

Long-term management effects on depth gradients of ^{13}C , ^{15}N and C/N ratio in agricultural soils

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ABSTRACT

Agricultural management practices influence the turnover and residence time of soil organic matter (SOM) and thus can contribute to carbon (C) removal from the atmosphere. However, advanced analytical techniques are needed to disentangle the interlinked processes of SOM stabilisation and destabilisation, as well as its built-up and decomposition. Stable isotopes of C ($\delta^{13}\text{C}$) and nitrogen (N; $\delta^{15}\text{N}$) as well as the carbon-to-nitrogen (C/N) ratio provide information about the quality and thus origin and turnover SOM. The aim of this study was to quantify the effect of different agricultural management practices (mineral fertilisation, farmyard manure (FYM) application, straw incorporation, crop rotations, liming, irrigation and reduced tillage) on these indicators in topsoil and subsoil. Ten German long-term experiments (LTEs) were sampled down to 100 cm depth. Changes in SOC and N content were assessed as indicators of SOM quantity and changes in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C/N ratios were assessed as indicators of SOM quality. Increases in C and N content were mainly affected in topsoil by mineral fertilisation, FYM, straw and irrigation. Changes in $\delta^{13}\text{C}$ were limited to crop rotations (+0.62 ‰) and FYM (−0.27 ‰) in topsoil, but liming effects also reached down to 70 cm (−0.46 ‰ on average). Mineral fertilisation reduced the $\delta^{15}\text{N}$ values in topsoil (−0.12 ‰), while the application of FYM increased them (+0.53 ‰), indicating different N sources. The $\delta^{15}\text{N}$ values in the subsoil changed due to crop rotations (+0.62 ‰) and reduced tillage (−0.96 ‰). Specific management practices did not change C/N ratios significantly, with the exception of NPK fertilisation in topsoil (−1.32). Variations in SOC and total N content were more than 50 % greater in subsoil than in topsoil, but ^{13}C and ^{15}N did not follow this trend, indicating a decoupling of changes in C and N content and changes in SOM quality. This study revealed challenges in the detection of subsoil effects due to the low SOM content that in some LTEs unexplained large spatial variability in $\delta^{15}\text{N}$ in subsoil. Since agricultural management practices change ^{13}C , ^{15}N and C/N ratios at least in topsoil, these variations need to be considered in isotope studies.

1. Introduction

Soil organic matter (SOM) is a key parameter for soil health and productivity. It is related to almost all soil functions, such as nutrient cycling, water retention and carbon (C) sequestration (Lal, 2016; Lehmann et al., 2020; Peichl et al., 2006; Wade et al., 2020). The quality of SOM determines its mineralisation rate and thus the release and supply of nutrients for plant growth (Bosatta and Ågren, 1999). Hence, careful management of SOM quantity and quality is key to sustainable agricultural management.

Numerous studies have documented the effects of different

agricultural management practices on soil organic C (SOC) stocks and SOM quality (see e.g. Bai et al., 2018; Haddaway et al., 2015; Osana et al., 2021; Söderström et al., 2014, for a review), but most of these studies focused on topsoil (e.g. Gocke et al., 2023). There is growing evidence that subsoil organic matter (OM) can also be affected by the fertilisation or tillage regime (Hobley et al., 2018; Skadell et al., 2023), and thus also the uptake of water and nutrients from lower depths (e.g. Gaiser et al., 2012; Kautz et al., 2013; Uhlig et al., 2023). Consequently, the effects of agricultural management practices on SOM storage and turnover in subsoils or even on a whole profile basis require greater attention, particularly given that subsoil is still

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underrepresented in soil science studies (Yost and Hartemink, 2020). Nevertheless, it remains unclear how different agricultural management practices affect the properties of SOM along the soil profile.

In general, the degree of SOM decomposition, and thus its age, advances with increasing soil depth (e.g. Balesdent et al., 1988; Kögel-Knabner and Amelung, 2021). The increased turnover of SOM usually results in isotope fractionation processes, leaving the heavier ^{13}C and ^{15}N isotopes behind and thus resulting in higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values with advancing stages of SOM decay (Boutton, 1996; Krüger et al., 2024). These processes can be influenced by environmental conditions such as the Suess effect, which describes the dilution of SOM formed from lighter ^{12}C from fossil fuel combustion (Graven et al., 2017), or a ^{13}C -depletion in plant leaves during droughts (Farquhar et al., 1989; Peters et al., 2018; Yi and Yano, 2022), which could contribute to lower $\delta^{13}\text{C}$ values in the surface soil. In agricultural soils, fractionation processes are also influenced by management (Desrochers et al., 2022; Gregory et al., 2016). Organic fertilisers, for example, increase $\delta^{13}\text{C}$ signatures as they are often enriched in ^{13}C due to enhanced degradation of OM (Bol et al., 2005). Naturally the most drastic changes in soil $\delta^{13}\text{C}$ values occur with C_3/C_4 vegetation changes (Balesdent et al., 1987; Boutton, 1996; Krüger et al., 2024).

Similar to the soil $\delta^{13}\text{C}$ values, the soil $\delta^{15}\text{N}$ signature of SOM also commonly increases with depth due to isotope fractionation with advancing SOM decomposition (Krull and Skjemstad, 2003; Nadelhoffer and Fry, 1988). As almost all processes involved in the cycling of N result in a loss of the lighter ^{14}N , such as nitrification, denitrification, ammonia volatilisation and leaching (Högberg, 1997), subsoils are commonly enriched in ^{15}N relative to topsoils (Krull and Skjemstad, 2003; Nadelhoffer and Fry, 1988). Similarly, upon fertilisation and with OM amendment, $\delta^{15}\text{N}$ values commonly rise with increasing N loss, making changes in soil $\delta^{15}\text{N}$ a suitable indicator of N-use efficiency and thus likely of the role of different N management on SOM dynamics as well (Kriszan et al., 2009; Kriszan et al., 2014).

Another indicator of such changes is the carbon-to-nitrogen (C/N) ratio, the depth profiles of which are to an extent specific for different soils and management regimes (Schneider et al., 2021). The C/N ratio generally decreases with the increasing transformation of plant-derived OM because microbes take up N while respiring atmospheric C dioxide (CO_2). Therefore, C/N ratios generally decrease with increasing soil depth (Lou et al., 2012). Moreover, external biomass inputs impact the soil C/N ratio. For instance, straw frequently exhibits higher C/N ratios than animal manure (Dannehl et al., 2017). The C/N ratio of plant residues remaining in the field can be influenced by the availability of N, and therefore decreases with increasing N fertilisation (Wang et al., 2021; Yang et al., 2022).

Isotopic signatures are used as tracers in soil science to reveal soil-related processes and different sources of OM and other inputs to soils (Krüger et al., 2024). Agricultural soils are open systems with large input and output fluxes, for example the input of OM or mineral N through fertilisation. These inputs have different isotopic fingerprints and can be traced by isotopes. Since agricultural management practices can affect these tracers in soils under agricultural use, their turnover and fate may bias the interpretations and results of isotopic studies if not given sufficient consideration. Consequently, a further aim of this study was to quantify the effects of agricultural management practices on isotopic tracers in order to interpret results correctly. Therefore, ten German LTEs were sampled at 0–100 cm with the same sampling design to determine the effects of common Central European agricultural management practices (e.g. mineral and organic fertilisation, irrigation, liming and reduced tillage) on isotopic signatures, C/N ratios, and SOC and total N contents. Furthermore, the aim was to show whether these effects are visible beyond the topsoil and how changes in isotopic signatures and C/N ratios are related to changes in C and N contents. However, this study should be considered as descriptive since it is only possible to attribute changes in the isotopic signature to specific underlying processes to a limited extent.

2. Material and Methods

2.1. The long-term experiments

Table 1 gives an overview of the ten sampled LTEs in Germany (see also Gocke et al. (2023) and Skadell et al. (2023) for details). The agricultural management practices investigated included the combination of NPK fertilisation, irrigation, crop rotations with either maize (C_4 crop) or legumes (C_3 crops: crimson clover and faba bean), incorporation of straw, application of FYM, liming, and reduced tillage depth (Table 2). All sampled soils were conventionally ploughed to a depth of 30 cm, except for the soils from the reduced tillage treatments, where the soils were tilled to 10 cm and 17 cm depth (Table 2). The soil samples were classified as Luvisols and Cambisols according to the *World Reference Base* system for soil classification. The average annual temperature was between 8.1 and 9.7 °C, and the average annual precipitation ranged from 510 to 820 mm. Soil samples were collected in 2016, 2017 and 2019, and the LTEs had been running for between 32 and 112 years, with a mean of 58 years.

2.2. Soil sampling and sample analysis

All the soil samples were taken with a percussion auger (6 cm diameter) from a depth of 0–100 cm. The soil cores were cut into four depth levels: 0–30 cm, 30–50 cm, 50–70 cm and 70–100 cm. Additional subdivisions if the soil depth increments were made when the horizon boundaries were visible within the specified depth intervals and an additional soil sample was collected. Soil material collected within a plot (one to three plot-level replicates) was composited by depth interval. The additional soil samples taken were aggregated into the four specified depth intervals using weighted mean values, with the fine soil stock of the respective layer used as a weighting factor. After drying at 40 °C and sieving to < 2 mm, the soil samples were milled and subsequently weighed to derive the fine soil mass. With an elemental analyser (EuroEA 3000, HEKAtech, Germany), total C and total N contents were determined via dry combustion. Inorganic C content was determined by adding 4 M hydrochloric acid to the soil which released carbon dioxide from carbonates. The volume of carbon dioxide released was measured by using calcimetry and converted to mass of inorganic C per fine soil mass following ISO 10693 (1995). The difference between total C and inorganic C content provided the SOC content of the soil samples. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were measured with an isotope-ratio mass spectrometer (IRMS, Thermo Fisher Scientific Delta plus). The isotope data are given in the usual δ notation as deviation in per mil (‰) from the Vienna Pee Dee Belemnite for $\delta^{13}\text{C}$ and from atmospheric N for $\delta^{15}\text{N}$. Modified from Walthert et al. (2010), soil samples with inorganic C were fumigated with 37 % (12 M) hydrochloric acid (HCl) prior to $\delta^{13}\text{C}$ measurement. Inorganic C removal was repeated, and $\delta^{13}\text{C}$ measured again if total C content determined by IRMS was higher than the total organic C (OC) determined by dry combustion. Thus, $\delta^{13}\text{C}$ values correspond to OC and $\delta^{15}\text{N}$ values to total N. Soil pH and electrical conductivity were measured in a suspension of soil and water at a ratio of 1:4. Sand, silt and clay contents were derived via visible near-infrared light reflectance spectroscopy (VNIR) by applying models that were trained on a representative subset of soil samples from all the LTEs (Hobley and Prater, 2019).

2.3. Coefficients of variation and response ratios for quantity and quality proxies

In order to compare the extent to which agricultural management practices influence variations in SOM quantity (SOC content [%], total N content [%]) and quality (^{13}C [atm%], ^{15}N [atm%] and C/N ratio), coefficients of variation (CVs) were calculated for each of the five variables across sites and depth intervals per treatment. First, only the reference sites were selected in order to calculate the natural variation (CV0) of

Table 1

Overview of the sampled long-term experiments (LTEs) with their site properties and environmental conditions. MAT = mean annual temperature; MAP = mean annual precipitation; WRB = World Reference Base system for soil classification. For further information on LTEs, sampling design and associated literature, see Skadell et al. (2023).

| LTE name used in this study | Official name of LTE | Latitude | Longitude | MAT [°C] | MAP [mm yr ⁻¹] | Major soil group (IUSS, 2015) | Texture (according to WRB) | Parent material | Start year |
|-----------------------------|---|----------|-----------|----------|----------------------------|-------------------------------|----------------------------|---------------------------------|------------|
| Dikopshof | Static Long-term Experiment DDV at Dikopshof | 50.81 | 6.95 | 9.7 | 634 | Luvisol | Silty loam | Loess | 1904 |
| Dahlem | Static Soil Management Experiment BDa_D3 at Berlin-Dahlem | 52.47 | 3.30 | 9.6 | 540 | Luvisol | Loamy sand | Periglacial sand | 1923 |
| Thyrow 1 | Static Fertilisation and Irrigation Experiment Thy_D1 at Thyrow | 52.25 | 13.23 | 9.2 | 510 | Luvisol | Sand | Periglacial sand | 1937 |
| Thyrow 2 | Static Nutrient Deficiency Experiment Thy_D41 at Thyrow | 52.25 | 13.24 | 9.2 | 510 | Luvisol | Sand | Periglacial sand | 1937 |
| Gießen 1 | Nutrient Depletion Experiment NDE at Gießen | 50.60 | 8.65 | 9.0 | 650 | Cambisol | Silty clay | Floodplain sediments | 1954 |
| Müncheberg | Fertilisation and Nutrient Gradient Experiment V140 at Müncheberg | 52.52 | 14.12 | 8.4 | 528 | Luvisol | Loamy sand | Aeolian sands over glacial till | 1963 |
| Göttingen | Soil Tillage Experiment Garte-Süd GS (Reinschhof) at Göttingen | 51.50 | 9.94 | 8.7 | 645 | Luvisol | Clayey loam | Loess | 1970 |
| Dürnast | Liming Experiment Dürnast D-II at Freising-Weihenstephan | 48.06 | 11.07 | 8.4 | 820 | Cambisol | Sandy loam to loam | Cover sand | 1978 |
| Gießen 2 | Biological Nitrogen Fixation Trial BNF at Gießen | 50.60 | 8.65 | 9.0 | 650 | Cambisol | Silty clay | Floodplain sediments | 1982 |
| Rauischholzhausen | International Organic Nitrogen Fertilisation Experiment IOSDV at Rauischholzhausen | 50.76 | 8.87 | 8.1 | 595 | Luvisol | Silty loam | Alluvial sediments | 1984 |

the sites without management effects (Eq. (1)). Subsequently, CVs for the combination of treatment and reference plots (CV01) were calculated in order to sum up the human-induced variation due to agricultural management practices (Eq. (2)). Finally, the natural logarithm of the response ratio (LRR) between CV01 and CV0 (Eq. (3)) was calculated in order to evaluate how changes in isotopic signature and C/N ratio are related to changes in C and N contents, and to compare the strength of management effects on SOM quantity and SOM quality.

$$CV_0 = \frac{sd_{Ref}}{\bar{x}_{Ref}} \times 100\% \quad (1)$$

$$CV_{01} = \frac{sd_{comb}}{\bar{x}_{comb}} \times 100\% \quad (2)$$

$$LRR = \log\left(\frac{CV_{01}}{CV_0}\right) \quad (3)$$

2.4. Statistical analysis

To analyse the relationship between agricultural management practice (= treatment) and SOC content, $\delta^{13}C$ signature, total N content, $\delta^{15}N$ signature and C/N ratio, linear mixed-effect models were fitted with the *lme4* package (Bates et al., 2015). To test a particular treatment (binary variable), plots were selected that had been fertilised with NPK and differed only in the treatment being tested. Plots fertilised with NPK were compared with plots without NPK. With an interaction term, treatment and depth were recorded as fixed effects. The various depth increments of the soil core were regarded as paired samples. To ensure independent observations, random intercept and slope models with random intercepts for sites and random slopes for depth levels were selected using the smallest AIC value. Normal quantile–quantile and residual plots were inspected visually and with the R package *DHARMA* (Hartig, 2016, version 0.4.6) to identify deviations from normality or homoscedasticity. The *emmeans* package (Lenth, 2017, version 1.8.6) (derived estimated marginal means of the tested treatments, as well as contrasts (treatment minus reference) per depth level (0–30 cm, 30–50 cm, 50–70 and 70–100 cm) and per target variable (SOC and total N content, $\delta^{15}N$ and $\delta^{13}C$ signatures, C/N ratio). Mean values are presented with their standard errors. Significance was set at a level of $\alpha =$

0.05. All statistical analyses were performed in R version 4.4.0.

3. Results

3.1. Carbon content and $\delta^{13}C$ values of soil organic matter

The $\delta^{13}C$ values increased with soil depth at all sites under all agricultural management practices (Fig. 1). Significant effects on $\delta^{13}C$ were detectable for the FYM treatment at 0–30 cm, with a significant reduction of -0.27% (Figs. 1 and 2). The comparison of crop rotations with either maize (C₄ crop) or legumes (crimson clover and faba bean, C₃ crops) included significantly increased $\delta^{13}C$ values in topsoil (0.62 ‰), but the changes in subsoil were non-significant with an increase of 0.17 ‰ on average (Fig. 2). The impact of liming on $\delta^{13}C$ was significant down to 70 cm, resulting in a decrease in $\delta^{13}C$ values of -0.57% (0–30 cm), -0.45% (30–50 cm) and -0.35% (50–70 cm). Nevertheless, these results demonstrated a positive effect at one of the four tested LTEs in one depth increment (Dahlem, 0–30 cm) where there was an increase of 0.38 ‰ (Fig. A1, Appendix A1). Irrigation significantly affected $\delta^{13}C$ values only at a depth of 30–50 cm, with a difference of -0.33% .

SOC content decreased with depth at all sites for all agricultural management practices (Fig. 1). Mineral fertilisation, irrigation, straw incorporation and manure resulted in significantly higher SOC contents in topsoil compared with the respective references, ranging from 0.7 g kg⁻¹ (NPK) to 1.5 g kg⁻¹ (FYM).

3.2. N content and $\delta^{15}N$ values of soil organic matter

Significant differences in the N content of topsoil were detectable for FYM application (0.13 g kg⁻¹), NPK fertilisation (0.11 g kg⁻¹), straw incorporation (0.07 g kg⁻¹) and irrigation (0.08 g kg⁻¹). Along with these changes, soil $\delta^{15}N$ values also changed in part, but the alterations to the $\delta^{15}N$ values depth profiles were non-continuous: they initially increased to a maximum between 30 cm and 70 cm, but then decreased again. This pattern was observed at all sites, regardless of the agricultural management practices (Fig. 1). Mineral NPK fertilisation significantly reduced the $\delta^{15}N$ values by -0.12% in topsoil, but did not alter the $\delta^{15}N$ values in subsoil. The application of FYM significantly increased $\delta^{15}N$ values in topsoil ($+0.53\%$) relative to the reference that had only been fertilised with NPK. In contrast, the $\delta^{15}N$ values in subsoil

Table 2

Agricultural management practices of the studied long-term experiments (LTEs) with application rates/treatment levels and respective references. N = nitrogen; P = phosphorus; K = potassium.

| Agricultural management practice | LTE | Reference | Treatment |
|---|---|---|--|
| NPK fertilisation | Dikopshof | N: 0 kg ha ⁻¹ yr ⁻¹ P ₂ O ₅ : 0 kg ha ⁻¹ yr ⁻¹ K ₂ O: 0 kg ha ⁻¹ yr ⁻¹ | N: 46, 70, 94 kg ha ⁻¹ yr ⁻¹ P ₂ O ₅ : 70, 100, 130 kg ha ⁻¹ yr ⁻¹ K ₂ O: 140, 200, 260 kg ha ⁻¹ yr ⁻¹ |
| | Thyrow 2 | | N: 60, 90 Mg ha ⁻¹ yr ⁻¹ P ₂ O ₅ : 55 kg ha ⁻¹ yr ⁻¹ K ₂ O: 125 kg ha ⁻¹ yr ⁻¹ |
| | Gießen 1 | | N: 180 kg ha ⁻¹ yr ⁻¹ P ₂ O ₅ : 45, 90 kg ha ⁻¹ yr ⁻¹ K ₂ O: 60, 120 kg ha ⁻¹ yr ⁻¹ |
| | Gießen 2 | | N: 180 kg ha ⁻¹ yr ⁻¹ P ₂ O ₅ : 45 kg ha ⁻¹ yr ⁻¹ K ₂ O: 60 kg ha ⁻¹ yr ⁻¹ |
| Irrigation | Thyrow 1 (Irrigation as experimental factor since 1969) | 0 mm yr ⁻¹ | 20–484 mm yr ⁻¹ (1971–2016), median 104 mm yr ⁻¹ |
| Liming | Dikopshof | CaO: 0 kg ha ⁻¹ yr ⁻¹ | CaO: 800 kg ha ⁻¹ yr ⁻¹ Liming to pH 6.0–6.4, liming to pH 6.8–7.0 |
| | Dürnast Dahlem Thyrow 2 | | CaO: 263 kg ha ⁻¹ yr ⁻¹ CaO: 157 kg ha ⁻¹ yr ⁻¹ |
| Crop rotation with C ₄ crops or legumes (= year-round C ₄ crop maize or legumes in rotation with cereals winter wheat, winter rye and summer barley) | Gießen 2 | Crimson clover (<i>Trifolium incarnatum</i>) and faba bean (<i>Vicia faba</i>), followed by three subsequent crops: winter wheat (<i>Triticum aestivum</i> ssp. <i>aestivum</i>), winter rye (<i>Secale cereale</i>) and summer barley (<i>Hordeum vulgare</i>) | Maize (<i>Zea mays</i>), followed by three subsequent crops: winter wheat (<i>Triticum aestivum</i> ssp. <i>aestivum</i>), winter rye (<i>Secale cereale</i>) and summer barley (<i>Hordeum vulgare</i>) |
| | Incorporation of straw | Thyrow 1 (Straw incorporation as experimental factor since 1978) | 0 Mg ha ⁻¹ yr ⁻¹ |
| Farmyard manure | Müncheberg | | 2 Mg ha ⁻¹ yr ⁻¹ dry matter |
| | Dikopshof Dahlem (FYM as experimental factor since 1939) | 0 Mg ha ⁻¹ yr ⁻¹ | 120 Mg ha ⁻¹ yr ⁻¹ 30 Mg ha ⁻¹ yr ⁻¹ |
| | Müncheberg Rauischholzhausen | | 10 Mg ha ⁻¹ yr ⁻¹ 3.2 Mg ha ⁻¹ yr ⁻¹ |
| Reduced tillage | Dahlem | 30 cm | 17 cm |
| | Göttingen | 28 cm | 10 cm |

were not significantly affected by FYM application. The comparison of crop rotations with either maize or legumes resulted in increased $\delta^{15}\text{N}$ values in 0–100 cm, with an average increase of 0.30 ‰, although only significantly so with an increase of 0.62 ‰ at 50–70 cm soil depth. Reduced tillage depth significantly lowered the $\delta^{15}\text{N}$ value at 30–50 cm depth by -0.96 ‰ (Fig. 2), while all other changes in $\delta^{15}\text{N}$ under the tillage treatment were not significant.

3.3. Carbon-to-nitrogen ratio

The C/N ratio generally decreased with soil depth at all sites and under all agricultural management practices (Fig. 1). However, differences between treatment and reference soils were inconsistent across the depth profiles, resulting in either positive or negative shifts, regardless of the agricultural management practice. Only NPK fertilisation showed a significant difference between treatment and reference in topsoil, with a -1.3 lower ratio in the plots fertilised with NPK (Fig. 2).

3.4. Comparison of the variation observed in proxies for quantity and quality

Fig. 3 illustrates the natural variability observed at every LTE using all reference samples of the seven analysed agricultural management practices. The mean CV0 of SOC was 15 % in topsoil and exhibited an increase in subsoil, reaching a mean value of 25 % at a depth of 30–100 cm. The CV0 for ¹³C remained constant at a value of 0.03 % up to a depth of 70 cm, before increasing to 0.04 % at the lowest depth increment.

Similarly, the CV0 of N showed a constant value of 21 % in subsoil, which was higher than the mean CV0 of 15 % observed in topsoil. The CV0 for ¹⁵N revealed minimal variation across the soil profile, with a mean value of 0.07 % across all depth intervals. However, a notable exception was observed in the 50–70 cm depth interval, where the CV0 reached 0.09 %. This value was 0.03 % higher than the mean of the other three depth intervals. The CV0 of the C/N ratio exhibited an increase from the topsoil (7 %) down to a depth of 50 cm, where it remained constant until 100 cm depth, reaching a value of 17 %.

Fig. 4 illustrates the management-induced variability observed at every LTE with all treatment and reference samples used to analyse the effects of agricultural management practices. The patterns of CV01 did not generally differ from those of CV0 (Fig. 3), but the mean values of CV01 were consistently higher, with two exceptions for N content in 30–70 cm and C/N ratio in 50–70 cm where the mean values of CV01 were lower. The CV01 values for SOC were on average 1.8 % points higher, with the greatest increase in variation in 70–100 cm (+3.8 % points). For ¹³C, CV01 values increased, but negligibly so by on average 0.004 % points. CV01 values of N content increased on average by 0.18 % points, while CV01 of ¹⁵N revealed no change in variation due to the management. The CV01 values for the C/N ratio increased by 1.3 % points.

With the LRRs shown in Fig. 5, the strength of the influence of agricultural management practices on the analysed proxies for quantity and quality can be compared, i.e. showing whether SOM quantity or SOM quality reacts more strongly to management.

The mean LRRs for ¹³C were highest in topsoil (0.35) and decreased

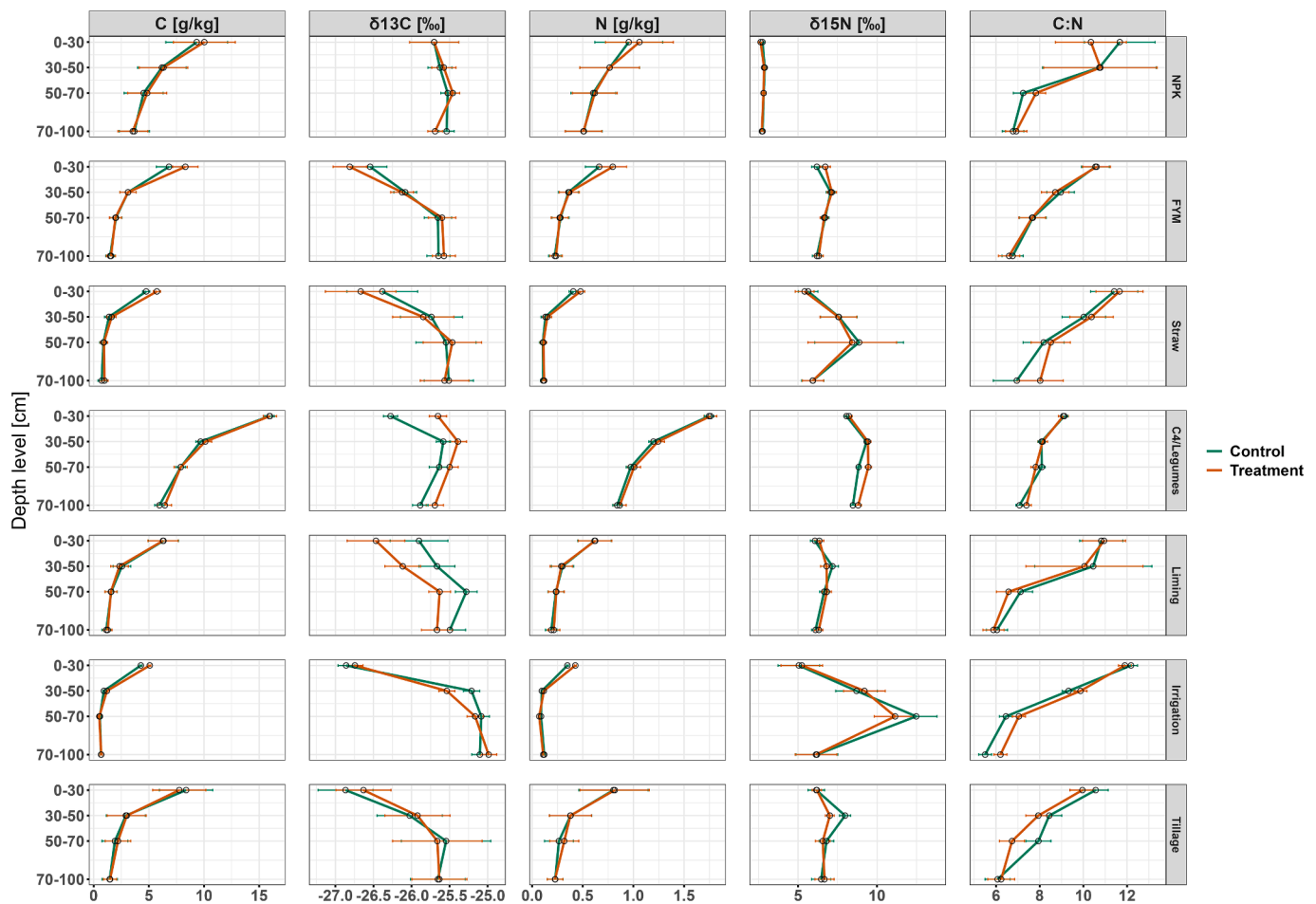


Fig. 1. Depth profiles of reference and treatment soils (mean \pm standard error) under different types of agricultural management [combined nitrogen, phosphorus and potassium (NPK) fertilisation ($n = 4$); irrigation ($n = 1$); comparison of crop rotations with either maize (C_4 crop) or legumes (crimson clover, faba bean) ($n = 1$); incorporation of crop residues left on the field (Straw) ($n = 4$); application of farmyard manure (FYM) ($n = 4$); liming ($n = 4$); reduced tillage depth (Tillage) ($n = 2$)] on soil organic carbon (C), $\delta^{13}\text{C}$ signature, total nitrogen (N) content, $\delta^{15}\text{N}$ signature and carbon-to-nitrogen (C/N) ratio per depth increment.

with depth to 0.08. For SOC, mean LRRs were constant in 0–50 cm and 70–100 cm with a mean LRR value of 0.16, but this figure was zero in 50–70 cm. ^{13}C exhibited a more pronounced response to agricultural management practices down to 70 cm depth, since mean LRRs were higher or zero (70–100 cm). When comparing mean LRRs of ^{15}N and N content, the results revealed no difference down to 70 cm. In 70–100 cm, N content responded more strongly (0.13) compared with ^{15}N . On average, LRRs for the C/N ratio were highest in 0–30 cm (0.2) and 70–100 cm (0.3), and slightly lower in 30–70 cm (0.13), thus also showing stronger responses of quality proxies to agricultural management practices. The underlying data used in Figs. 3, 4 and 5 can be found in Appendix A2.

3.5. Site-specific variability

There was high subsoil variability at the sandy sites of Dahlem and Thyrow 1. Increments at 30–40 cm in Dahlem and 59–70 cm depth in Thyrow 1 showed disproportionately high $\delta^{15}\text{N}$ values of 42 ‰ and 25 ‰ respectively. Thus, these values far exceeded average $\delta^{15}\text{N}$ values of 5.6 ‰ to 6.6 ‰ in mineral soils (Schneider et al., 2021). At Dahlem, in particular, nine out of 48 plots showed $\delta^{15}\text{N}$ values of between 10 ‰ and 42 ‰ (mean: 19 ‰), especially in 30–40 cm depth (Fig. 4). The $\delta^{15}\text{N}$ values of most of the nine extreme plots were also elevated at shallower depths (0–15 cm and 15–30 cm depth), as shown in Figs. A3 and A4 (Appendix A1). The high $\delta^{15}\text{N}$ values in Dahlem were limited to the north-eastern edge of the field experiment (Fig. 6). A high variability in

subsoil $\delta^{15}\text{N}$ values was also detected at the other sandy site at Thyrow 1, especially at a depth of 50–70 cm (Fig. 7). Half of the 18 sampled and analysed plots showed $\delta^{15}\text{N}$ values above 14 ‰ (mean: 19 ‰). The mean $\delta^{15}\text{N}$ value of the other nine plots was 7 ‰. High $\delta^{15}\text{N}$ values (> 20 ‰) were observed in Thyrow 1 in the eastern side of the field and in one plot (plot number 211) on its western side. The distribution of these high values was not as concentrated in a specific area as it was in Dahlem. To avoid analytical uncertainties and potential artefacts of low isotopic signals, the samples with unexpectedly high $\delta^{15}\text{N}$ values were measured multiple times (two to five times) with varying preparation weights and on different dates. In addition to depth gradients and the effects of long-term agricultural management practices, heterogeneity at field scale was found to strongly influence $\delta^{15}\text{N}$ values. No such heterogeneity was found in the other target variables of $\delta^{13}\text{C}$ and C/N ratio or total N and SOC contents at either site. Thus, there was also no correlation between $\delta^{15}\text{N}$ values and any of the aforementioned variables at Dahlem or Thyrow 1 within the respective depth intervals ($R^2 \leq 0.2$, n.s., for all correlations tested).

4. Discussion

4.1. Management effects on ^{13}C , ^{15}N and C/N ratio

On average, mineral fertilisation with NPK did not affect the $\delta^{13}\text{C}$ signature in the soil, which is contrary to other results in the literature. Other studies (e.g. Bol et al., 2005; Liu et al., 2018) have indicated that

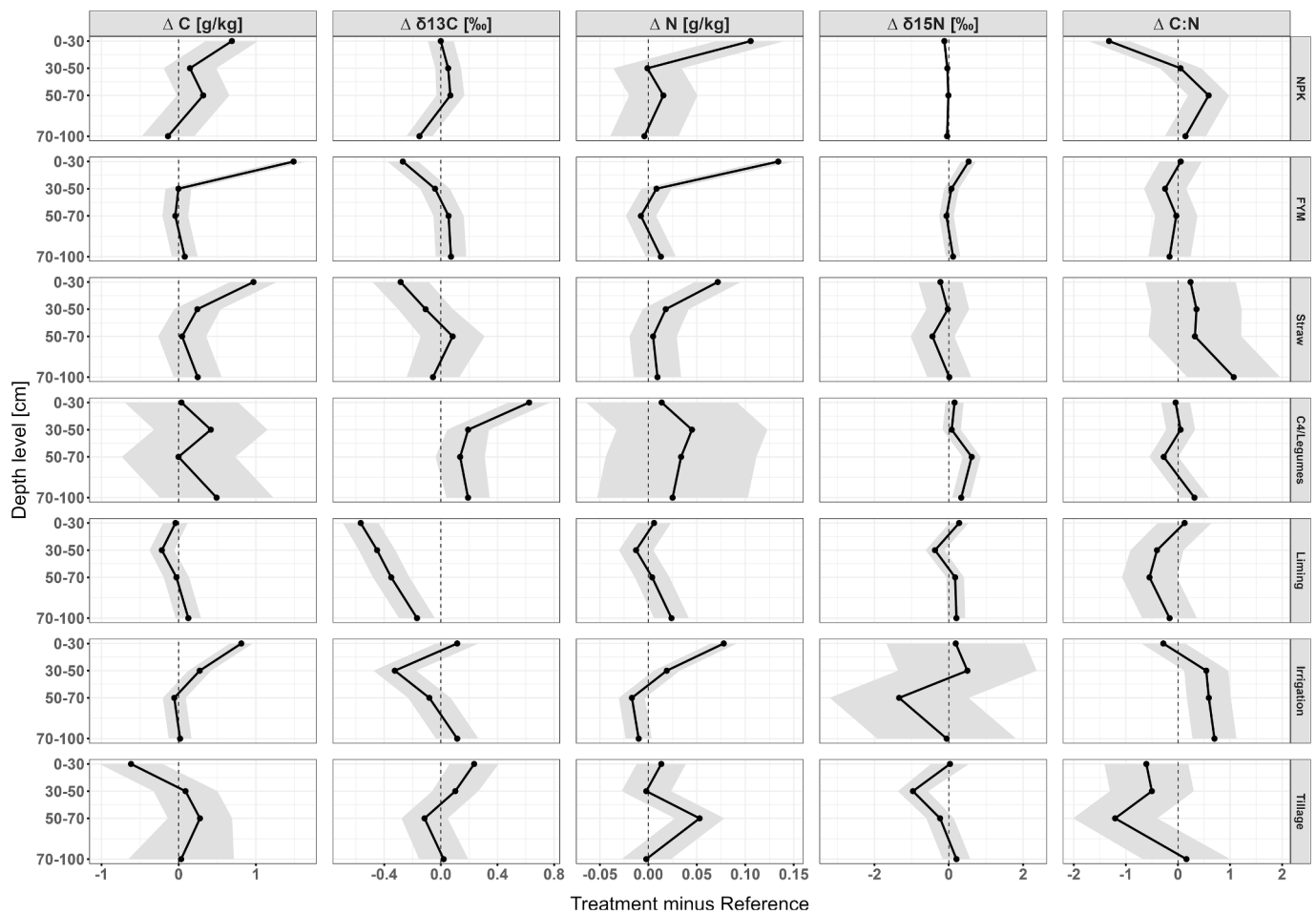


Fig. 2. Changes in soil organic carbon (C), $\delta^{13}\text{C}$ signature, total nitrogen (N) content, $\delta^{15}\text{N}$ signature and carbon-to-nitrogen (C/N) ratio under different types of agricultural management [combined nitrogen, phosphorus and potassium (NPK) fertilisation ($n = 4$); irrigation ($n = 1$); comparison of crop rotations with either maize (C_4 crop) or legumes (crimson clover, faba bean) ($n = 1$); incorporation of crop residues left on the field (Straw) ($n = 4$); application of farmyard manure (FYM) ($n = 4$); liming ($n = 4$); reduced tillage depth (Tillage) ($n = 2$)] on soil organic carbon (C), $\delta^{13}\text{C}$ signature, total nitrogen (N) content, $\delta^{15}\text{N}$ signature and carbon-to-nitrogen (C/N) ratio per depth increment. Grey shaded area represents the standard error.

$\delta^{13}\text{C}$ values are higher in the presence of mineral fertilisation, given that the addition of N to SOM results in a higher SOM turnover, thus leading to ^{13}C -enrichment in the soil. The site-specific results indicated that this was the case at three of the four tested LTEs, where $\delta^{13}\text{C}$ increased by an average of 0.2 ‰ in topsoil. However, at Gießen 2, the difference was negative (-0.3 ‰), which was the reason for the overall non-significant change in the $\delta^{13}\text{C}$ signature.

The stable isotopic signature of $\delta^{15}\text{N}$ was significantly reduced upon NPK fertilisation. As mineral N fertiliser produced using the Haber-Bosch process is derived from atmospheric N with a $\delta^{15}\text{N}$ value of 0 ‰ (Brand et al., 2014; Manaigo et al., 2024), the reduction in the $\delta^{15}\text{N}$ value in the soil reflected the increased incorporation of mineral N in topsoil. Gurmesa et al. (2017) also observed a decline in ^{15}N content following the application of N. At the same time, lower $\delta^{15}\text{N}$ values can also point to efficient N use, whereas excessive N losses would have resulted in elevated soil $\delta^{15}\text{N}$ values compared to the reference (Högberg, 1997; Kriszan et al., 2009).

Mineral NPK fertilisation, FYM application, straw incorporation and irrigation have been found to be most effective at sequestering SOC (Gocke et al., 2023) and thus also total N (Fig. 2). However, the C/N ratio was significantly lower for the NPK treatment only, because with SOC increase, also the quality of SOM changed to one that retained more N in the system.

The application of organic amendments, such as FYM, has been demonstrated to result in a reduction of the $\delta^{13}\text{C}$ signature in the soil

(Bol et al., 2005; Busari et al., 2016; Gerzabek et al., 1997; Sudhakaran et al., 2020). Indeed, FYM was also found to decrease the topsoil $\delta^{13}\text{C}$ signatures, here by an average of -0.27 ‰. Unlike the other studies, where only topsoil samples were analysed, the present study also analysed the subsoil, but changes in the 30–50 cm depth were not significant. To transport the more negative signal of FYM to lower depths, OM must first reach greater soil depths, for example through leaching of dissolved OC or bioturbation (Rumpel and Kögel-Knabner, 2011). However, the data in the present study did not provide evidence that this occurred to a degree that significantly affected soil $\delta^{13}\text{C}$ natural abundance values.

Unlike C, losses of N from organic amendments can be severe, particularly due to ammonia volatilisation, but also due to nitrate leaching, nitrous oxide emissions and the harvesting of crops with greater N uptake (Giordano et al., 2021). All these processes result in the fractionation of N isotopes, predominantly leading to an enrichment of ^{15}N in the soil (Högberg, 1997; Kriszan et al., 2014; Schneider et al., 2021). The $\delta^{15}\text{N}$ signature of slurry- and manure-derived N commonly ranges between 6 and 13 ‰ (Kriszan et al., 2014), i.e. larger than the soil $\delta^{15}\text{N}$ value of the reference topsoil. As a result, the $\delta^{15}\text{N}$ signature of topsoil increased following the long-term application of FYM in the present study (see also Bol et al., 2005; Busari et al., 2016; Sudhakaran et al., 2020), while FYM application again did not significantly affect $\delta^{15}\text{N}$ values in subsoil. Nevertheless, the results indicated that the $\delta^{15}\text{N}$ signature in cropland topsoils can be used to identify the type of

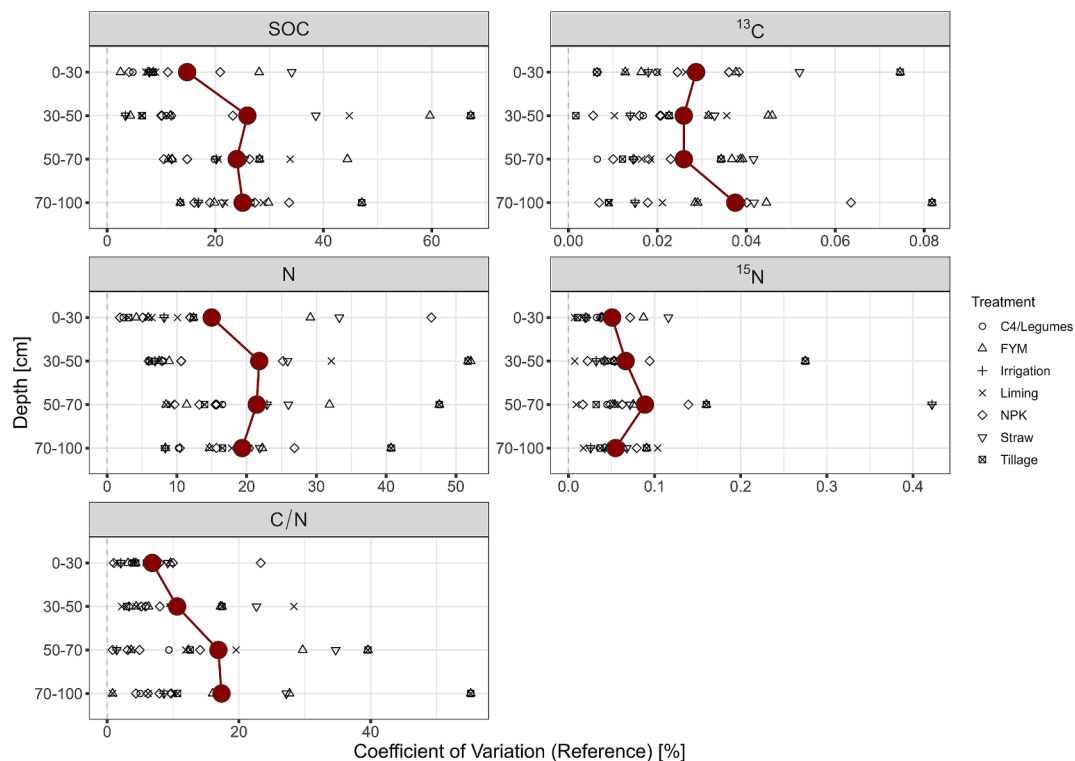


Fig. 3. Natural variability at every long-term experiment (not differentiated in the plot) by agricultural management practice (= treatment) and analysed variables per depth interval. Black symbols represent the coefficients of variation [%] for the content of soil organic carbon (SOC), ^{13}C , nitrogen (N), ^{15}N and the carbon-to-nitrogen (C/N) ratio using all reference samples of the respective treatments listed in the legend. C4/legumes = comparison of crop rotations with maize (C₄ crop) or legumes (crimson clover and faba bean, C₃ crops); FYM = application of farmyard manure; NPK = combined N, phosphorus (P) and potassium (K) fertilisation. Red dots represent the mean variation coefficients across sites and treatments per depth interval. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fertiliser, i.e. organic or mineral, since the observed effects contrast with those of NPK fertilisation.

One striking result was that the C/N ratio was not affected by FYM, in contrast to mineral NPK fertilisation. Given that NPK represents the reference system, it is assumed that the C/N ratio of the added FYM is similar to that of plant input in mineral fertilised plots. This results in a comparable soil C/N ratio of the different fertiliser treatments.

Harvest residues (e.g. straw) that were left on the field resulted in significantly higher C and N contents but, unlike the additions of FYM, the differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were not significant in topsoil. The values even tended to be more negative, as could be expected from the lower $\delta^{13}\text{C}$ value of the incorporated straw that contains less processed C. However, this absence of significance was in line with changes in soil C/N ratios, which were also not significantly affected by the straw treatments. We therefore conclude that the removal or application of harvest residues affects the quantity of SOM but not the quality of SOM.

Planting of C₄ crops, which were probably not grown prior to the start of the LTE Gießen 2 in 1982, resulted in a shift in the isotopic signature of the soil towards less negative values. C₃ crops have a $\delta^{13}\text{C}$ value of between -32 and -20 ‰, while C₄ crops have a value of between -17 and -9 ‰ (Boutton, 1991). This explains why a crop rotation with maize, the most common and dominant C₄ crop in German agriculture, resulted in a significantly higher $\delta^{13}\text{C}$ value in topsoil compared with soil where only C₃ crops were grown. The respective reference to the C₄ crop plots replaced maize with legumes. Legumes are plants that form symbioses with bacteria capable of fixing atmospheric N, thus also changing the $\delta^{15}\text{N}$ signature of soil (Cox et al., 2022). The results of the present study align with this process as $\delta^{15}\text{N}$ decreased, but with significant effects only in subsoil (50–70 cm; Fig. 1).

Hobley et al. (2018) also found significantly higher $\delta^{15}\text{N}$ values in subsoil than in topsoil, explaining that this soil depth is crucial for N

processing (van Groenigen et al., 2015). This is probably due to the loss of lighter isotopes during denitrification, which enriches the remaining N in ^{15}N (Kriszan et al., 2014; Robinson, 2001). Since denitrification is an anaerobic process, it predominantly occurs in subsoil, further contributing to the enrichment of ^{15}N at these depths. This explains the non-continuous depth gradients of ^{15}N with the highest values in the upper subsoil, as described in section 4.2 below.

In contrast to the other treatments, liming had significant effects into the subsoil down to a depth of 70 cm. However, it resulted in lower $\delta^{13}\text{C}$ values of SOC relative to the non-limed plots (except for the Dahlem site, where positive effects were observed; see Appendix A1, Fig. A1). As carbonate typically has a $\delta^{13}\text{C}$ value of approximately 0 ‰ (Brand et al., 2014), the possibility of a methodological artefact had to be discounted. Incomplete fumigation of inorganic carbonate would appear unlikely after prolonged acid treatment, and applying fumigation to non-carbonate samples did not significantly alter the soil $\delta^{13}\text{C}$ values (Appendix A1, Fig. A5 and A6). Hence, it was concluded that the decline in $\delta^{13}\text{C}$ values instead reflected enhanced biomass production, and thus greater accrual of OM with a small degree of alteration. Similarly, in subsoil the decline in $\delta^{13}\text{C}$ values upon liming might be attributed to the presence of more roots in the limed trials, with lower $\delta^{13}\text{C}$ values in the roots than in bulk SOM, even if on a whole profile basis soil $\delta^{13}\text{C}$ values increased with depth (Fig. 1) and thus overall advanced stages of SOM decomposition. Furthermore, these effects were observed in subsoil down to a depth of 70 cm, which could be explained by the influence of roots or at least by more lignified roots that are lighter in ^{13}C than those consisting of cellulose (Amelung et al., 2008). As demonstrated by Skadell et al. (2023), the above- and belowground OC inputs of these LTEs were only slightly higher after liming, with an average increase of $0.1 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ due to liming, which was equivalent to a relative increase of 7 %. This leads to two possible scenarios: i) the slightly higher

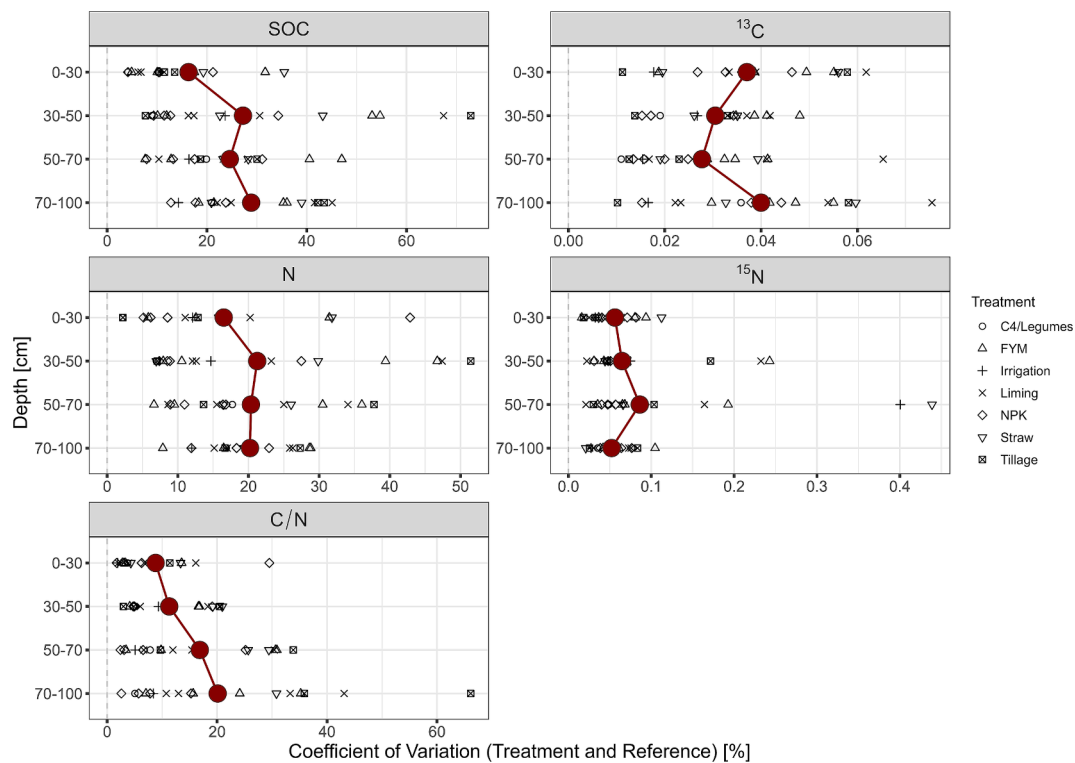


Fig. 4. Agricultural management-induced variability at every long-term experiment (not differentiated in the plot) by agricultural management practice (= treatment) and analysed variables per depth interval. Black symbols represent the coefficients of variation [%] for the content of soil organic carbon (SOC), ^{13}C , nitrogen (N), ^{15}N and the carbon-to-nitrogen (C/N) ratio using all reference AND treatment samples of the respective treatments listed in the legend. C4/legumes = comparison of crop rotations with maize (C₄ crop) or legumes (crimson clover and faba bean, C₃ crops); FYM = application of farmyard manure; NPK = combined N, phosphorus (P) and potassium (K) fertilisation. Red dots represent the mean variation coefficients across sites and treatments per depth interval. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

OC input was sufficient to result in lower $\delta^{13}\text{C}$ values of the limed soil due to enhanced biomass production, leading to a negative effect, or ii) too much OC was destroyed with the fumigation method.

Irrigation increased C and N contents down to 50 cm depth, but reduced them in the lower subsoil (> 50 cm). In topsoil, these results might be interpreted as increased turnover of SOM, leading to elevated $\delta^{13}\text{C}$ values due to isotope discrimination processes (Krüger et al., 2024) and to elevated $\delta^{15}\text{N}$ values probably due to larger relative N losses (Högberg and Johansson, 1993). In addition, $\delta^{13}\text{C}$ values of crop plants can also be altered by the effect of drought stress or water availability on C uptake and fixation processes during photosynthesis (e. g., Farquhar et al., 1989). Drought stress typically induces closing of stomata, which decreases C isotope discrimination during CO₂ uptake for photosynthesis and thus results in increased (less negative) $\delta^{13}\text{C}$ values in plant biomass. In contrast, sufficient water supply leads to opening of stomata and increased discrimination of the heavier ^{13}C isotope, resulting in lower (more negative) plant $\delta^{13}\text{C}$ values (Krüger et al., 2024). However, these processes are in contrast to the observed changes in $\delta^{13}\text{C}$ values in topsoil. Therefore, the decrease in plant $\delta^{13}\text{C}$ values observed in the irrigated treatments was probably offset by the stimulation of SOM turnover during litter decomposition. In subsoil, however, irrigation reduced the soil $\delta^{13}\text{C}$ compared to the reference treatment. This is probably due to greater discrimination under improved water supply combined with increased root growth into the subsoil for irrigated plants, similar to the mechanisms described for the limed trials. The C/N ratio was also lower in topsoil of the irrigation trial compared to the other treatments, possibly reflecting lower N use efficiency upon watering. The fertilised N remains available in the soil for a longer period of time due to irrigation. Consequently, a higher amount of N reaches the plants through irrigation and is then returned to the soil as OM with a low C/N ratio. In contrast, the soil in the non-irrigated

plots (which also received N) quickly becomes too dry for the fertilised N to be absorbed by the plants.

The effects of tillage on SOM were ambiguous. Soil $\delta^{13}\text{C}$ values increased with reduced tillage depth, in contrast to what would be expected from decreased SOM turnover with reduced tillage practice. However, site-specific results showed that this pattern was not observed at Göttingen, where the $\delta^{13}\text{C}$ signature decreased by -0.64% . Instead, the soil $\delta^{15}\text{N}$ values declined with reduced tillage, though mainly in 30–50 cm depth. Busari et al. (2016) found a significant depletion of ^{15}N in zero-tilled soil compared with conventionally tilled soil due to lower C and N mineralisation and volatilisation.

4.2. Comparison of changes in quality and quantity indicators

The human-induced variation of the proxies for SOM quantity and quality exceeded their natural site variation, as expected and as displayed by the LRRs. The LRRs revealed that SOC and stable isotopes of C responded more strongly to the agricultural management practices than ^{15}N and total N content. Moreover, in subsoil the effects on SOC and ^{13}C were greater than on total N and ^{15}N . This indicates that the changes induced by biomass input and turnover have a greater effect on the quantity and quality of C than they do on the quantity and quality of N. The comparison between the LRRs for SOC content and ^{13}C revealed that the proxies for quality responded more strongly than the proxies for quantity, with higher LRRs for ^{13}C than for SOC. In contrast, the response of agricultural management practices was the same for total N content and ^{15}N (Fig. 5). The variation in C/N ratio in response to agricultural management practices increased with depth, in contrast to the expectation that human-induced variation decreases with depth. Here, the increased variation was driven by a few sites with a large natural variation: the sites of Dahlem and Müncheberg showed an

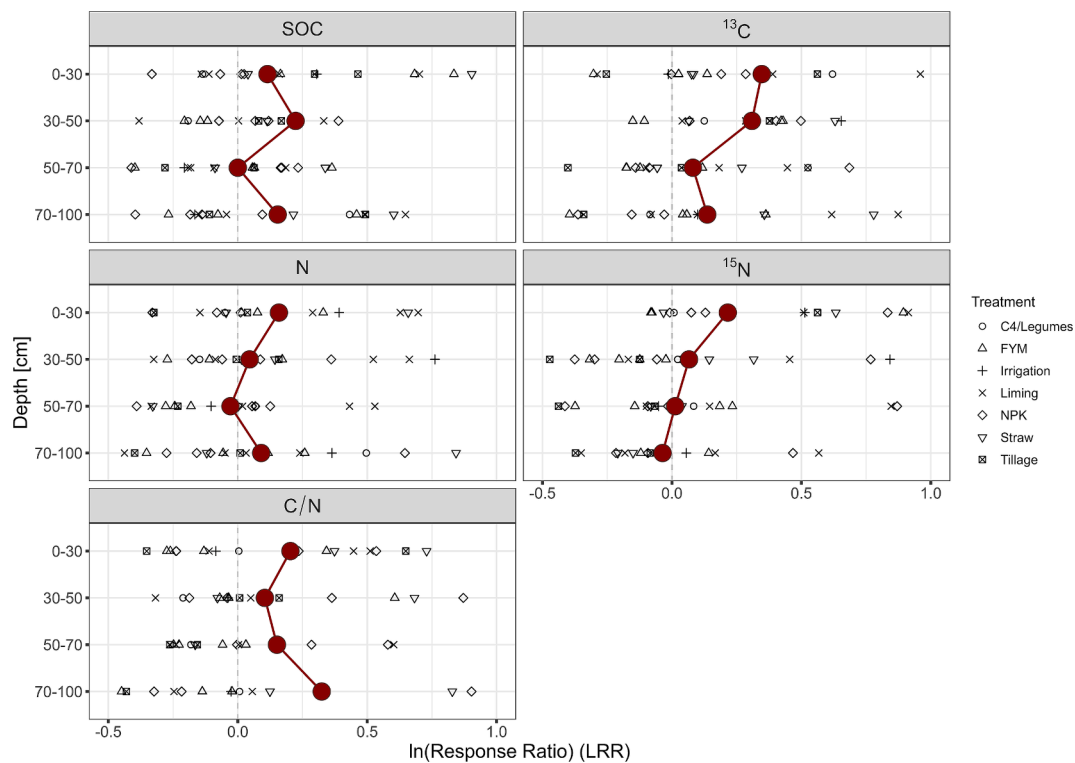


Fig. 5. Natural logarithm of the response ratio (LRR) for every agricultural management practice (= treatment) and variable analysed per depth interval. Black symbols represent the LRRs for the content of soil organic carbon (SOC), ^{13}C , nitrogen (N), ^{15}N , and the carbon-to-nitrogen (C/N) ratio of all sites (not differentiated in the plot) and treatments listed in the legend. C4/legumes = comparison of crop rotations with maize (C₄ crop) or legumes (crimson clover and faba bean, C₃ crops); FYM = application of farmyard manure; NPK = combined N, phosphorus (P) and potassium (K) fertilisation. Red dots represent the mean LRRs across sites and treatments per depth interval. The x-axis was cut at $-0.5 < x < 1$ for improved visibility of mean LRRs. The original figure with all data points can be obtained from [Appendix A1](#), Fig. A2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

extremely high variation in C/N ratio in subsoil, with CVs above 50 %. Natural variations together with very low SOM contents with increasing depth, close to the limit of detection, lead to a greater probability of analytical errors, which is discussed in more detail below.

4.3. Challenges in analysing stable isotopic signatures in subsoil

Analysing stable isotopes in subsoil can be challenging due to the lower C and N contents there than in topsoil (Schneider et al., 2021). The heterogeneity of subsoil is greater than that of topsoil due to the lack of ploughing and other homogenising processes, which leads to a variability that makes it challenging to detect management differences. In the present study, the $\delta^{15}\text{N}$ signature in soil generally increased with depth before decreasing again, which is consistent with Schneider et al. (2021) who found that mineral cropland soils have a mean $\delta^{15}\text{N}$ value of 6.0 ‰ at 0–10 cm, increasing to a mean value of 6.6 ‰ at 50 cm depth, before decreasing again to an average of 5.6 ‰ at 70–100 cm. The present study, however, revealed some extreme cases, e.g. at Dahlem where maximum values above 40 ‰ were measured. The variability of $\delta^{15}\text{N}$ at Dahlem is an order of magnitude higher than the general effects of agricultural management. Furthermore, since these high values were limited to a specific area of the field and were neither homogeneously distributed nor assignable to a specific management practice, they cannot be caused by management. This was also confirmed by the corresponding CV values for natural variation, which were already high without the influence of agricultural management (Fig. 3). One possible reason for hotspots with high $\delta^{15}\text{N}$ values is the presence of more clay minerals that are able to fix ammonia (Nieder et al., 2011), but clay content did not correlate with $\delta^{15}\text{N}$ values at a given soil depth. Other reasons might be unreported labelling experiments in the field or contamination with fossil fuels such as petroleum directly in the field,

which can be enriched in ^{15}N with values above 18 ‰ (Quan and Adeboye, 2021) and could, at least in part, have caused these high $\delta^{15}\text{N}$ values in the confined area. The presence of this spatial variability, especially in subsoil, has implications for the detection of changes in temporal trends or management effects on ^{15}N . It also shows the value of stable isotopes in detecting soil heterogeneity that is not detectable with SOM content alone. However, these peaks in $\delta^{15}\text{N}$ values in Dahlem and Thyrow cannot be explained by any natural mechanism.

5. Conclusions

Stable isotopes are tracers of sources of C and N in soils, as indicated by increases in ^{13}C in crop rotations that contain C₄ crops compared with pure C₃ crop rotations, and also by decreasing ^{15}N content due to mineral fertilisation. At the same time, the input of OM to soils and its turnover time are affected by different agricultural management practices and are reflected in the isotopic values and C/N ratio. The additional OM input with FYM and straw resulted in lower $\delta^{13}\text{C}$ values. These effects were mainly restricted to topsoil. In subsoil, agricultural management effects were small and heterogeneity across the LTEs sites also hampered the detection of management effects. This was particularly surprising with a subsoil variation in ^{15}N at two LTEs that was beyond what could be described as a natural variation. This study thus shows the challenges of detecting management-induced changes in topsoil and subsoil, and illustrates the various factors that affect ^{13}C , ^{15}N and C/N ratios. When using the natural abundance of stable isotopes in agricultural soils, management-induced changes should be taken into consideration in order to avoid the misinterpretation of data.

This study showed the complementarity of C and N content, isotope values and C/N ratio as indicators of SOM dynamics. All five parameters were affected differently by agricultural management practices, mostly

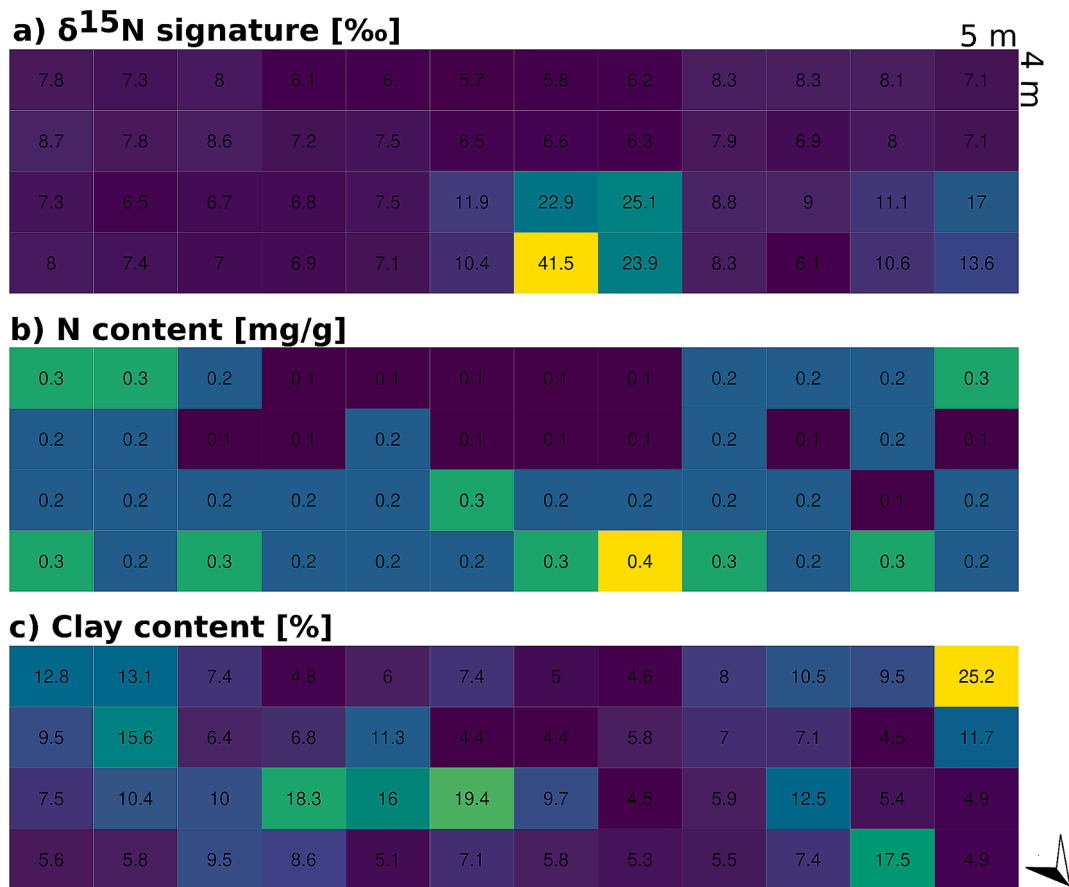


Fig. 6. Each of the three facets represents the arrangement of the sampled plots (rectangles) of the LTE Dahlem (BDa_D3) in 30–40 cm depth. The measured value of a) $\delta^{15}\text{N}$ value [‰], b) N content [mg g^{-1}] and c) clay content [%] is shown in each plot.

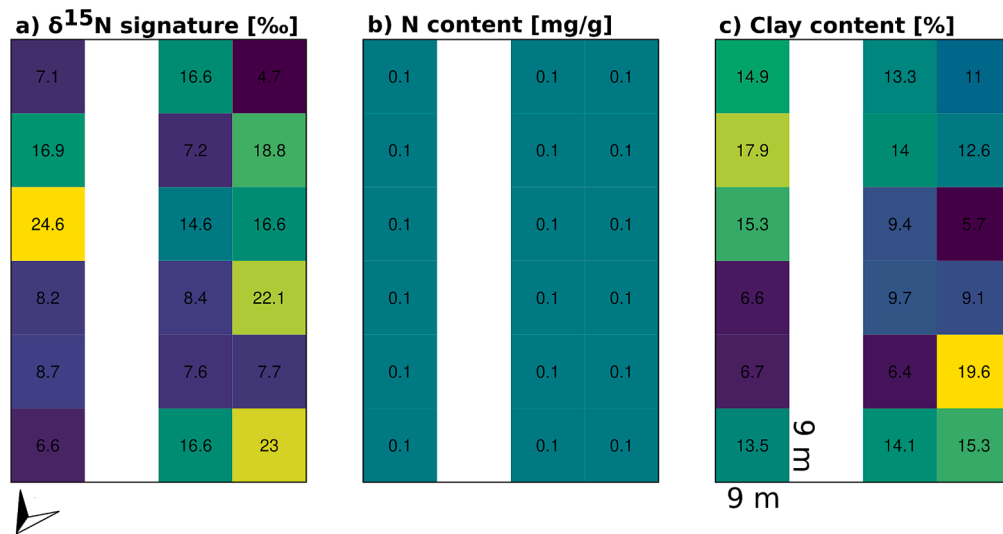


Fig. 7. Each of the three facets [a-c)] represents the arrangement of the sampled plots (squares) of the LTE Thyrow 1 (Thy_D1) in 59–70 cm depth. The measured value of the a) $\delta^{15}\text{N}$ value [‰], b) N content [mg g^{-1}] and c) clay content [%] is shown in each plot.

with a greater impact in topsoil. Even in LTEs running for between 32 and 112 years, subsoil ^{15}N was barely affected, while subsoil ^{13}C was only affected by irrigation and liming.

Attributing changes in SOM quality and quantity to the agricultural management practices in the LTEs rather than to natural variations within the site remains difficult in the case of subsoil and should be undertaken with care. Specifically, future research on these LTEs should

focus on directly measuring C and N inputs along with their stable isotopic signatures at different soil depths to better understand the mechanisms driving the vertical distribution patterns observed in this study. More targeted and detailed studies, such as lab-scale mesocosm experiments, could provide clearer insights into these underlying processes, particularly short-term N transformation pathways.

CRedit authorship contribution statement

Laura E. Skadell: Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation. **Florian Schneider:** Writing – review & editing, Formal analysis, Data curation. **Sara L. Bauke:** Writing – review & editing, Data curation. **Wulf Amelung:** Writing – review & editing, Project administration, Data curation. **Axel Don:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Data curation, 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. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2025.117341>.

Data availability

All data used in this study can be found in the supplement files.

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