}N in the portion of the fertiliser entering the soil system (Bedard-Haughn et al., 2003; Bouwman et al., 2002). Given that, with a maximum of 6.89 ‰, the $\delta^{15}N$ signatures we found in our bulk soil samples were well below the average values of animal excreta given above it is reasonable to assume that adding organic fertiliser would largely increase the soil $\delta^{15}N$ signature. This also connects back to the larger changes in $\delta^{15}N$ we found in sites with higher LUI and LUI F (avg. + 0.72 ‰) where the main N source is organic fertiliser and the much smaller changes we found in less managed sites (avg. -0.21 ‰) where the main external N sources would be BNF and atmospheric N deposition.

Mineral fertiliser on the other hand is atmospherically derived and therefore has a $\delta^{15}N$ signature of ca. 0 ‰ (Sieve et al., 2021). Still organic and mineral fertilisers both indirectly also lead to an enrichment in soil ^{15}N by providing more readily available N (Klaus et al., 2013; Watzka et al., 2006). This increased N availability leads to a higher N turnover rate with increased losses which fractionate and thereby enrich the $\delta^{15}N$ signature in the soil (Booth et al., 2005; Elrys et al., 2021a, 2021b; Wang et al., 2016). In their global analysis Elrys et al. (2021a, 2021b) found that increased N availability leads to an increase in microbial biomass (MB) and thereby also an increase in nitrogen turnover processes and losses. Additionally, with fertilisation induced higher N availability, plants also fractionate more thereby contributing to an enrichment of soil ^{15}N (Mudge et al., 2013).

The accumulation of ^{15}N in soils with higher fertilisation rates is linked to the openness of their N cycle due to increased turnover losses and losses during fertiliser application (Bouwman et al., 2002; Craine et al., 2015; Stevenson et al., 2010). The observation that increasing $\delta^{15}N$ was related to decreasing TN confirms recent observations in pre-alpine grassland that intense management with organic fertilisers promotes N mining compared to extensive management (Schlingmann et al., 2020; Zistl-Schlingmann et al., 2020).

We also observed that sites with an increase in $\delta^{15}N$ exhibited more of a decline in SOC concentrations. This can also at least partially be attributed to the higher N availability through fertilisation as well as carbon and phosphorus availability-induced priming effects, which stimulate microbial activity leading to increased decomposition of soil organic matter (SOM) and increased soil respiration (Apostolakis et al., 2022; Elrys et al., 2021a; Kuzyakov et al., 2000). This can lead to a net loss of SOC, especially when nitrogen is added in high amounts (Jesmin et al., 2021; Khan et al., 2007). Another effect which comes into play here is that high N availability can lead to an increase in N allocation to above-ground biomass and simultaneously a decrease in N allocation to below-ground biomass (Li et al., 2019; Menge et al., 2024). This shift can reduce root-derived C inputs to the soil, leading to lower overall SOC stocks (McGranahan et al., 2014).

4.2.2. Mowing

We did not find a direct effect of mowing on the soil $\delta^{15}N$ signature which is in line with other reports (Watzka et al., 2006). However, in our study mowing does strongly correlate with fertilisation and all sites which are intensively fertilised are also intensively mown. This can be attributed to farmers fertilising more to increase yield via increased biomass growth and more frequent mowing. Therefore, the fertilisation effect observed statistically cannot be considered entirely independent of mowing.

Like fertilisation mowing also has an effect on soil N and C concentrations. Firstly, it contributes to the above-mentioned SOM mining by removing N via biomass harvest. Secondly, mowing also removes C from the system by removing biomass and litter, and reducing the input of C from root exudates (Li et al., 2022; Qiu et al., 2015). Still, our data indicate that it is mainly fertilisation which is triggering the extent of $\delta^{15}N$ changes.

4.2.3. Grazing

We found that grazing led to an increase in soil $\delta^{15}N$ over time. This effect can be seen as separate from the effects of mowing and fertilisation in our data, as areas are either mostly grazed or mostly fertilised and mown, though a mix of some grazing and some mowing and fertilisation certainly exists. The low to moderate grazing and the resulting movement of animals on grasslands (referred to as trampling) are known to aid the incorporation of organic matter such as plant litter into the soil, to stimulate plant and root growth, and increase microbial biomass and plant species richness (Gilmullina et al., 2020; Leriche et al., 2001; Robson et al., 2007; Rumpel et al., 2015). All of this induces microbial N turnover which again fractionates N and leaves more ^{15}N in the soil (Booth et al., 2006; Denk et al., 2017; Elrys et al., 2021b).

When grassland is used for grazing, animals also consume aboveground biomass and thereby remove N from the system. However, N is also returned to the system via manure which has a ^{15}N signature which exceeds that of the biomass consumed by about 2-5 ‰ due to fractionation during metabolic processes (Bedard-Haughn et al., 2003; Sieve et al., 2021; Steele and Daniel, 1978). Urine is usually ^{15}N depleted compared to diet, however, as with organic fertiliser its addition leads to an increase in soil ^{15}N (Bedard-Haughn et al., 2003; Steele and Daniel, 1978). Furthermore, urine and manure patches are particularly susceptible to ^{15}N fractionation processes (particularly ammonia volatilization) enriching the portion of N which goes into the soil organic nitrogen pool even further (Chalk et al., 2019; Frank et al., 2004).

Additionally, we found that with higher MAP the positive effect of grazing on the soil $\delta^{15}N$ signature lessened. This possibly indicates that precipitation buffers the effect of grazing through faster incorporation of manure and urine into the wet soil matrix via improved soil infiltration (Simelane et al., 2024). This faster incorporation leads to less exposure of manure and urine patches to air and direct sunlight which decreases losses via ammonia volatilization and thereby decreases ^{15}N enrichment. We also investigated whether higher MAP induced higher plant growth and thereby plant N uptake, but our data did not show any correlation between MAP and yield.

Multiple studies have found MAP to correlate positively with MB and gross nitrogen mineralisation which is a driver of N losses (Elrys et al., 2021b; Liu et al., 2009). In our comprehensive dataset, the mitigation of ammonia volatilization via MAP seems to compensate for this effect. Leaching losses of N are also reported to be higher with higher precipitation, however, leaching is the N loss pathway which fractionates least (Chen et al., 2019; Zheng et al., 2020).

Kleinebecker et al. (2014) identified PSR as a major driver of soil $\delta^{15}N$ based on a single sampling of bulk soil $\delta^{15}N$ in the same German temperate grassland regions we investigated. We - like Klaus et al. (2018) who also investigated the same regions - did not find a direct effect of PSR on soil $\delta^{15}N$. This discrepancy likely stems from the differences in available data on management, PSR and past soil $\delta^{15}N$. Whereas (Kleinebecker et al., 2014) had only 4 years of management data, one single sampling of PSR and no prior $\delta^{15}N$ data, we had reference data from 2011 (for $\delta^{15}N$) and PSR and management data for the 12 years between 2011 and 2023. In fact, most studies investigating soil properties in relation to past management lack long-term, high-quality and high-resolution data on past land management whereas PSR data is much more easily quantified and obtained. Additionally, PSR also changes at much slower rates than management which can differ substantially from one year to the next. This variability in data availability and precision could easily lead to statistical models selecting PSR over management. The validity of the dominating effect of PSR on soil $\delta^{15}N$ observed by Kleinebecker et al. (2014) is also limited by the fact that high PSR is a consequence of little to no fertilisation. A study comparing only unfertilised sites with varying PSR would be needed to shed more light on the effect PSR alone has on soil $\delta^{15}N$. In our study, both management and PSR are documented equally well for the same time span and we were able to demonstrate that the management effects far outweigh the effects of PSR on soil $\delta^{15}N$ changes over this 12-year study period.

Nonetheless, we found an interaction of PSR with the effect grazing has on soil $\delta^{15}N$. At low PSR (< 38.34 which applies to 45 of 59 sites) the positive effect of grazing on soil $\delta^{15}N$ increased with decreasing PSR, whereas for sites with higher PSR, no interaction between PSR and the grazing effect was found. At higher PSR grazing intensity is mostly relatively low and its positive effect on soil $\delta^{15}N$ might be buffered through the decreased susceptibility of extensively managed species-rich grasslands to N losses (de Vries et al., 2012). Furthermore, PSR has been observed to decrease $\delta^{15}N$ as interspecific

N competition by plants decreases fractionation (Mudge et al., 2013). Typically, grasslands have high PSR because they are managed extensively and are not fertilised (Allan et al., 2015; Midolo et al., 2019). This also means that BNF with a $\delta^{15}N$ signature near 0 ‰ contributes a larger portion to the total N input in species-rich unfertilised grasslands than in fertilised and less species-rich grasslands (Craine et al., 2015; Qiu et al., 2015). Additionally, with a lack of fertilisation, we also tend to have a more closed N cycle with fewer losses and therefore less ^{15}N enrichment (Craine et al., 2015; Wang et al., 2021). Overall, our results confirmed our hypotheses and showed, that, across Germany, management activities (particularly grazing and fertilisation) increase bulk soil $\delta^{15}N$ signature in grassland topsoil and this effect interacts with but outweighs those of climate and PSR.

5. Conclusion

Our 12-year study on measured $\delta^{15}N$ changes revealed that fertilisation and grazing were the primary drivers of isotopic shifts, while biodiversity and climate had no significant impact. Biodiversity-induced changes occurred much more slowly than those driven by management. The gradual shifts in soil $\delta^{15}N$ observed at extensively managed sites indicate that this isotopic marker is only suitable for detecting changes over much longer time spans than commonly assumed. In contrast, at more intensively managed sites, $\delta^{15}N$ can serve as a management indicator over shorter periods, ranging from a few years to several decades. However, without an initial $\delta^{15}N$ baseline or detailed management history, such analyses remain speculative.

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Data availability

This work is based on data collected by the BE_BioMON project of the Biodiversity Exploratories program (DFG Priority Program 1374). The datasets with the following IDs are either publicly available or available on request in the Biodiversity Exploratories Information System (http://doi.org/10.17616/R32P9Q): 331973 (Hinderling and Keller, 2023), 19007 (Hänsel et al., 2024), 14446 (Schöning and Trumbore, 2012), 15306 (Klaus et al., 2012), 17086 (Schöning, 2018), 32058 (Kepp and Schwärzler, 2025). The datasets are listed in the references section.

Glossary

C – Carbon
LUI – Land-use Intensity Index
MB – Microbial biomass
N – Nitrogen

MAP – Mean annual precipitation
MAT – Mean annual temparature
PSR – Plant species richness
SOC – Soil organic carbon
SOM – Soil organic matter
TN – Total nitrogen

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Figure Captions

Figure 1: Average $\delta^{15}N$ values for the 59 mineral soil sites grouped by study region and management category. The category mainly grazed includes all sites with LUI G > 1 and LUI F. Mainly fertilised includes all sites with LUI F > 1 and LUI G. Extensive is comprised of the remaining sites where both LUI G and LUI F are < 1. For the SCH region, none of the sites were fertilisation dominated.

Figure 2: Average $\delta^{15}N$ values for the 13 organic soil sites grouped by study region and management category. The category mainly grazed includes all sites with LUI G > 1 and LUI F. Mainly fertilised includes all sites with LUI F > 1 and LUI G. Extensive is comprised of the remaining sites where both LUI G and LUI F are < 1. For the SCH region, none of the sites were fertilisation dominated.

Figure 3: All mineral soil sites grouped into either an increase or a decrease in $\delta^{15}N$ from 2011 to 2023 plotted against the average land-use intensity index (LUI), and the LUI for fertilisation (LUI F) from 2011 to 2023.

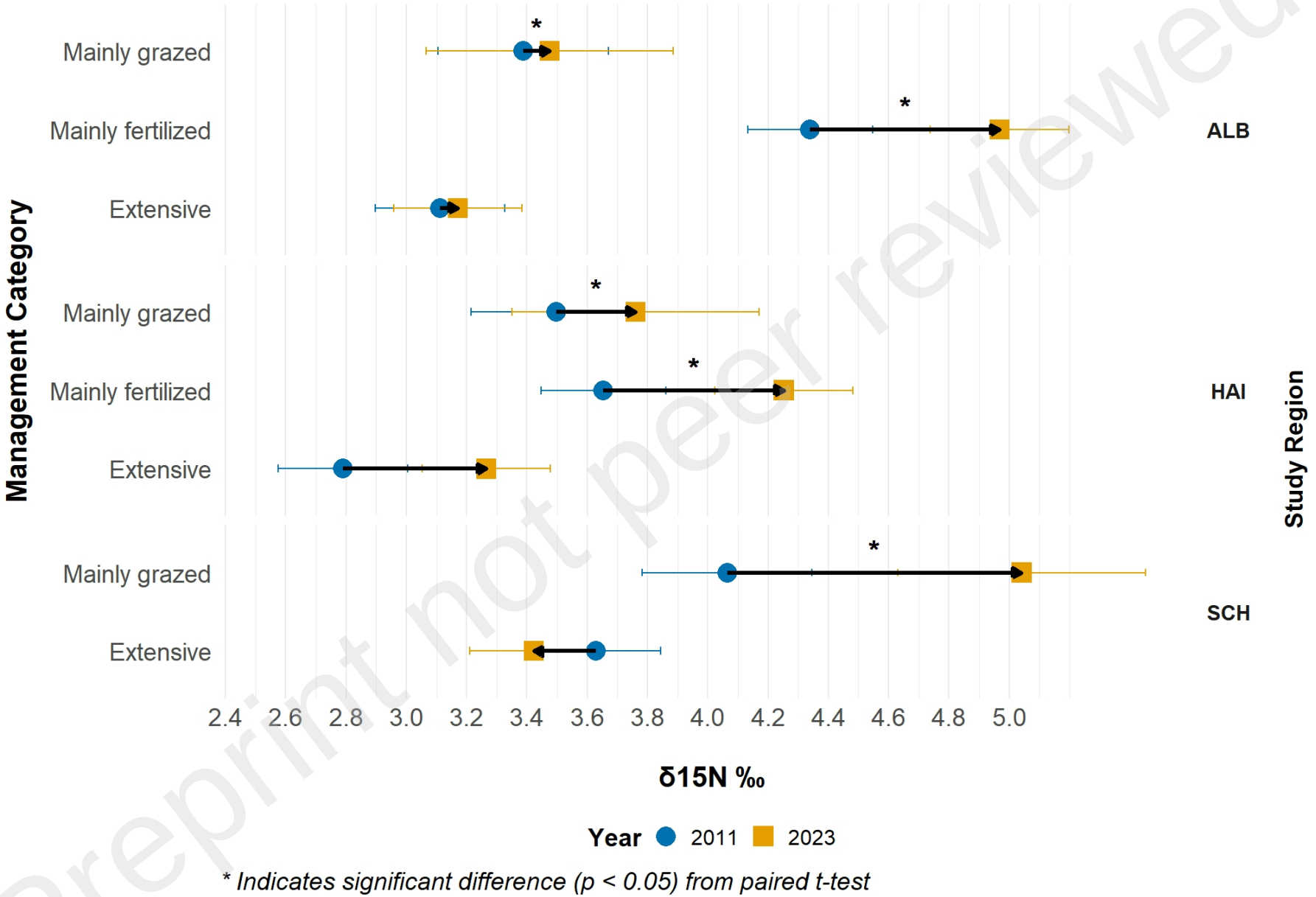
Figure 4: All mineral soil sites grouped into either an increase or a decrease in $\delta^{15}N$ from 2011 to 2023 plotted against their change in soil total nitrogen concentration (ΔN%) and soil organic carbon concentration (ΔC%) from 2011 to 2023.

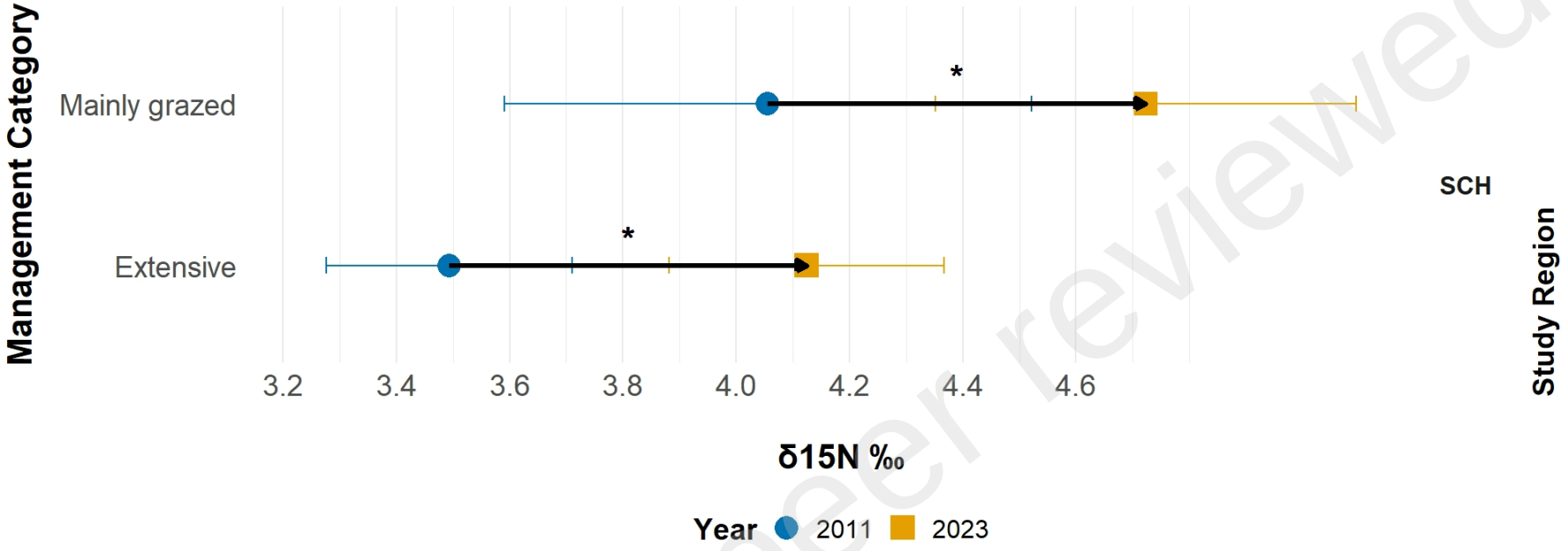
Figure 5: Effects of the land-use intensity index for fertilisation (LUI F) and the change in soil total nitrogen concentration from 2011 to 2023 (ΔN%) on changes of bulk soil $\delta^{15}N$ ‰ between 2011 and 2023 with the shaded area representing the 95% confidence interval.

Figure 6: The Johnson-Neyman plot showing the slope of the effect of the land-use intensity index for grazing (LUI G) on changes in bulk soil $\delta^{15}N$ ‰ from 2011 to 2023 ($\Delta\delta^{15}N$) plotted against the mean annual precipitation in that period (MAP). The shaded represents the 95% confidence interval and the thicker part of the y-axis indicates the range of observed MAP values.

Figure 7: The Johnson-Neyman plot showing the slope of the effect of the land-use intensity index for grazing (LUI G) on changes in bulk soil $\delta^{15}N$ ‰ from 2011 to 2023 ($\Delta\delta^{15}N$) plotted against the average plant species richness during that time (PSR). The shaded area represents the 95% confidence interval and the thicker part of the y-axis indicates the range of observed PSR values.

Figure S1: Model fit plotted as the predicted values for the change in $\delta^{15}N$ ‰ from 2011 to 2023 ($\Delta\delta^{15}N$) against the actual observed values. The colour and shape of the data points indicate the study region.





** Indicates significant difference ($p < 0.05$) from paired t-test*