



Management rather than climate and biodiversity shapes long-term $\delta^{15}\text{N}$ dynamics in grassland soils

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ABSTRACT

The bulk soil $\delta^{15}\text{N}$ signature in grassland soils serves as a fingerprint of nitrogen inputs, turnover and losses, reflecting the influence of land management, climate, plant biodiversity and abiotic soil properties. Although natural abundance $\delta^{15}\text{N}$ has been proposed as an indicator for these factors, previous studies yielded mixed results regarding the importance of its drivers. In this study, we quantified changes in soil $\delta^{15}\text{N}$ across 72 German grassland sites after 12 years (2011 vs. 2023) and analysed relationships with detailed records of land use intensity, plant biodiversity, climate, and soil properties. Land use intensity was quantified on a continuous scale based on mowing, grazing and fertilisation. In the top 10 cm of soil, $\delta^{15}\text{N}$ changes ranged from -0.86 to $+2.49\text{‰}$, equating to an average increase of $+0.35\text{‰}$ per decade. Over two-thirds of our study sites showed a long-term increase in $\delta^{15}\text{N}$ associated with high land use intensity. Our findings indicate that fertiliser application and grazing are the dominant drivers of $\delta^{15}\text{N}$ increases, while a biodiversity-mediated decrease via reduced N losses was not observed. The slow decadal-scale shifts suggest that single soil $\delta^{15}\text{N}$ measurements without known initial values are only informative for long-term management processes and not for assessing recent management impacts.

1. Introduction

Grasslands make up one-third of Germany's agricultural area and are primarily used for the production of plant 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 sustain and protect these valuable ecosystems and the ecosystem services they provide, it is crucial to understand how land use in interaction with plant biodiversity and soil properties shapes 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 extent of N fluxes and losses as well as for tracing individual pathways within the N cycle (Chalk et al., 2019). The isotopic signature refers to the

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concentration of the heavier ^{15}N isotope 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. Due to fractionation for the lighter ^{14}N isotope which is preferentially metabolised or lost, the heavier ^{15}N can accumulate 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 $\delta^{15}\text{N}$ given in ‰ and calculated in relation to the atmospheric natural abundance of ^{15}N (Watzka et al., 2006).

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

Bulk soil $\delta^{15}\text{N}$ has also been investigated as an indicator of past land management, climate, vascular plant biodiversity, and soil properties, since each of these factors influences N input, turnover, and losses (Kleinebecker et al., 2014; Michener and Lajtha, 2008; Watzka et al., 2006). Grassland management mainly consists of mowing, grazing, and fertilisation (Blüthgen et al., 2012), all of which impact soil organic carbon (SOC), soil total nitrogen (TN) and the plant species 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_2O) and nitric oxide (NO), ammonia volatilisation (NH_3) and nitrate (NO_3^-) leaching into the groundwater (Dittert et al., 1998; Estavillo et al., 1997). These N-loss processes increase the $\delta^{15}\text{N}$ signature by preferentially removing ^{14}N thereby inducing an accumulation of ^{15}N in the soil (Denk et al., 2017; Mudge et al., 2013; Stevenson et al., 2010; Wang et al., 2021). Moreover, grasslands under agricultural use are often fertilised with organic fertiliser such as slurry or farmyard manure, which in itself typically has a high positive $\delta^{15}\text{N}$ signature and is additionally susceptible to gaseous losses (Bedard-Haughn et al., 2003; Bouwman et al., 2002). Mowing mainly impacts the grassland N cycle by removing N and C from the system with biomass harvest (Li et al., 2022; Qiu et al., 2015). Grazing also removes biomass as it is taken up by the animals, however, the animals also return N in manure and urine which has a higher $\delta^{15}\text{N}$ than the plant material the animals eat as their metabolic processes also fractionate (Bedard-Haughn et al., 2003; Chalk et al., 2019; Frank et al., 2004; Sieve et al., 2021; Steele and Daniel, 1978).

Grassland management also affects the plant species composition and biodiversity with low management intensity, particularly less fertilisation and mowing, resulting in higher plant biodiversity (Allan et al., 2015; Midolo et al., 2019). A grassland ecosystem with higher plant species richness has a more closed N cycle with fewer losses compared to a less species-rich grassland, which leads to less ^{15}N enrichment in the soil (de Vries et al., 2012; Mudge et al., 2013). Another way in which plant species composition can influence the soil $\delta^{15}\text{N}$ signature is via the abundance of legumes, which add N via BNF with a ^{15}N signature at atmospheric natural abundance (Watzka et al., 2006).

Climatic conditions such as temperature and precipitation further impact the soil N turnover by providing more or less favourable conditions for microbes involved in different N-transformation processes. Higher temperatures and higher precipitation can lead to higher overall microbial biomass and activity and thereby a higher N turnover with more fractionating processes and N losses (Elrys et al., 2021b; Kou et al., 2020; Liu et al., 2009). Additionally, ammonia volatilisation, which is

the N-loss pathway with the highest fractionation rates, increases with higher temperatures (Bouwman et al., 2002). Precipitation and soil physical properties together also impact the share of water and air filled pore space in the soil, favouring either aerobic or anaerobic N processes (Elrys et al., 2021b; Tall et al., 2019). Soil texture also relates to the soil $\delta^{15}\text{N}$ signature since the soil organic matter (SOM) in the clay-sized particle fraction was found to be characterised by higher $\delta^{15}\text{N}$ values than the coarser silt or sand fractions (Tieszen et al., 1984). This $\delta^{15}\text{N}$ enrichment of the clay fraction SOM is due to the higher degree of microbial processing of its organic N, which led to an accumulation of ^{15}N during transformation processes (Ledgard et al., 1984; Liang, 2020; Whalen et al., 2022).

Studies using $\delta^{15}\text{N}$ as an indicator share the assumption that the bulk soil signature is enriched by fractionation during N turnover and losses as well as by organic fertiliser input, while it is depleted by BNF and mineral fertiliser input. However, conclusions on the main drivers determining the soil $\delta^{15}\text{N}$ signature vary widely. Some identified the relationship between plant species richness and soil N cycling as a key driver of $\delta^{15}\text{N}$ values in grassland soils (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). Two methodological limitations of the prevailing retrospective approach may explain these diverging results, as both lead to large uncertainties and unknowns, and thus potentially to misinterpretation. Firstly, $\delta^{15}\text{N}$ values have typically been measured only once and then used as an indicator of management, climate or plant biodiversity history without knowing initial soil $\delta^{15}\text{N}$ values. Without such a baseline, an observed value cannot be separated into its initial state and the subsequent change driven by management, climate or biodiversity, leaving the actual signal of interest unresolved. Secondly, there are large differences in the uncertainties of these potential drivers of $\delta^{15}\text{N}$ 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 plant biodiversity is usually measured at the time of sampling and may have been influenced by management at unknown time scales. Because drivers are known at different precisions and timescales, explanatory power gets misattributed toward whichever driver happens to be best measured rather than whichever is most causally important. The lack of precise management data over long time spans has also prevented an actual long-term quantification of $\delta^{15}\text{N}$ changes in managed grassland systems under real farming conditions.

Our study was designed to quantify the speed and direction of grassland bulk soil $\delta^{15}\text{N}$ changes at decadal time scales while addressing both problems directly by providing initial values and the change between them, alongside equally precise, long-term data for all drivers so that the attribution of explanatory power is not biased. This provides urgently needed knowledge on the potentials and constraints of $\delta^{15}\text{N}$ as an indicator of past land management, climate, vascular plant biodiversity, and soil properties. We quantified changes in soil $\delta^{15}\text{N}$ in 72 different German agricultural grassland sites over 12 years (2011 and 2023) and linked these changes to precisely quantified management, plant species richness, legume cover, climate and soil properties in this time span. Specifically, we aimed to (I) quantify the speed and direction of soil $\delta^{15}\text{N}$ changes, to identify which time span is fingerprinted, and (II) identify the main drivers, in order to constrain what information $\delta^{15}\text{N}$ changes contain. This will support future studies using $\delta^{15}\text{N}$ as an indicator of management, plant biodiversity, climate, and soil properties. We hypothesised that (1) $\delta^{15}\text{N}$ changes in grassland bulk soil occur at a pace of well below 1‰ per decade so that typical variability of $\delta^{15}\text{N}$ across soils is a consequence of exposure to different drivers over decades rather than years. (2) given the notion that $\delta^{15}\text{N}$ is an indicator of land management in the past, we expect $\delta^{15}\text{N}$ increases over time to be higher at higher land use intensity. (3a) $\delta^{15}\text{N}$ increases are driven primarily by intensive management, while $\delta^{15}\text{N}$ decreases occur under low management intensity combined with higher plant biodiversity; and (3b) soil properties and climate play only a minor role in these changes.

2. Methods

2.1. Study design

We studied 72 grassland sites spread across three distinct regions in Germany: 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. Coordinates for all sites can be accessed via the BExIS database (Ostrowski et al., 2021). 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 the 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 $\delta^{15}\text{N}$ 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).

Land use intensity was assessed annually via questionnaires sent to land managers in which they reported the number of mowing events, and type and amount of fertiliser-N applied (Franke et al., 2025; Lorenzen et al., 2025; Ostrowski et al., 2020; Vogt et al., 2019). From this data, an overall land use intensity index (LUI) was calculated for each site and each year. 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 $\text{ha}^{-1} \text{yr}^{-1}$), M_p is the frequency of mowing events per year, and F_p is the fertilisation level ($\text{kg N ha}^{-1} \text{yr}^{-1}$) 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 standardisation 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).

Fertilisation rates were calculated from the same questionnaires and showed that between 2011 and 2023 on average more organic than mineral fertiliser was applied on the study sites (average of $22.1 \text{ kg N ha}^{-1} \text{yr}^{-1}$ from organic fertiliser versus $12.7 \text{ kg N ha}^{-1} \text{yr}^{-1}$ for mineral fertiliser) (Lorenzen et al., 2025). Detailed fertilisation practice varied considerably among plots. Therefore, we do not distinguish between mineral and organic fertilisation in our analysis but expect the isotopic signal of the organic fertilisers to determine the overall effect of fertilisation in our study.

Plant species richness (PSR; here defined as the number of different plant species) and legume cover percentage (LC), are recorded annually for a 16 m^2 subplot on each site and documented in BExIS (Hinderling and Keller, 2023). For this study we used the mean value of the annual records of PSR and LC between 2011 and 2023.

2.2. Fieldwork and laboratory analysis

To allow for a second time point of $\delta^{15}\text{N}$ bulk soil data 12 years after the sampling in 2011 (for methods see Kleinebecker et al., 2014), we sampled soil at 25 different grassland sites in HAI, 24 sites in ALB and 23 sites in SCH in early May 2023. SCH is the only area where 13 grassland

sites were located on organic soils, classified as Niedermoor (fen peat soils, specifically Erdniedermoor and Mulmiedermoor subtypes) according to the German Soil Classification System (Bodenkundliche Kartieranleitung, KA5; AG Bodenkunde, 2005). These soils contain > 30 mass-% organic matter in the upper horizons and were analysed separately due to a lack of comparability with mineral soils. The sites were chosen to represent a full spectrum of 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. Right after sampling, the samples from each depth on every site were combined into one composite sample, homogenised manually and sieved once 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 TN and SOC concentrations, as well as $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ concentration was calculated using the equation below with the standard being atmospheric natural abundance of ^{15}N (Mariotti, 1983).

$$\delta^{15}\text{N}\text{‰}_0 = \left(\frac{R(^{15}\text{N}/^{14}\text{N})_{\text{sample}}}{R(^{15}\text{N}/^{14}\text{N})_{\text{standard}}} - 1 \right) \times 1000$$

Each sample was measured against a working standard N_2 gas calibrated against secondary isotope standards (IAEA-N1, -N2 and -N3; accuracy of calibration $< 0.1\text{‰}$). Each sample was measured only once. The precision of measurement runs was estimated via a solid laboratory standard (a finely ground wheat flour with $\delta^{15}\text{N}$ 2.54‰) run after every tenth sample to account for possible instrument drift. Mean standard deviation of laboratory standard measurements across the individual measurement runs was 0.17‰.

For this analysis, we focus on the top 10 cm of soil, as data from 2011 were collected only to this depth. Detailed data on soil properties, such as soil clay content, were recorded and stored in BExIS for 2011 (see Baumann et al., 2022; Schöning et al., 2012) but not for 2023. All data recorded in 2023 have also been made available in the BExIS database (Kepp and Schwärzler, 2025).

2.3. Data analysis

We first used linear models to analyse how the difference in $\delta^{15}\text{N}$ between 2011 and 2023 ($\Delta\delta^{15}\text{N}$) was influenced by management, soil chemistry, vegetation, and climate. Predictors included LUI and its components (LUI F, LUI G, LUI M; defined above); the differences in SOC, TN, and C to N ratio ($\Delta\text{C}\%$, $\Delta\text{N}\%$, and $\Delta\text{C/N}$); plant species richness (PSR) and mean legume cover percentage (LC); and mean annual precipitation (MAP) and mean annual air temperature at 2 m above ground (MAT). In addition to the 12 year means of MAT, MAP, PSR, LC, LUI, LUI F, LUI M, and LUI G we examined whether these variables exhibit temporal trends during this time span using regression analyses. For each continuous variable, we fitted a simple linear regression model with year as the predictor variable and the annual values (or annual mean for MAT and MAP) of the variable as the response variable. The slope of the regression line was used to indicate the direction and magnitude of change through time. Statistical significance of the slope was assessed using a t -test for variables with two categorical levels and a one-way analysis of variance (ANOVA) for those with three categorical levels. Variables were considered to exhibit a significant trend if $p < 0.05$; slopes were interpreted as positive, negative, or non-significant trends accordingly. MAP did not show any significant trends. To assess whether the trends found in MAT, PSR, LC, LUI, LUI F,

LUI M, and LUI G influenced $\Delta\delta^{15}\text{N}$ we compared the $\Delta\delta^{15}\text{N}$ for sites with a positive trend, with a negative trend and without a trend but found no significant differences. We also calculated a decadal change for each trend using the slope of any significant trends and setting those which were not found significant to 0.

The impact of soil clay content (SCC) and soil sand content (SSC) on $\Delta\delta^{15}\text{N}$ was also assessed based on data from the sampling in 2011. The most parsimonious model was selected based on the corrected Akaike information criterion (AICc) using the MuMIn::dredge function in R.

Several predictor pairs were strongly correlated ($|r| > 0.5$); in each case we retained the variable with higher explanatory power and/or lower variance inflation factor (VIF). LUI F was retained over LUI M ($|r| > 0.73$), as LUI F had a stronger impact on the $\delta^{15}\text{N}$ signature through predominant organic fertiliser use, while LUI M additionally correlated with LUI G ($|r| > 0.51$) and had the highest VIF of the three. The correlation between LUI F and LUI M reflects linked fertilisation-mowing cycles, particularly in intensively managed grasslands. MAP was retained over MAT ($|r| > 0.64$) because of its higher coefficient of variation across sites ($\text{cv} = 0.308$ vs. 0.076). SCC was retained over SSC ($|r| > 0.73$) based on higher explanatory power and lower VIF. $\Delta\text{N}\%$ was retained over $\Delta\text{C}\%$ ($|r| > 0.67$) based on higher explanatory power. However, because N and C play distinct roles in the N cycle, we ran the full model selection process twice (once with each), which confirmed the similarity of their effects and showed no major impact on other variables. The AICc comparison favoured the $\Delta\text{N}\%$ model (AICc = 99.79 vs. 112.77 for $\Delta\text{C}\%$). $\Delta\text{C}/\text{N}$ was near zero throughout, reflecting the synchronous changes in N% and C%, and was therefore excluded.

As $\Delta\text{N}\%$ showed a non-linear effect on $\Delta\delta^{15}\text{N}$, we fitted a Generalised Additive Model (GAM) with a smoothed term for $\Delta\text{N}\%$ and compared a set of candidate specifications by AICc: a linear baseline, GAMs with and without LC, a $\Delta\text{C}\%$ smooth in place of $\Delta\text{N}\%$, the addition of MAP and PSR, and a study-region random effect to account for spatial clustering. Their estimated degrees of freedom, deviance explained and AICc are reported in Table A3. The most parsimonious specification was retained; in particular, the random effect was dropped because the AICc indicated minimal variability attributable to differences between study areas. Model diagnostics confirmed that the assumptions were met, with residuals approximately normally distributed and homoscedastic (Figure A2).

Furthermore, we applied a Random Forest regression (randomForest R package) to assess the relative importance of LUI F, LUI G, $\Delta\text{N}\%$, and SCC on $\Delta\delta^{15}\text{N}$. This four-predictor set was reached by reducing the full set of candidate predictors. The reduction improved out-of-bag predictive performance (Table A4). We grew 5001 trees with the package defaults for regression ($\text{mtry} = \lfloor p/3 \rfloor = 1$, $\text{nodesize} = 5$, unrestricted depth, bootstrap samples of size n with replacement) and quantified variable importance as the percentage increase in mean squared error (%IncMSE) under out-of-bag permutation. Model fit was evaluated using the OOB R^2 ($= 0.24$). A sensitivity analysis confirmed that the default mtry was near-optimal and that variable rankings were stable (Figure A3). LC was removed as it showed no significant effect, and AICc suggested it did not improve the model.

We also divided our data into two groups: one with sites that showed an overall increase in $\delta^{15}\text{N}$ 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, LUI G, PSR and LC between 2011 and 2023, decadal trends for LUI, LUI F, LUI M, LUI G, LC, PSR, and MAT, as well as C%, N% and C/N in 2011 and 2023, $\Delta\text{C}\%$, $\Delta\text{N}\%$ and $\Delta\text{C}/\text{N}$ and SSC in 2011 were analysed. Numeric variables were tested using Welch's t -tests, and for categorical decadal-trend variables (positive/negative/no-trend) using one-way ANOVA of $\Delta\delta^{15}\text{N}$. To address multiple comparisons across the 18 tests, p -values were adjusted using the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995). In addition to comparing the increase and decrease groups, we regressed the continuous $\Delta\delta^{15}\text{N}$ on each of these explanatory factors individually using simple linear regression, with Benjamini-Hochberg correction

applied across the regressions. This avoids dichotomising the response and provides greater statistical power than the group comparison.

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 $\delta^{15}\text{N}$ value for each group for each exploratory for 2011 and 2023. SCH was the only region with no fertilisation-dominated management.

All statistical analyses and their corresponding visualisations were performed using RStudio (R Version 4.4.2) using the packages dplyr, MuMIn, mgcv, ggplot2, gratia, car, randomForest and effects.

3. Results

3.1. $\delta^{15}\text{N}$ change and management categories

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

For the sites on mineral soils, the differences in $\delta^{15}\text{N}$ changes from 2011 to 2023 were significant and positive in all mainly grazed and mainly fertilised sites (Fig. 1). For extensive sites, both an increase and a decrease in $\delta^{15}\text{N}$ from 2011 to 2023 were observed, but differences between the $\delta^{15}\text{N}$ values at the two different sampling events were not significantly different from zero in any of the study regions (Fig. 1). The organic soil sites showed a significant difference between bulk soil $\delta^{15}\text{N}$ in 2011 and in 2023 under both extensive and grazing-dominated management (Fig. 2, Table 2). For both mineral and organic soil sites, average annual fertilisation rates in kg N per hectare (kg N ha^{-1}) and grazing intensities in livestock units (LU) and days per hectare ($\text{LU} \cdot \text{d ha}^{-1}$) between 2011 and 2023 for each management category are given in Table 1.

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

The same grouping was applied to the 13 remaining organic soil sites. Similar to the SCH $\delta^{15}\text{N}$ values on mineral soils, the average $\delta^{15}\text{N}$ signature in organic soils in 2011 was 3.49‰ ($\text{SE} \pm 0.22$) for the 8 extensively managed sites and 4.05‰ ($\text{SE} \pm 0.47$) for the 5 grazed sites. In 2023, extensively managed sites had a mean $\delta^{15}\text{N}$ signature of 4.13‰ ($\text{SE} \pm 0.24$), which is higher than the value observed for sites on mineral soils. The grazed sites, on the other hand, had a $\delta^{15}\text{N}$ signature of 4.72‰ ($\text{SE} \pm 0.47$), which is lower than that of the sites on mineral soils.

In 40 out of the 59 mineral soil sites, we found an increase of $\delta^{15}\text{N}$ from 2011 to 2023 and in 19 sites, we found a decrease (Table 3). This was relatively consistent across the three study areas with an increase found in 17 out of 24 sites in ALB, 19 out of 25 in HAI and 6 out of 10 in SCH. For the organic soil sites in SCH, only 1 out of 13 showed a decrease in $\delta^{15}\text{N}$. 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 soils from further more

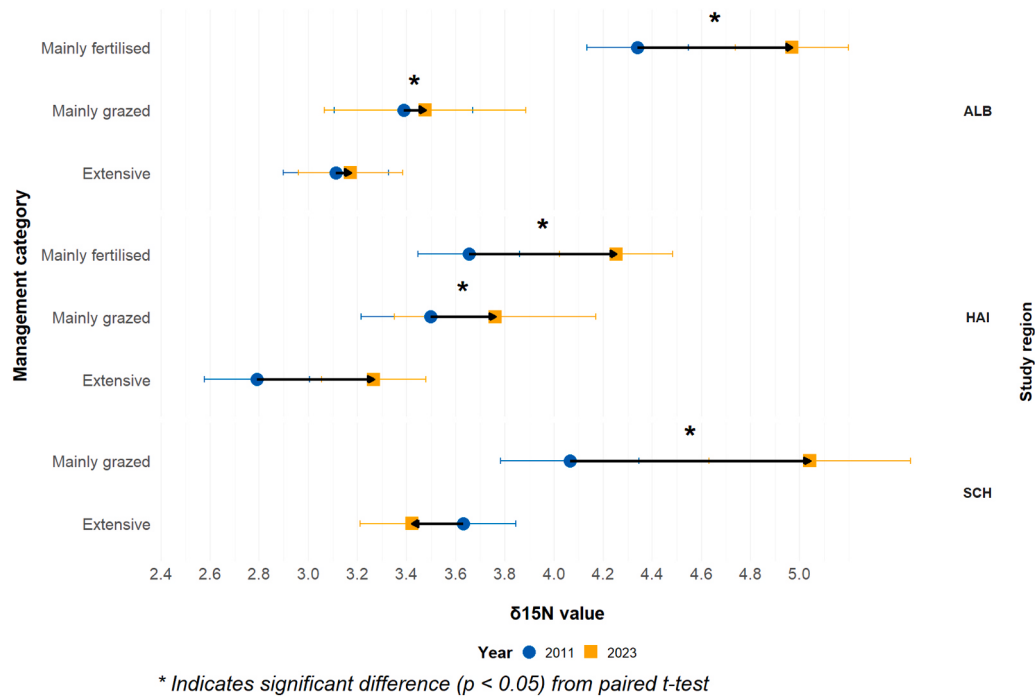


Fig. 1. Average $\delta^{15}\text{N}$ values for the 59 mineral soil sites grouped by study region and management category. Study regions are the Swabian Alb (ALB), Hainich-Dün (HAI) and Schorfheide-Chorin (SCH). The category mainly grazed includes all sites with a land use intensity index for grazing (LUI G) > 1 and LUI G > the land use intensity index for fertilisation (LUI F) ($n = 16$). Mainly fertilised includes all sites with LUI F > 1 and LUI F > LUI G ($n = 22$). Extensive is comprised of the remaining sites where both LUI G and LUI F are < 1 ($n = 21$). For the SCH region, none of the sites were fertilisation dominated.

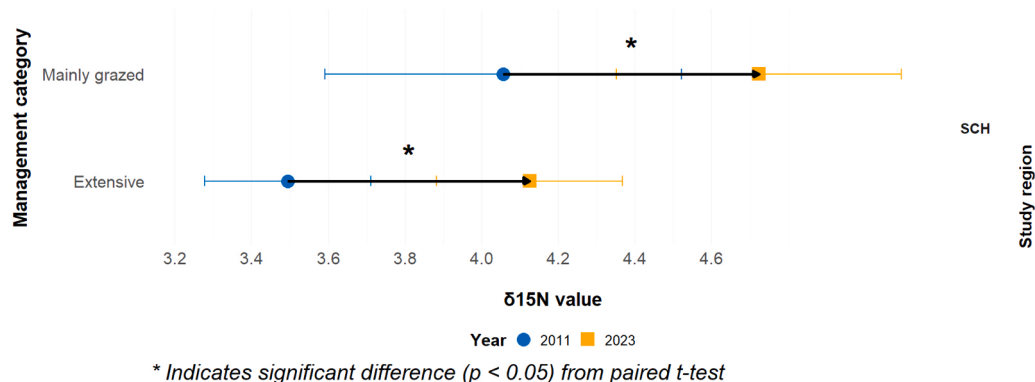


Fig. 2. Average $\delta^{15}\text{N}$ values for the 13 organic soil sites in the Schorfheide-Chorin (SCH) region grouped by management category. The category mainly grazed includes all sites with a land use intensity index for grazing (LUI G) > 1 and LUI G > the land use intensity index for fertilisation (LUI F) ($n = 5$). Extensive is comprised of the sites where both LUI G and LUI F are < 1 ($n = 8$). None of the organic soil sites were fertilisation dominated (LUI F > 1 and LUI F > LUI G).

detailed analysis.

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

Soils with increasing $\delta^{15}\text{N}$ had slightly higher SOC and TN concentrations compared to those with decreasing $\delta^{15}\text{N}$ in 2011 and 2023, though none of these differences were statistically significant (Table A1). However, soils with increasing $\delta^{15}\text{N}$ showed decreasing SOC and TN concentrations between 2011 and 2023, while decreasing soil $\delta^{15}\text{N}$ was related to unchanged or slightly increasing SOC and TN concentrations over time (Fig. 4, Table 3). This indicates a higher increase in SOC and TN concentrations in the soil at sites with a decrease in soil $\delta^{15}\text{N}$ over time. The observation that $\Delta\text{C}\%$ and $\Delta\text{N}\%$ show the same

trend is also reflected in the similarity in the $\Delta\text{C}/\text{N}$ of both the $\delta^{15}\text{N}$ increase and decrease groups. The LC was higher for soils showing a decrease in $\delta^{15}\text{N}$ compared to those with an increase, though this tendency was not statistically significant (Table 3). SCC was slightly higher in soils with an increase in $\delta^{15}\text{N}$. However, there was no significant difference between the increase and decrease group (Table 3).

We found significant decadal trends for management, MAT, PSR and LC. Only MAP showed no significant trend. Comparing sites with a $\delta^{15}\text{N}$ increase and those with a $\delta^{15}\text{N}$ decrease, we found no significant differences in any of the decadal trends (Table 3). Thus, it was the differences in management, vegetation and climate across sites rather than the gradual changes of these factors within a single site that mediated changes of $\delta^{15}\text{N}$.

To avoid the loss of power associated with splitting $\Delta\delta^{15}\text{N}$ into increase and decrease groups, we additionally regressed the continuous $\Delta\delta^{15}\text{N}$ on each explanatory factor individually (Table A2). LUI was the

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				
Mainly fertilised	33.3–258.4	94.1	0.0–135.1	42.5
Mainly grazed	0.0–76.5	12.2	128.6–730.9	387.1
Extensive	0.0–24.4	5.4	2.6–126.4	51.0
Organic soils				
Mainly grazed	0.00–19.4	5.1	255.4–496.9	324.3
Extensive	0.00–24.8	7.9	0.0–94.7	33.3

Table 2

Mean values of $\delta^{15}\text{N}$ 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 region. Study regions are the Swabian Alb (ALB), Hainich-Dün (HAI) and Schorfheide-Chorin (SCH). Sample size of each group is also given.

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

only factor significantly related to $\Delta\delta^{15}\text{N}$ after Benjamini–Hochberg correction (slope = 0.48, $p_{\text{BH}} = 0.009$), with its components LUI F and LUI G significant at the uncorrected level. PSR, LC, SCC, MAT and the decadal-trend variables showed no significant relationship after correction. $\Delta\text{N}\%$ showed no significant linear relationship with $\Delta\delta^{15}\text{N}$, unlike in the group comparison in Table 3; the non-linear form of this relationship is examined in the following section (Fig. 6).

3.2. Generalised additive and random forest models

The GAM confirmed that management was the dominant control of $\Delta\delta^{15}\text{N}$. The final GAM selected was: $\Delta\delta^{15}\text{N} \sim \text{LUI F} + \text{LUI G} + s(\Delta\text{N}\%) + \text{SCC}$. The model revealed that LUI F had a significant positive effect on $\Delta\delta^{15}\text{N}$ (Estimate = 0.166, SE = 0.040, 95% CI [0.087, 0.244], $t = 4.128$, $p = 0.0001$), (Fig. 5). LUI G also showed a significant positive effect on $\Delta\delta^{15}\text{N}$ (Estimate = 0.145, SE = 0.051, 95% CI [0.045, 0.244], $t = 2.850$, $p = 0.0063$) (Fig. 5). Changes of TN in bulk soil ($\Delta\text{N}\%$) showed a significant smooth (non-linear) effect on $\Delta\delta^{15}\text{N}$ (edf = 3.14, $F = 6.79$, $p < 0.001$) (Fig. 6). At negative $\Delta\text{N}\%$, the model predicted an

increase in $\delta^{15}\text{N}$. As $\Delta\text{N}\%$ approached zero and became slightly positive, the partial effect became increasingly negative, reaching a minimum around $\Delta\text{N}\% \approx 0.07$ –0.1. At higher levels of $\Delta\text{N}\%$, the effect began to increase again, though this was associated with fewer observations and greater uncertainty. The estimated degrees of freedom (edf > 1) suggest a moderately complex non-linear relationship. This non-linear form also reconciles the $\delta^{15}\text{N}$ increase/decrease comparison with the regressions. $\Delta\text{N}\%$ differed between groups (Table 3) yet showed no significant linear association with $\Delta\delta^{15}\text{N}$ (Table A2), because the binary split captures the descending limb of the relationship whereas a single linear slope fitted across the full range averages to near zero.

SCC had a negative effect on $\Delta\delta^{15}\text{N}$ (Estimate = -0.001, SE < 0.001, 95% CI [-0.0017, -0.0002], $t = -2.551$, $p = 0.0137$) (Fig. 5). The GAM explained 49% of the deviance in $\Delta\delta^{15}\text{N}$ (adjusted $R^2 = 0.43$, $n = 59$). Plotting the predicted $\Delta\delta^{15}\text{N}$ 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 A1). Overall, these findings highlight the significant role of grazing and fertilisation and soil TN in shaping nitrogen isotope dynamics over time.

The Random Forest model included LUI F, LUI G, SCC and $\Delta\text{N}\%$ as predictors and $\Delta\delta^{15}\text{N}$ as the response variable. It explained 24% of the variation in $\Delta\delta^{15}\text{N}$, with an out-of-bag mean squared error of 0.24. The lower R^2 compared to the GAM likely stems from the small sample size of 59, which is relatively small for a random forest analysis with four predictors. LUI F was the most important predictor, with %IncMSE values of 31.1. This was followed by $\Delta\text{N}\%$ and LUI G with %IncMSE 27.2 and 25.4, respectively. Lastly, SCC had a %IncMSE of 14.7 (Fig. 7).

4. Discussion

4.1. Speed and direction of δN changes over time

A main result of our long-term and large-scale study of temperate grassland soils was that two-thirds showed an increase in $\delta^{15}\text{N}$ (average of 0.72 ± 0.10), while a third of all sites showed a decrease in $\delta^{15}\text{N}$ (average of -0.21 ± 0.05). $\delta^{15}\text{N}$ changes were small and ranged from -0.86 to +2.49‰, equating to an average increase of +0.35‰ per decade. The larger change in the $\delta^{15}\text{N}$ signal observed in the increase group compared to the decrease group suggests that the drivers responsible for increasing the $\delta^{15}\text{N}$ signal (fractionation during N turnover and losses and organic fertiliser inputs) are acting faster and/or are more prevalent than those responsible for decreasing it (BNF and mineral fertiliser input). Accordingly, sites with a $\delta^{15}\text{N}$ increase outnumber those with a decrease by almost two to one.

As soon as management is not extensive its effect outweighs that of other factors and bulk soil $\delta^{15}\text{N}$ increases. Only for extensively managed sites, the observed changes over 12 years were marginal, hardly changing the initial $\delta^{15}\text{N}$ values in 2011. Intensively grazed sites, as they are found in SCH and intensively fertilised sites in HAI and ALB, show higher $\delta^{15}\text{N}$ starting values and much larger increases in $\delta^{15}\text{N}$ over time. The larger changes in $\delta^{15}\text{N}$ over time highlight the dominance of N inputs and turnover processes increasing $\delta^{15}\text{N}$ in grazed and fertilised sites. The higher initial $\delta^{15}\text{N}$ signature of the more intensively managed sites in 2011 further indicates that the differences in management intensity documented in this study reach further into the past (Fig. 8).

Other studies examining the impact of land use on $\delta^{15}\text{N}$ have yielded widely varying results with regard to direction, speed and magnitude of changes in $\delta^{15}\text{N}$ (Chalk et al., 2019; Marriott et al., 1997; Mudge et al., 2013; Sieve et al., 2021; Stevenson et al., 2010; Watzka et al., 2006). Studies investigating different management effects showed similar results to what we found in more intensively managed sites. The overall magnitude of changes in $\delta^{15}\text{N}$ in our study 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, $\delta^{15}\text{N}$

Table 3

Mean values and standard error (SE) of all variables used for all mineral soil sites grouped by increase or decrease in $\delta^{15}\text{N}$ from 2011 to 2023, and the raw p value as well as the Benjamini-Hochberg (BH) adjusted p value of the difference between these groups. The variables considered are the difference in organic carbon concentration ($\Delta\text{C}\%$), the difference in total nitrogen concentration ($\Delta\text{N}\%$) and the difference in carbon to nitrogen ratio ($\Delta\text{C}/\text{N}$) between 2011 and 2023. Additionally, the mean values as well as decadal trends of plant species richness (PSR), land use intensity index (LUI), LUI for fertilisation (LUI F), LUI for mowing (LUI M), LUI for grazing (LUI G), and legume cover given in % (LC) were calculated over the period 2011–2023. Mean annual temperature decadal trend (MAT trend) is given in $^{\circ}\text{C}$. Soil clay content (SCC) is given in g/kg and was estimated in 2011.

Variable	Mean all sites (SE)	Mean group increase $\delta^{15}\text{N}$ (SE)	Mean group decrease $\delta^{15}\text{N}$ (SE)	Significance of group differences (p value)	BH adjusted p value
Sample size	59	40	19		
$\Delta\delta^{15}\text{N}\%$	0.42 (± 0.09)	0.71 (± 0.10)	-0.21 (± 0.05)	< 0.0001	< 0.0001
$\Delta\text{N}\%$	0.00 (± 0.01)	-0.02 (± 0.01)	0.03 (± 0.01)	0.00129**	0.00579**
$\Delta\text{C}\%$	-0.29 (± 0.12)	-0.47 (± 0.15)	0.08 (± 0.14)	0.01093*	0.03935*
$\Delta\text{C}/\text{N}$	-0.17 (± 0.11)	-0.19 (± 0.14)	-0.12 (± 0.19)	0.769	0.866
SCC 2011	422.02 (± 24.71)	441.35 (± 30.25)	381.32 (± 41.28)	0.259	0.465
PSR	33.42 (± 1.40)	32.19 (± 1.60)	36.01 (± 2.63)	0.234	0.465
PSR trend ^{1,†}	4.03 (± 0.65)	4.39 (± 0.86)	3.30 (± 0.92)	0.956	0.956
LC	8.47 (± 0.78)	7.37 (± 0.82)	10.76 (± 1.56)	0.072	0.215
LC trend ^{1,†}	-3.77 (± 1.10)	-3.64 (± 1.20)	-4.05 (± 2.28)	0.882	0.934
LUI	1.71 (± 0.08)	1.87 (± 0.10)	1.36 (± 0.09)	0.00069***	0.00414**
LUI trend ^{1,†}	-0.02 (± 0.06)	-0.04 (± 0.06)	0.02 (± 0.13)	0.416	0.624
LUI F	1.24 (± 0.23)	1.66 (± 0.31)	0.35 (± 0.12)	0.00034***	0.00304**
LUI F trend ^{1,†}	-0.07 (± 0.12)	-0.05 (± 0.16)	-0.11 (± 0.14)	0.594	0.713
LUI M	0.98 (± 0.11)	1.10 (± 0.13)	0.73 (± 0.17)	0.103	0.266
LUI M trend ^{1,†}	-0.06 (± 0.04)	-0.01 (± 0.04)	-0.17 (± 0.10)	0.233	0.465
LUI G	1.09 (± 0.19)	1.16 (± 0.26)	0.94 (± 0.25)	0.545	0.701
LUI G trend ^{1,†}	0.18 (± 0.19)	0.06 (± 0.18)	0.44 (± 0.45)	0.487	0.675
MAT trend ^{1,†}	0.41 (± 0.09)	0.44 (± 0.12)	0.35 (± 0.15)	0.369	0.604

* significant,

** highly significant,

*** very highly significant

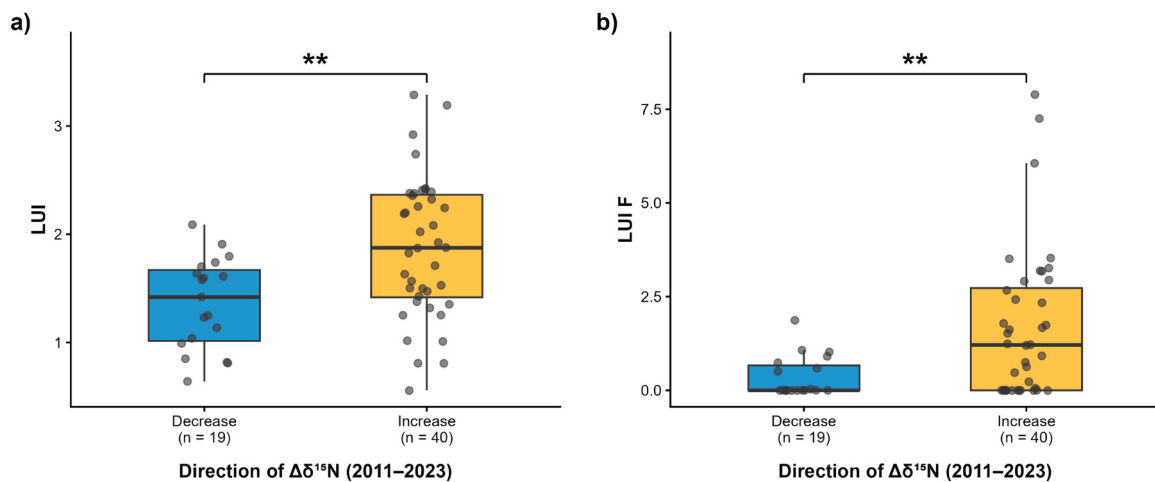
¹ Mean decadal change estimated based on trends between 2011 and 2023 (see Section 2.3 for details).[†] Trends are statistically significant.

Fig. 3. Comparison of the a) land use intensity index (LUI) and b) LUI from fertilisation (LUI F) between all mineral soil sites with an increase (orange, $n = 40$) and a decrease (blue, $n = 19$) in bulk soil $\delta^{15}\text{N}$ between 2011 and 2023 ($\Delta\delta^{15}\text{N}$). Boxes show the interquartile range (IQR) with the median as a horizontal line; whiskers extend to $1.5 \times \text{IQR}$. Individual sites are shown as overlaid points. Asterisks indicate statistical significance from Welch's t -tests between the two groups after Benjamini-Hochberg correction for multiple comparisons across all 18 tests: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, n.s. = not significant.

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 fertilisation and mowing regimes. They only sampled once, but management regimes had been the same since the grasslands were established. In line with our findings, $\delta^{15}\text{N}$ of both vegetation and soils closely mirrored management differences, with higher values observed under increased fertilisation. Sieve et al. (2021) did find short-term trends of $\delta^{15}\text{N}$ 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, emphasising the need for long-term studies to discern broader and more detailed trends. Contrasting results by Marriott et al. (1997) demonstrated a decrease from 6.1‰ to 5.1‰ $\delta^{15}\text{N}$ over one year under grazing and mineral fertilisation and no significant changes in the ungrazed and unfertilised controls. However, fertilisation and grazing had stopped on those unmanaged sites only 3 years prior to their first sampling. The use of mineral fertilisers, a potentially altered plant species composition favouring inputs via BNF, and the short duration of their study may explain these rather unexpected changes. Once these methodological deviations are accounted for, these management studies broadly agree with our finding that intensive management enriches bulk soil $\delta^{15}\text{N}$ and multi-year studies are

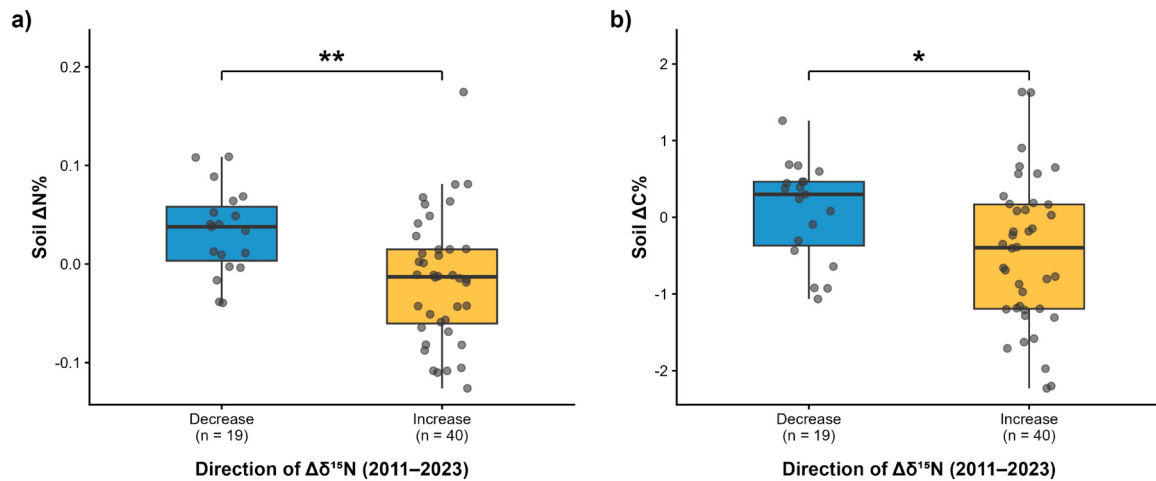


Fig. 4. Comparison of change in a) soil total nitrogen concentration ($\Delta\text{N}\%$) and b) soil organic carbon concentration ($\Delta\text{C}\%$) from 2011 to 2023 between all mineral soil sites with an increase (orange, $n = 40$) and a decrease (blue, $n = 19$) in bulk soil $\delta^{15}\text{N}$ between 2011 and 2023 ($\Delta\delta^{15}\text{N}$). Boxes show the interquartile range (IQR) with the median as a horizontal line; whiskers extend to $1.5 \times \text{IQR}$. Individual sites are shown as overlaid points. Asterisks indicate statistical significance from Welch's t -tests between the two groups after Benjamini-Hochberg correction for multiple comparisons across all 18 tests: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, n.s. = not significant.

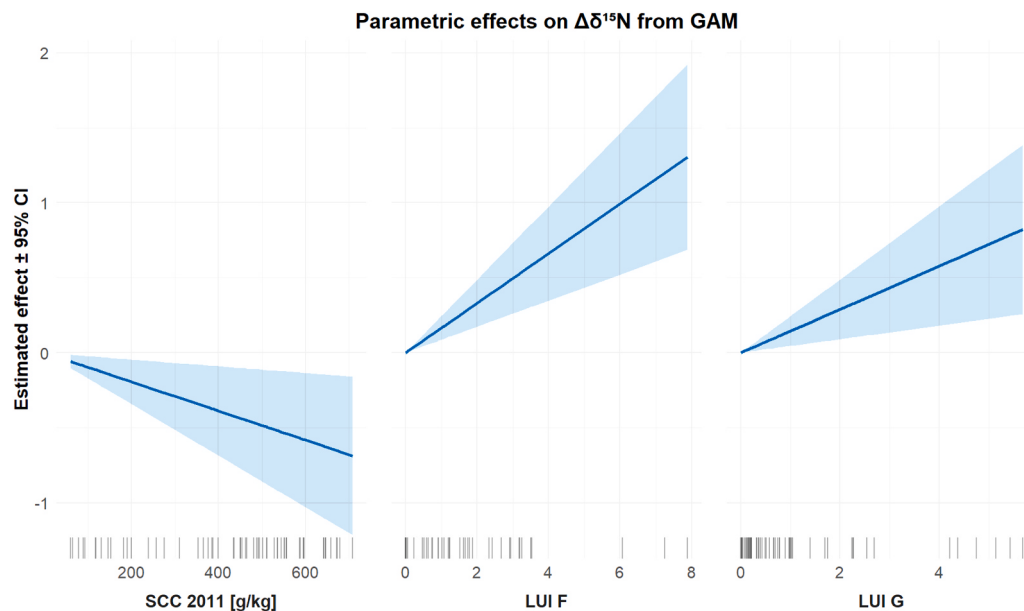


Fig. 5. The effects of fertilisation intensity (LUI F), grazing intensity (LUI G) and soil clay content in 2011 (SCC) on the difference in $\delta^{15}\text{N}$ between 2011 and 2023 ($\Delta\delta^{15}\text{N}$) from the generalised additive model (GAM) $\Delta\delta^{15}\text{N} \sim \text{LUI F} + \text{LUI G} + s(\Delta\text{N}\%) + \text{SCC}$. The light blue area around the line shows the 95% confidence interval (CI). Tick marks along the x-axis (rug) indicate the distribution of observed values for each predictor. Model diagnostics are provided in Fig. A3.

required to adequately capture this increase.

The effects of overall land use changes on the bulk soil $\delta^{15}\text{N}$ signature have also been explored in other studies. When investigating soil nitrogen dynamics after the transformation of an area of native forest to croplands, Jones and Dalal (2017) found an increase of 2.1‰ $\delta^{15}\text{N}$ in the top soil (10 cm) over two decades which is slightly higher than what we observed. Neither the forest nor the later cropland was ever fertilised, so the increase in $\delta^{15}\text{N}$, which went along with a decrease in total N, reflects the removal of N (particularly the preferentially plant assimilated ^{14}N) through cropping. This demonstrates that more drastic changes in land use also reflect faster changes in the natural abundance of soil $\delta^{15}\text{N}$. Even more drastic land use changes were investigated by Stevenson et al. (2010), who found that $\delta^{15}\text{N}$ values increased with land use intensity in a single-time sampling: from $+2.1\text{‰}$ under native forest, to $+2.8\text{‰}$ under plantation forestry, $+3.8\text{‰}$ in pasture under drystock, $+5.4\text{‰}$ in

pasture under dairy management, and $+6.2\text{‰}$ under intensive cropping. Similarly, Qiu et al. (2015) observed an increase of approximately 1.5‰ in $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 and composition. In contrast, management-related changes, particularly through fertilisation and intensive grazing, are associated with higher rates of $\delta^{15}\text{N}$ change over time.

These findings underscore the value of long-term data collection, particularly with comparative historical records. Our findings reveal

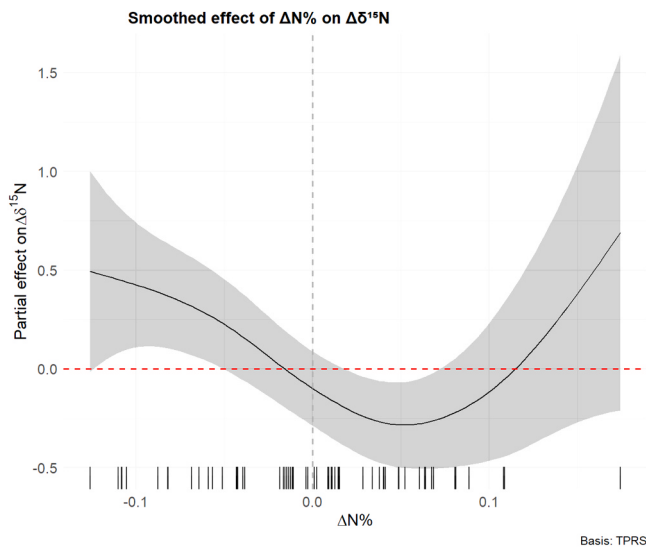


Fig. 6. The non-linear smoothed effect of the difference in soil N concentration between 2011 and 2023 ($\Delta N\%$), on the difference in $\delta^{15}N$ between 2011 and 2023 ($\Delta \delta^{15}N$) from the generalised additive model $\Delta \delta^{15}N \sim \text{land use intensity index for fertilisation (LUI F)} + \text{land use intensity index for grazing (LUI G)} + s(\Delta N\%) + \text{soil clay content in 2011 (SCC)}$, with vertical lines on the x-axis indicating the spread of our data. The grey area around the line shows the 95% confidence interval.

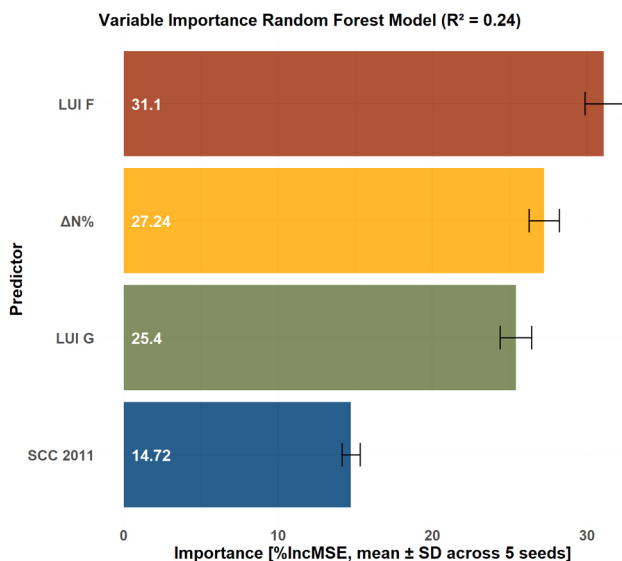


Fig. 7. The variable importance from the random forest model assessing the impact of four variables (fertilisation intensity (LUI F), difference in soil N concentration between 2011 and 2023 ($\Delta N\%$), grazing intensity (LUI G) and soil clay content in 2011 (SCC)) on the difference in $\delta^{15}N$ between 2011 and 2023 using the percentage increase in mean squared error (%IncMSE). Error bars indicate standard deviation across 5 seeds.

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 of ^{15}N in bulk soil. The slow rates of $\delta^{15}N$ changes in extensively managed systems necessitate multi-decade studies to discern patterns beyond the variability observed among treatments. In our study, for such sites, the 12-year changes in $\delta^{15}N$ were much smaller than the variation across similar sites observed in 2011 and 2023, so that for a meaningful analysis of natural abundance $\delta^{15}N$ in these sites, long-term treatment and ideally historic $\delta^{15}N$ values of the same sites for comparison are needed. This highlights that

single-time bulk soil $\delta^{15}N$ sampling in extensively managed systems may be limited in reflecting short term changes in vegetation and climate conditions. With regard to management, $\delta^{15}N$ changes are faster and, thus, can better capture more recent changes (Fig. 8). Nonetheless, if the target areas are also extensively 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 identified management effects as the main drivers of changes the $\delta^{15}N$ signature of grassland top soils. Earlier studies conducted at different scales also found that increasing land use leads to an increase in the soil $\delta^{15}N$ signature (Mudge et al., 2013; Sieve et al., 2021; Stevenson et al., 2010). Other studies based on single sampling found that other parameters such as PSR effects on N retention and losses seemed to dominate over management effects (Kleinebecker et al., 2014). However, in this study, it has also become evident that the different aspects of management, namely mowing, fertilisation, and grazing, affect the soil $\delta^{15}N$ signature to different extents.

4.2.1. Fertilisation, soil TN, and SOC

We found that fertilisation has a strong positive effect on $\delta^{15}N$ (Fig. 8), which is in line with the findings of other studies (Mudge et al., 2013; Schimel and Bennett, 2004; Watzka et al., 2006). On our sites, average fertilisation between 2011 and 2023 ranged from 0 to 258 kg N ha⁻¹ yr⁻¹ (mean 34.8 kg N ha⁻¹ yr⁻¹). Most of the fertiliser applied was organic fertiliser with up to 205 kg N ha⁻¹ yr⁻¹ (mean 22.1 kg N ha⁻¹ yr⁻¹), whereas mineral fertiliser had a maximum of 88 kg N ha⁻¹ yr⁻¹ (mean 12.7 kg N ha⁻¹ yr⁻¹).

Organic fertiliser consists largely of animal excreta, which generally show a strong positive $\delta^{15}N$ signature with studies finding between 7 and 20‰ (Bedard-Haughn et al., 2003; Choi et al., 2020). 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 (Kriszan et al., 2009). Additionally, organic fertilisers are particularly prone to N losses via ammonia volatilisation, which further enriches ^{15}N in the portion of the fertiliser entering the soil (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 intensively 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 about 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 against ^{14}N 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 N cycling processes and losses. Additionally, with fertilisation-induced higher N availability, plants also fractionate more via higher uptake rates, thereby contributing to an enrichment of soil ^{15}N (Mudge et al., 2013). The combined effect of these fractionating processes induced by increased N availability are reflected in our findings where at higher

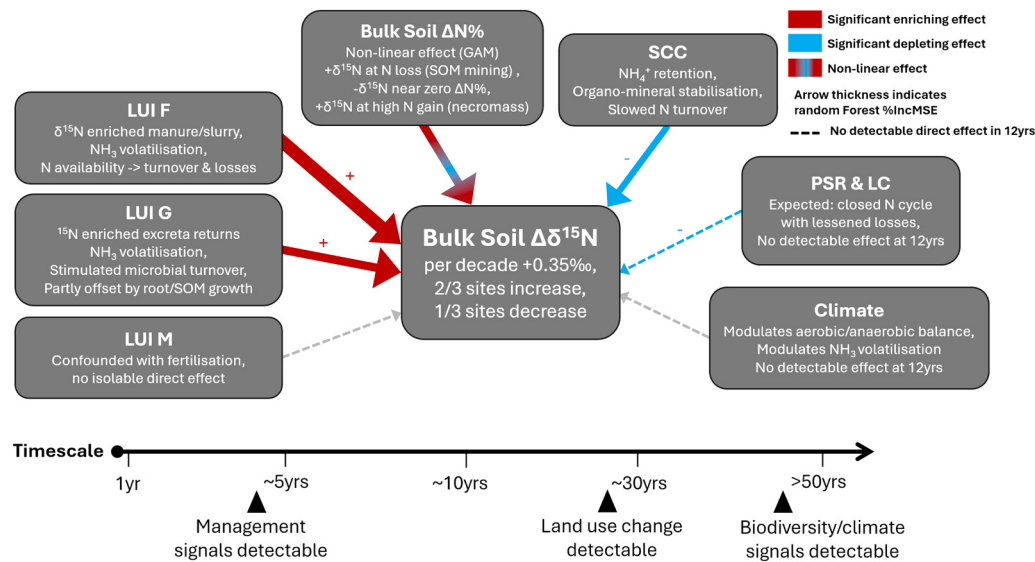


Fig. 8. Synthesis of drivers of bulk soil $\delta^{15}\text{N}$ change between 2011 and 2023 ($\Delta\delta^{15}\text{N}$) in temperate German grasslands. The difference in bulk soil N content between 2011 and 2023 ($\Delta\text{N}\%$) exhibits a non-linear effect (generalised additive model (GAM) smooth, $\text{edf} = 3.14$): $\delta^{15}\text{N}$ increases at negative $\Delta\text{N}\%$ (attributed to soil organic matter (SOM) mining), decreases at moderate positive $\Delta\text{N}\%$, and increases again at higher positive $\Delta\text{N}\%$ (attributed to accumulation of ^{15}N -enriched microbial necromass in mineral-associated organic matter). Land use intensity mowing (LUI M) was confounded with land use intensity fertilisation (LUI F) ($|r| > 0.73$) and could not be isolated. Predictors are ranked by Random Forest %IncMSE (LUI F = 31.1, $\Delta\text{N}\% = 27.2$, land use intensity grazing (LUI G) = 25.4, soil clay content 2011 (SCC) = 14.7). Plant species richness (PSR) and legume cover (LC) as well as climate variables were not statistically detectable at the 12-year resampling interval; their expected mechanistic roles over longer timescales are given inside each box. $\Delta\text{N}\%$ and PSR and LC are themselves partly downstream of management; the figure shows direct relationships with $\Delta\delta^{15}\text{N}$ rather than the full causal web. The timescale axis indicates approximate observation windows over which each class of driver becomes detectable in bulk soil $\delta^{15}\text{N}$, synthesised from this study and from Liao et al. (2006), Mudge et al. (2013), and Stevenson et al. (2010). Quantitative results: Section 3.2; mechanistic interpretation: Section 4.

fertilisation rates an increase in the soil $\delta^{15}\text{N}$ signature was observed.

The accumulation of ^{15}N in soils with higher fertilisation rates is linked to the openness of the N cycle due to increased losses during N turnover and fertiliser application (Bouwman et al., 2002; Craine et al., 2015; Stevenson et al., 2010). Despite an increased N input in intensively managed systems, recent observations in pre-alpine grassland have shown that intensive management with organic fertilisers promotes soil organic N mining compared to extensive management (Schlingmann et al., 2020; Zistl-Schlingmann et al., 2020). In our study, we also found that increasing $\delta^{15}\text{N}$ was related to decreasing TN, suggesting that soil organic N mining and subsequent ^{15}N enrichment associated with higher N losses occurs here as well.

We also observed that sites with an increase in soil $\delta^{15}\text{N}$ exhibited a stronger 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 N (and P) 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). The observed decrease in SOC occurs in parallel to the increase in $\delta^{15}\text{N}$ since both are a result of higher N inputs in intensively managed systems.

In apparent contrast to the argument that SOM mining drives bulk soil ^{15}N accumulation, we also observed an increase in $\delta^{15}\text{N}$ at higher rates of soil TN increase. This occurred across all management categories which indicates that multiple factors are at play here. When SOM is formed and soil TN (and SOC) increase, the stabilising pool is increasingly derived from microbial necromass and processed organic matter.

This material is enriched in ^{15}N relative to plant inputs by $\sim 3\text{--}5\%$: microbial N metabolism preferentially releases ^{14}N -depleted compounds through ammonification and subsequent gaseous or leaching losses, while immobilisation retains the heavier residue in newly formed SOM (Dijkstra et al., 2008; Liang et al., 2017). Repeated mineralisation-immobilisation turnover therefore progressively concentrates ^{15}N in the residual soil pool. This internal fractionation provides a baseline mechanism for coupled increases in TN, SOC, and $\delta^{15}\text{N}$ that operates regardless of management. In fertilised and grazed sites ^{15}N enrichment with SOM formation is reinforced through ^{15}N enriched inputs. Slurry, manure, and faecal returns are strongly ^{15}N -enriched due to volatilisation of ^{14}N -rich ammonia during excretion, storage, and surface application (Dittert et al., 1998; Frank et al., 2004; Watzka et al., 2006). Combined with high gross mineralisation rates and associated fractionating losses, this drives $\delta^{15}\text{N}$ upward as TN accumulates (Denk et al., 2017; Mudge et al., 2013; Stevenson et al., 2010; Wang et al., 2021) (Fig. 8).

4.2.2. Mowing

We did not find a direct effect of mowing on the soil $\delta^{15}\text{N}$ signature, which is in line with other studies (e.g. 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 fertilisation induced increases in biomass production, which in turn results in more frequent mowing events. Therefore, the fertilisation effect observed statistically cannot be considered entirely independent of mowing.

Like fertilisation, mowing 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 reducing the input of C from root exudates (Li et al., 2022; Qiu et al., 2015). We cannot exclude, that the removal of biomass from plants, which preferentially take up ^{14}N (Mudge et al., 2013), impacts the soil $\delta^{15}\text{N}$ signature, however, our data

indicates that fertilisation has the greater effect on $\delta^{15}\text{N}$ changes masking any possible effects mowing might have by itself.

4.2.3. Grazing

We found that grazing led to an increase in soil $\delta^{15}\text{N}$ over time (Fig. 8). This effect can be investigated separately from the effects of mowing and fertilisation, as in our study, grasslands were either predominantly grazed or predominantly fertilised and mown, though a mixture of some grazing and some mowing with fertilisation certainly exists.

Grazing animals consume aboveground biomass, thereby removing N from the system. However, 50–70% of the N removed is returned to the system via manure and urine (Gilmullina et al., 2020). Due to fractionation during metabolic processes, the $\delta^{15}\text{N}$ signature of manure exceeds that of feed (by about 2–5‰), whereas that of urine is usually depleted compared to the diet (Bedard-Haughn et al., 2003; Sieve et al., 2021; Steele and Daniel, 1978). Still, the $\delta^{15}\text{N}$ signature of both exceeds atmospheric natural abundance at 0‰, and with both compounds being very susceptible to gaseous losses, especially ammonia volatilisation, their N input into the system is enriched (Chalk et al., 2019; Frank et al., 2004). Via the return of ^{15}N enriched excreta, grazing contributes to an increase in the bulk soil $\delta^{15}\text{N}$ signature.

Low to moderate grazing and the trampling of animals are also known to stimulate the incorporation of organic matter such as plant litter into the grassland soil, promote plant and root growth, and increase microbial biomass (Cao et al., 2024; Gilmullina et al., 2020; Leriche et al., 2001; Robson et al., 2007; Rumpel et al., 2015; Tälle et al., 2016). All of this leads to increased 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).

Our study confirmed that grazing overall leads to an increase in the soil $\delta^{15}\text{N}$ signature, though the effect was less pronounced than that of fertilisation.

One reason for lower increasing effect of grazing on the soil $\delta^{15}\text{N}$ signature compared to fertilisation is that the above-mentioned incorporation of organic matter into the soil through grazing animals also aids plant growth, both of which lessen losses and thereby subsequent enrichment of soil $\delta^{15}\text{N}$ (Cao et al., 2024; Leriche et al., 2001; Robson et al., 2007; Rumpel et al., 2015; Tälle et al., 2016). Another reason might be the dependence of N inputs from grazing animals on the productivity of the system. Whereas only part of the fodder N is returned in grazing via animal excreta, N inputs in fertilised systems are not dependent system productivity and can even exceed the N removal with mowing.

We may have also observed smaller changes in the soil $\delta^{15}\text{N}$ signature from grazing compared to fertilisation and mowing due to underrepresentation of intensive grazing regimes on our sites. Of the three areas we investigated, only the Schorfheide-Chorin area showed very high levels of grazing. This was reflected in similarly high $\delta^{15}\text{N}$ signatures and temporal changes in $\delta^{15}\text{N}$ in mainly grazed areas there and mainly fertilised areas in the Swabian Alb and Hainich-Dün areas.

4.2.4. Soil clay content

The clay mineral fraction in soil tends to be enriched in ^{15}N compared to the sand fraction, due to its strong association with SOM, which is highly microbially processed and, therefore, enriched in ^{15}N (Ledgard et al., 1984; Liang, 2020; Tiessen et al., 1984; Whalen et al., 2022). In our study, however, we observed a negative relationship between soil clay content and changes of soil $\delta^{15}\text{N}$ over time (Fig. 8). While soil $\delta^{15}\text{N}$ generally increased in most sites due to the much stronger management effect, the rate of increase was reduced with greater clay content. This pattern may partly be explained by the higher capacity of clay-rich soils to retain ammonium (NH_4^+). Soil aggregate formation, promoted in clay-rich soils, enhances the fixation of ammonium within soil microenvironments, thereby restricting its availability for microbial turnover processes such as nitrification and ammonia volatilisation

(Kome et al., 2019). Additionally, the positively charged ammonium ion is strongly adsorbed on the negatively charged surfaces of clay minerals (Govindasamy et al., 2023). Through these combined mechanisms, both ammonium adsorption and fixation in clay-rich, well-aggregated soils reduce the proportion of nitrogen subject to fractionating losses (e.g., nitrification consecutive denitrification and volatilisation) (Govindasamy et al., 2023; Kome et al., 2019). Because these N loss processes preferentially release ^{14}N , diminished turnover leads to a slower rate of ^{15}N enrichment in soil nitrogen pools (Högberg, 1997), which is consistent with the trend observed in our data.

Furthermore, Lu et al. (2025) reported that in humid-climate soils, clay can inhibit nitrogen mineralisation by fostering the formation of stable organo-mineral associations. Similarly, Craine et al. (2015) observed that such associations stabilise organic nitrogen, slowing its mineralisation and reducing the nitrogen losses that would otherwise lead to ^{15}N enrichment via fractionation. The formation of organo-mineral associations with clay has also been shown to correlate with elevated SOC concentrations in soil (Georgiou et al., 2025; Matus, 2021). This finding was confirmed by our study, where soils with higher clay content were also characterised by higher SOC concentrations.

A key mechanism underlying N retention in these systems is the accumulation of microbial necromass which is readily stabilised in mineral-associated organic matter pools (Buckeridge et al., 2022; Zhao et al., 2025). Microbial processing tightly couples carbon and nitrogen cycling, such that both C and N in microbial necromass can become efficiently retained as part of mineral-associated organic matter, especially in clay-rich soils. This form of stabilisation slows overall N mineralisation rates and the associated fractionating losses that contribute to soil ^{15}N enrichment (Buckeridge et al., 2022; Wu et al., 2023).

4.2.5. Plant species richness and legume cover

Gubsch et al. (2011) found a lower soil $\delta^{15}\text{N}$ correlated to sites with higher PSR four years after sowing. However, within-treatment changes over those four years did not show a correlation between PSR and $\delta^{15}\text{N}$. Similarly, Kleinebecker et al. (2014) identified PSR as a major driver of soil $\delta^{15}\text{N}$ in the same German temperate grassland regions we studied, based on a single bulk soil $\delta^{15}\text{N}$ measurement campaign in 2011. In contrast, our analysis of changes in soil $\delta^{15}\text{N}$ from 2011 to 2023 did not reveal a direct effect of long-term mean PSR on these changes (Fig. 8). This discrepancy likely arises from differing approaches: Kleinebecker et al. (2014) compared differences between sites in a single year whereas our study examined temporal changes within the same sites over a 12-year period. While Kleinebecker et al. (2014) relied on five years of management history and one-time PSR and $\delta^{15}\text{N}$ measurements without prior $\delta^{15}\text{N}$ baseline data, we analysed changes in $\delta^{15}\text{N}$ from 2011 to 2023 and used both PSR and management data spanning those 12 years. Furthermore, land use intensity itself influences PSR, as intensive management typically reduces PSR through competitive exclusion and disturbance (Blüthgen et al., 2012; Neuenkamp et al., 2025; Socher et al., 2012), meaning that PSR may partly reflect the legacy of management rather than acting as an independent driver of soil $\delta^{15}\text{N}$.

Differences in available data may have further contributed to the discrepancy between our findings and those of Kleinebecker et al. (2014). 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 readily available. Such variability in data availability and precision could easily lead to statistical models selecting PSR over management. Because models tend to assign greater weight to variables that are measured more precisely and have fewer missing values, PSR data can end up appearing more influential simply due to its higher quality. In contrast, the noisier and less complete management histories introduce uncertainty that attenuates their estimated effects, making them less likely to be selected even when they are biologically important. 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 $\delta^{15}\text{N}$ changes over this 12-year study period.

Despite the fact that higher legume cover leads to more BNF and therefore a higher input of N with $\delta^{15}\text{N}$ 0‰ into the system (Gubsch et al., 2011), we did not find a significant effect of legume cover on the change in $\delta^{15}\text{N}$ (Fig. 8). However, we found a higher mean legume cover in the group with decreasing soil $\delta^{15}\text{N}$ though the difference was not statistically different ($p = 0.084$) from the increase group. The decrease group showed a higher mean legume cover than the increase group. This also connects to the group's lower fertilisation rate, at which BNF (with a $\delta^{15}\text{N}$ signature near 0‰) contributes a larger portion to the total N input as compared to the fertilised and less species-rich grasslands (Craine et al., 2015; Qiu et al., 2015).

Moreover, at high fertilisation rates both legume cover and symbiotic N_2 fixation by legumes decrease, leading to potential indirect effects of fertilisation on N isotopic composition of organic matter input to the soil (Nyfeler et al., 2011). This underlines that fertiliser N inputs have a higher and much more prominent impact on $\delta^{15}\text{N}$ changes in the bulk soil, which, if present, lessen and outweigh $\delta^{15}\text{N}$ decreases from BNF.

4.3. Study limitations

Several limitations of this study should be acknowledged. First, our resampling interval of 12 years captures decadal-scale change but cannot resolve the multi-decade trajectories at which biodiversity- or climate-driven signals are expected to emerge; extrapolation of the rates we report to substantially longer or shorter timescales should therefore be made with caution. Second, mowing and fertilisation were strongly correlated across our sites, reflecting linked management cycles in intensively managed grasslands, which means that the statistical effect we attribute to fertilisation cannot be considered entirely independent of mowing. Third, our study is restricted to three regions of temperate German grasslands; while these span a substantial climatic and management gradient, generalisation to grasslands under markedly different climates, soil types, or management traditions requires further work. Fourth, while we identified management as the dominant driver of $\delta^{15}\text{N}$ changes, we did not directly measure the underlying mechanistic pathways such as gross mineralisation, gaseous losses, ammonia volatilisation, microbial necromass accumulation, or root-derived C inputs. Nor did we quantify the interactions among drivers that shape how these pathways operate in the field. Our inferences regarding these processes rest on the integration of our results with mechanisms established in the literature. Direct process measurements alongside $\delta^{15}\text{N}$ tracing ideally across the full web of coupled management, vegetation, climate, and soil processes, would strengthen the causal attribution in future studies. A fifth, related limitation is that the dominance of management we report does not preclude meaningful interactions with the other drivers we examined. Land use intensity itself shapes plant species richness and legume cover, with lower intensity (particularly less fertilisation and mowing) supporting higher biodiversity (Allan et al., 2015; Midolo et al., 2019) and higher legume cover, which in turn modulates N inputs via BNF (Nyfeler et al., 2011; Watzka et al., 2006). Climate further conditions how management effects translate into $\delta^{15}\text{N}$ changes: higher precipitation alters the balance of aerobic and anaerobic N processes through its effect on water- and air-filled pore space (Elrys et al., 2021b; Tall et al., 2019), while higher temperatures accelerate microbial N turnover and ammonia volatilisation which are the loss pathway with the strongest fractionation (Bouwman et al., 2002; Elrys et al., 2021b; Kou et al., 2020). Our analysis isolates the dominant signal but should not be read as evidence that these interactions are absent.

5. Conclusion

This work precisely quantifies the direction and magnitude of changes of soil nitrogen isotopic signatures in differently managed

temperate grasslands of Germany. Overall, our results confirmed that management activities - particularly fertilisation and grazing - significantly increase bulk soil $\delta^{15}\text{N}$ signatures in grassland top soils, but at relatively slow rates. Fertilisation and grazing effects overrule the influences of climate, vegetation, and soil clay content. Plant biodiversity-induced changes in soil $\delta^{15}\text{N}$ observed on extensively managed sites were very gradual and can only be detected at time spans covering several decades.

These findings carry three practical implications for future studies using bulk soil $\delta^{15}\text{N}$ as an indicator. First, baseline $\delta^{15}\text{N}$ measurements or detailed long-term management records are a prerequisite for interpretation: without them, single-time $\delta^{15}\text{N}$ values cannot be separated into their initial state and the change driven by management or other factors. Second, $\delta^{15}\text{N}$ is most useful as a short- to medium-term indicator (several years to a decade) in intensively managed systems or following drastic management changes, where signals are detectable on these timescales. Third, in extensively managed systems and for biodiversity-mediated effects, $\delta^{15}\text{N}$ changes are too slow to resolve at decadal timescales, and multi-decade observation periods together with historical baselines are required to detect them robustly.

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CRedit authorship contribution statement

Michael Schlöter: Writing – review & editing, Project administration, Funding acquisition. **Rudi Schäufele:** Writing – review & editing, Methodology, Data curation. **Marion Schruppf:** Writing – review & editing, Project administration. **Ingo Schöning:** Writing – review & editing, Data curation. **Pacay-Barrientos Narda Lucia:** Writing – review & editing, Data curation. **Till Kleinebecker:** Writing – review & editing, Data curation. **Valentin H. Klaus:** Writing – review & editing, Data curation. **Theresa Schwärzler:** Writing – review & editing, Formal analysis, Data curation. **Julia Kepp:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation, Conceptualization. **Stefanie Schulz:** Writing – review & editing, Project administration, Funding acquisition. **Dannemann Michael Ulrich:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Steffen A. Schweizer:** Writing – review & editing, Project administration, Funding acquisition. **Ralf Kiese:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, 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.2026.110651](https://doi.org/10.1016/j.agee.2026.110651).

Data availability

Data will be made available on request.

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