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Increased soil carbon storage through plant diversity strengthens with time and extends into the subsoil

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Abstract

Soils are important for ecosystem functioning and service provisioning. Soil communities and their functions, in turn, are strongly promoted by plant diversity, and such positive effects strengthen with time. However, plant diversity effects on soil organic matter have mostly been investigated in the topsoil, and there are only very few long-term studies. Thus, it remains unclear if plant diversity effects strengthen with time and to which depth these effects extend. Here, we repeatedly sampled soil to 1 m depth in a long-term grassland biodiversity experiment. We investigated how plant diversity impacted soil organic carbon and nitrogen concentrations and stocks and their stable isotopes ^{13}C and ^{15}N, as well as how these effects changed after 5, 10, and 14 years. We found that higher plant diversity increased carbon and nitrogen storage in the topsoil since the establishment of the experiment. Stable isotopes revealed that these increases were associated with new plant-derived inputs, resulting in less processed and less decomposed soil organic matter. In subsoils, mainly the presence of specific plant functional groups drove organic matter dynamics. For example, the presence of deep-rooting tall herbs decreased carbon concentrations, most probably through stimulating soil organic matter decomposition. Moreover, plant diversity effects on soil organic matter became stronger in topsoil over time and reached subsoil layers, while the effects of specific plant functional groups in subsoil progressively diminished over time. Our results indicate that after changing the soil system the pathways of organic matter transfer to the subsoil need time to establish. In our grassland system, organic matter storage in subsoils was driven by the redistribution of already stored soil organic matter from the topsoil to deeper soil layers, for example, via bioturbation or dissolved organic matter. Therefore, managing plant diversity may, thus, have significant implications for subsoil carbon storage and other critical ecosystem services.

KEYWORDS
ecosystem functioning, land-use change, soil carbon storage, soil nitrogen, soil organic matter, stable isotopes, subsoil

1 | INTRODUCTION

Plant diversity increases ecosystem functions and services (Cardinale et al., 2012; Isbell et al., 2011). This positive biodiversity–ecosystem functioning (BEF) relationship is particularly important for below-ground processes, such as soil organic carbon (C) and nutrient cycles and storage, as well as soil fertility (Hooper et al., 2012), which then feedback on ecosystem productivity (Wardle et al., 2004). This positive BEF relationship even strengthens over time in experimental settings (Cardinale et al., 2007; Eisenhauer et al., 2012; Guerrero-Ramírez et al., 2017; Reich et al., 2012). However, most studies investigating C storage and nutrient cycling in the BEF context focus on topsoil (Cong et al., 2014; Lange et al., 2015), cover a relatively short period, often less than 5 years (e.g., Steinbeiss et al., 2008; Xu et al., 2020), or consider C storage and nutrient cycling over longer periods, as processes in steady state (but see Yang et al., 2019). There are only a few long-term studies examining the BEF relationships in the topsoil (Furey & Tilman, 2021; Lange et al., 2019). Studies on the long-term plant diversity effects on C and nitrogen (N) dynamics in the subsoil are even rarer. Thus, the temporal dynamics and the depth extent of the effects of plant diversity and community composition on the soil organic matter and its key components (C and N) have been rarely, or not at all, considered so far.

The main sources of soil organic matter in natural terrestrial systems are leaf litter inputs to the soil surface and root-derived inputs belowground (Amundson, 2001; Jobbagy & Jackson, 2000). The distributions of root and soil organic matter are highly correlated and decline exponentially with soil depth (Jobbagy & Jackson, 2000). Diversity and composition of plant communities strongly impact the production of shoots and roots (Marquard et al., 2009; Mueller et al., 2013; Ravenek et al., 2014; Tilman et al., 2001) and thus, the fresh plant-derived C inputs into the soil (Eisenhauer et al., 2017; Mellado-Vazquez et al., 2016). It is assumed that in more diverse plant mixtures roots grow denser and to deeper soil layers (Fargione & Tilman, 2005; Mommer et al., 2010; Mueller et al., 2013), potentially impacting the cycling of organic C and N in the subsoil. However, whether diversity and rooting depth of the plant community are related to each other likely depend on environmental factors, such as the presence of legumes or the fertility of soils. Specifically, in the N-limited, sandy soil of the Cedar Creek biodiversity experiment high plant diversity led to increased root biomass deeper in the soil profile (Mueller et al., 2013), while no significant plant diversity effects were found on the root distribution in the soil profile of the fertile soil of the Jena Experiment (Ravenek et al., 2014).

Plant-derived C inputs fuel the soil microbial community, with higher plant diversity leading to a more abundant, more diverse, and more active microbial community (Eisenhauer et al., 2010; Lange et al., 2014; Lange et al., 2015; Zak et al., 2003). The microbial community, in turn, not only respires major amounts of the plant-derived C but also converts the easily decomposable plant C into forms that are better retained in soil, such as metabolic end products and microbial necromass (Bradford et al., 2013; Gleixner, 2013; Lange et al., 2015; Liang et al., 2019). While consistent positive effects of plant diversity on soil C and N storage were reported for upper soil layers (e.g., De Deyn et al., 2011; Fornara & Tilman, 2008; Steinbeiss et al., 2008), subsoil C and N stocks are suggested to be stable with slow element turnover. This is in line with the depletion of modern C in soil organic matter of deeper soil layers (Trumbore, 2009). However, soil organic matter in deeper soil layers is very heterogeneous with patches having accelerated elemental cycling, which is related to rhizodeposition and the activity of the soil fauna (Kuzyakov & Blagodatskaya, 2015).

It has been proposed that roots contribute to soil organic matter sequestration in subsoils (Balesdent & Balabane, 1996; Rasse et al., 2005) and that plant diversity and increased root production lead to more storage of C and N down to 60 cm soil depth (Fornara & Tilman, 2008). On the other hand, fresh and labile compounds exuded by roots may stimulate the decomposition of existing soil organic matter in subsoils, causing the so-called "priming effect" (Fontaine et al., 2007; Kuzyakov, 2010). In addition to plant species richness, particular plant functional groups and their composition within the plant community are assumed to impact soil element cycling in different layers of the soil profile (Fornara & Tilman, 2008) as, for instance, the roots of tall herbs reach much deeper soil layers than those of grasses (Ebeling et al., 2014).

Additionally, the quality of root inputs (e.g., C/N ratios) is affected by plant diversity and plant functional group composition, in particular, by the presence of legumes (Bessler et al., 2012). In turn, the quality of plant-derived organic matter influences its decomposition (Chen et al., 2017). For example, root turnover is higher in herbs than in grasses, which putatively increases the C and N inputs to the soil (Fornara & Tilman, 2009). This in turn results in increased N availability in soils (Oelmann et al., 2007; Spehn et al., 2005) with potential effects on the microbial transformation of soil organic matter (Lange et al., 2019; Leimer et al., 2016). However, so far it is not clear how plant diversity impacts subsoil dynamics of C and N. For instance, do the enhanced plant C inputs to subsoil with higher diversity lead to more C sequestration or to more C losses due to the priming effect? Furthermore, it is unclear if higher plant diversity leads to more N exploitation of the deeper soil layers due to higher nutrient demands of more diverse and more productive plant communities, or if plant diversity influences the soil nutrient retention via other mechanisms, for example, related to soil microbial communities and activity (Leimer et al., 2016).

For investigating processes and dynamics of soil organic matter, it is insightful to assess C and N changes together with their stable isotopes ^{13}C and ^{15}N, respectively (Balesdent & Balabane, 1996; Ehleringer et al., 2000). Generally, soil organic matter is enriched in the ^{13}C and ^{15}N signatures (increase in δ^{13}C and δ^{15}N) compared with plant material due to fractionation during microbial mineralization and processing of organic C and N (Hobley et al., 2017; Högberg, 1997; Kramer et al., 2003; Nadelhoffer & Fry, 1988). This causes an increased enrichment of ^{13}C and ^{15}N with soil depth, where a higher mineralization rate and more processed soil organic matter can be found (Acton et al., 2013; Balesdent et al., 1993; Garten, 2006). However, it is uncertain how plant diversity and plant community composition affect

the isotopic composition of soil organic matter below the topsoil. Increased plant diversity might affect the dynamics of stable isotopes in both directions: enrichment due to a more active microbial community or depletion due to more fresh plant-derived inputs. The combined assessment of the concentration of C or N together with their respective stable isotopes enables to draw conclusions on processes that take place and impact changes in the soil organic matter (Nel et al., 2018). For instance, an increase of the C concentrations accompanied by a depletion of ^{13}C (decrease in δ^{13}C values) indicates that input of fresh plant material mainly contributes to soil organic matter formation and sequestration. In contrast, the decrease of C concentrations and the enrichment of ^{13}C indicates enhanced decomposition and loss of soil organic matter, while the decrease of C concentrations and the depletion of ^{13}C indicates priming of stored soil organic C and its replacement with fresh plant-derived C.

This study investigated the effects of plant diversity and plant functional group composition on C and N storage and cycling within the soil profile down to 1 m. Taking advantage of a long-term grassland biodiversity experiment, the Jena Experiment, we were able to assess the effects of the plant diversity and functional group composition on the changes of soil organic matter in regular intervals over 14 years. Specifically, we asked (i) whether the stocks, concentrations, and stable isotope ratios of organic C and N changed similarly in depth segments of the soil profile after the conversion from an arable land to a grassland; (ii) whether the effects of plant diversity and/or functional group composition on the stocks, concentrations and stable isotope ratios of organic C and N were similar in the subsoil and the topsoil; (iii) whether plant community effects strengthened over time; and (iv) what processes determined the changes of soil organic C and N stocks, concentrations and stable isotope ratios?

2 | MATERIALS AND METHODS

2.1 | Study site of the Jena Experiment

This study was carried out as a part of the Jena Experiment, a large-scale grassland diversity experiment (Roscher et al., 2004; Weisser et al., 2017). The Jena Experiment is located on the floodplain of the Saale River near the city of Jena (Thuringia, Germany; 50°57′ N, 11°35′ E). The soil of the field site is classified as Eutric Fluvisol (FAO, 1998), developed from loamy fluvial sediments. The texture ranged from sandy loam to silty clay with increasing distance to the river Saale reflecting the sedimentation process. For 40 years prior to the establishment of the experiment, the field site was a cropland with inputs of mineral fertilizer. In the spring of 2002, 82 experimental grassland plots with a size of 20×20 m were established. Plots are arranged in four blocks to account for changes in soil texture. In this study, we focused on the most intensively investigated block 2. While initial soil pH was similar among plots (7.2–7.4), soil texture in the top 20 cm varied within block 2. Specifically, the sand portion increased in the upper 20 cm of the soil profile from 10% in the north to 38% in the south of the block, while silt decreased from 70% to

44%. Clay showed almost no spatial trend and was in the range of 17%–27%. In contrast, in 20–100 cm depth, soil texture was homogenous containing 16% of sand, 59% of silt, and 25% of clay.

The experimental plots were assembled by randomly selecting plant species from the 60 species pool with consideration of species richness and functional group richness and composition. The experimental plots spread gradients of plant species richness from 1 to 60 (i.e., 1, 2, 4, 8, 16, and 60) and the plant functional group richness from 1 to 4, including grasses, legumes, small herbs, and tall herbs based on morphological, phenological, and physiological traits (for details see Roscher et al., 2004). The block 2 contained four replicates of monocultures, 2-, 4-, and 8-species mixtures as well as three replicates of 16-species mixtures ($n = 19$). Experimental communities are weeded manually twice to three times per year to maintain the plant diversity levels and community composition, and mown twice per year in June and September, as is typical for hay meadows in Central Europe.

2.2 | Soil sampling and laboratory analysis

Soil samples were taken in April 2002 before sowing and 5, 10, and 14 years after in April of each sampling year. Three independent 1-m soil cores per plot were collected using a machine-driven soil corer (Cobra, Eijkelkamp Agrisearch Equipment) with an inner diameter of 8.7 cm. Soil cores were segmented into 5 cm sections, resulting in 20 segments per soil core. Soil samples were air-dried sieved to 2 mm and milled. After each sampling subsamples were analysed for organic C and total N concentrations with a Vario Max and a Vario EL (Elementar Analysensysteme GmbH), respectively. Organic C was determined as the difference between the total C concentration and the inorganic C concentration; the latter was measured after removing the organic C by heating the sample to 450°C for 16 h in a muffle furnace. This method is highly reliable (Steinbeiss et al., 2008) and even outperformed methods using acids to remove the inorganic C in terms of accuracy in our laboratory (Bisutti et al., 2004; Schreider-Goidenko, 2015). In 2002, soil bulk density was determined at six plots on block 2. The depth segments for density measurements ranged from 0 to 10 cm, 10–20 cm, 20–30 cm, 30–40 cm, 40–60 cm, 60–80 cm, and 80–100 cm. Samples were taken with a metal bulk density ring of 10 cm height, sieved to 2 mm, and dried at 105°C. Soil density was calculated by weight (Hartge & Horn, 1992). The chosen plots represented a spatial gradient across the block and resulted in an average soil bulk density value at the beginning of the experiment. In the later soil sampling campaigns in 2007, 2012, and 2016, changes in bulk density were measured for every plot in block 2 with 5 cm depth resolution using the inner diameter of the soil corer for volume calculation. To adapt the depth resolution of 2002 to the later sampling campaigns in 0–30 cm depth a logarithmic and in 30–100 cm depth a linear regression was applied (.8 ≤ r ≤ 1 and see Table S1).

Further δ^{13}C values of organic C and δ^{15}N values of total N were measured with a DeltaPlus isotope ratio mass spectrometer (Thermo Fisher), coupled via a ConFlowIII open-split to an elemental

analyser (Carlo Erba 1100CE analyser; Thermo Fisher Scientific) after carbonates in soil being removed with 120mL of 5%–6% H_2SO_3 (Steinbeiss et al., 2008).

To calculate the changes in the concentrations and stocks of organic C and total N as well as changes in the $\delta^{13}C$ values (=$\Delta\delta^{13}C$) and $\delta^{15}N$ values (=$\Delta\delta^{15}N$) over time, values measured in 2002 were averaged first for each depth segment of each plot and subtracted from the values measured per core and layer in the later years (2007, 2012, and 2016), resulting in periods of 5, 10, and 14 years. Similarly, we calculated the mean annual changes in organic C and total N stocks and concentrations as well as $\delta^{13}C$ values, $\delta^{15}N$ values, and C:N ratios for each sampling period.

2.3 | Statistical analyses

All statistical analyses were conducted with the statistical software R (2018). Linear mixed-effects models applying the "lme" function in the R library "nlme" (Pinheiro et al., 2016) were applied to test for plant diversity effects and their changes over time on the concentrations and stocks of C, N, their isotopic values ($\delta^{13}C$, $\delta^{15}N$) and the C/N ratios. Starting from a constant null model, with plot identity as random effect, sown plant species richness (log-linear term) was fitted first, followed by plant functional groups richness (linear term); and in alternative models, the presence of all individual plant functional groups was fitted, as they are not independent of each other (Roscher et al., 2004). The maximum likelihood method and likelihood ratio tests were applied to assess the statistical significance of stepwise model improvement. The effect size of every tested variable was determined as marginal R^2 (i.e., the effect size of the random effect plot identity was not considered) using the "r.squaredGLMM" function in the R library "MuMIn" (Bartoń, 2013). Plant species richness, functional groups richness, and the presence of the functional groups were varied as orthogonally as possible in the experimental design (Roscher et al., 2004). To account for the possible correlations between plant species richness and functional group richness, and to test which of the both factors was more important, all models were run twice with a changed sequence of fitting, that is, plant species richness versus functional group richness (Eisenhauer et al., 2010). Here we reported the results of the model that explained best the variance of the response variables. In most models, plant species richness was a better predictor in comparison with functional group richness when fitted first.

3 | RESULTS

3.1 | Soil organic matter changes within the soil profile after land-use change

In 2002, before the grassland was established, soil organic matter was homogeneously distributed within the ploughing horizon: concentrations of C and N, the C/N ratio, as well as the isotopic ratios of $\delta^{13}C$ and $\delta^{15}N$ were at similar levels among soil sections and plots in the upper 30 cm (Figure 1; average values of C: 20.9±2.6 g kg^{-1} [mean±standard deviation], N: 2.2±0.2 g kg^{-1}, C/N ratio: 9.4±0.4, $\delta^{13}C$: −26.8±0.3‰, $\delta^{15}N$: 6.3±0.4‰). Accordingly, the decrease of the C and N stocks in the first 20 cm of the soil profile was driven by increased bulk density with increasing soil depth. Below the plough horizon, concentrations and stocks of C and N strongly decreased between 30 and 45 cm, followed by a moderate decrease, resulting in very low concentrations of C and N at 1-m soil depth (C: 6.7±1.4 g kg^{-1}, N: 0.8±0.2 g kg^{-1}). Below the plough horizon, ^{13}C and ^{15}N became slightly enriched with increasing soil depth, up to values of $\delta^{13}C$: −25.6±0.3‰ and $\delta^{15}N$: 6.8±0.3‰ at 1-m depth (Figure 1).

After 14 years since the establishment of the Jena Experiment, soil organic matter has developed toward a typical grassland soil profile; that is, in particular in the top 5 cm, C and N concentrations strongly increased (C: +6.4±4.2 g kg^{-1}, N: +0.6±0.4 g kg^{-1}), whereas the concentrations largely decreased in soil sections between 10 and 40 cm (C: −1.9±2.7 g kg^{-1}, N: −0.1±0.3 g kg^{-1}, Figure 1a,b). The decrease of the C and N stocks was less pronounced but significant between 10 and 40 cm soil depth (C: −1.8±52.0 g m^{-2}, N: −0.8±5.0 g m^{-2}). Thus, the C and N stock gains in the top 10 cm of the soil (C: 9.6±25.3 g m^{-2}, N: 0.9±2.8 g m^{-2}) exceed the losses below that soil depth. Below 40 cm, the changes in C and N stocks and concentrations were marginal (Figure 1d,e) but still significant for C concentrations in most subsoil depth layers and for N stocks down to a depth of 80 cm.

After 14 years, the ^{13}C values and ^{15}N values were strongly depleted in the uppermost 5 cm ($\Delta\delta^{13}C$: −0.9±0.5‰, $\Delta\delta^{15}N$: −1.2±0.6‰). Below a soil depth of 10 cm, $\delta^{13}C$ values did not change considerably, and ^{15}N got slightly enriched to a depth of 80 cm. Below 80 cm, the enrichment in ^{15}N increased, being highest at 1-m soil depth ($\Delta\delta^{15}N$: 0.3±0.5‰, Figure 1f,g).

After land-use change, C and N concentrations and their isotopic compositions developed differently across the soil profile. C and N concentrations increased continuously in the upper 10 cm, with the accompanied depletion of the respective stable isotopes ^{13}C and ^{15}N, in particular the depletion of ^{13}C in the first 5-year period (Figure 2a,b,f,g). In the lower layers of the former ploughing horizon (10–30 cm), the concentrations of C decreased in the first 10 years and increased afterward, while N concentrations decreased in the first 5-year period and remained stable in the following 9 years. In the subsoil below 40 cm depth, the changes of C and N concentrations showed the same patterns as in the deeper ploughing horizon, but they were less pronounced (Figure 2a,b). The changes of ^{13}C below the upper 10 cm were relatively small and variable among the periods without a temporal direction. The $\delta^{15}N$ values below the upper 10 cm slightly decreased in the first 10 years after land-use change and did not change afterward.

The changes in C and N stocks among the sampling periods were mainly driven by the high bulk density observed in 2007 and the decreasing bulk densities in 2012. This pattern was observed within the entire soil profile (Table S2). This resulted in increased C and N

FIGURE 1 Depth profiles of (a) organic carbon and (b) nitrogen concentrations, (d, e) their stocks, their stable isotope ratios (f) $\delta^{13}C$ and (g) $\delta^{15}N$ values, as well as the (c) carbon-to-nitrogen ratio (C/N ratio) before the experimental field site was established in 2002 (yellow) and 14 years later in 2016 (blue). Circles through the depth profile represent the means, and the error bars show 2× the standard error of the mean. Asterisks indicate significant differences ($\alpha = .05$, based on likelihood ratio tests) of soil organic matter means between 2002 and 2016 in specific soil depth layers.
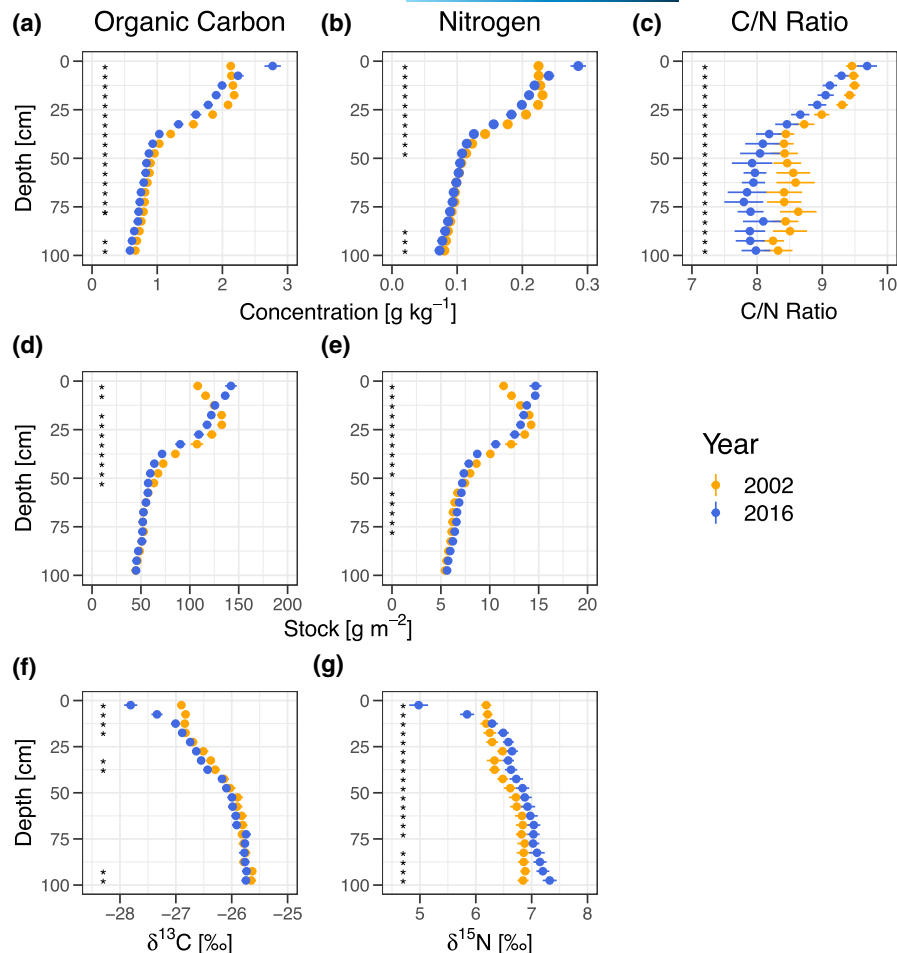
stocks in all soil depths in the first period from 2002 to 2007 and a decrease in the stocks from 2007 to 2012 (Figure 2d,e).

3.2 | Impact of plant diversity on soil organic matter changes within the soil profile

Changes in C and N and their isotopic composition were significantly impacted by plant diversity and plant community composition at all depth increments in the first meter of the soil profile. Plants impacted the concentration and stock changes in a similar way, but such effects were more pronounced for the concentration changes (Figure 3). Therefore, only the significant effects ($p < .05$) on concentration changes are described below. In the first 5 years after the land-use change, higher plant species richness led to increased C and N concentrations in the upper 5 cm of the soil (monocultures, C: -0.5 ± 2.3 g kg^{-1}, N: 0.0 ± 0.2 g kg^{-1}; 16-species mixtures, C: $+7.1 \pm 4.8$ g kg^{-1}, N: 0.7 ± 0.5 g kg^{-1}) with an accompanied depletion of the ^{13}C and ^{15}N values (monocultures, $\Delta\delta^{13}C$: -0.5 ± 0.3‰, $\Delta\delta^{15}N$: 0.1 ± 0.5‰; 16-species mixtures, $\Delta\delta^{13}C$: -1.0 ± 0.5‰, $\Delta\delta^{15}N$: -1.2 ± 0.5‰). This positive effect of plant species richness strengthened over time in the upper 5 cm and expanded over time to deeper soil layers (Figure 3). Therefore, in plots with higher species richness, the gains of soil C in the first

10 cm increased, while the losses in the deeper ploughing horizon after the land-use change were mitigated. Specifically, the plant species richness explained 27.7% and 23.8% of the changes in C and N concentrations, respectively in the top 5 cm soil layer 5 years after the establishment of the experiment. The explained variance by plant species richness increased to 54.4% and 50.2% for C and N concentrations, respectively, after 14 years. Within the soil profile, the effects of plant species richness after 14 years weakened with soil depth, though being significant for both C and N concentrations to the soil depths of 45 and 55 cm, respectively (Figure 3). In contrast, almost no effects were observed in soil layers below the top 10 cm earlier in the experiment.

Similar to plant species richness, plant functional group richness positively impacted the changes in soil N concentrations below the upper 10 cm, that is, the N losses after the land-use change were reduced. However, the mitigated loss of N by higher plant functional group richness was only observed in the first 10 years of the experiment. During the extended 14-year period, the mitigation was more driven by plant species richness (Figure 3). Furthermore, with increased functional group richness, ^{13}C was depleted between 20 and 75 cm, and ^{15}N was depleted between 85 and 100 cm soil depth. In contrast, plant functional group richness did not affect the C concentration in soil but led to increasingly depleted $\delta^{13}C$ values over time in soil depths between 25 and 75 cm.
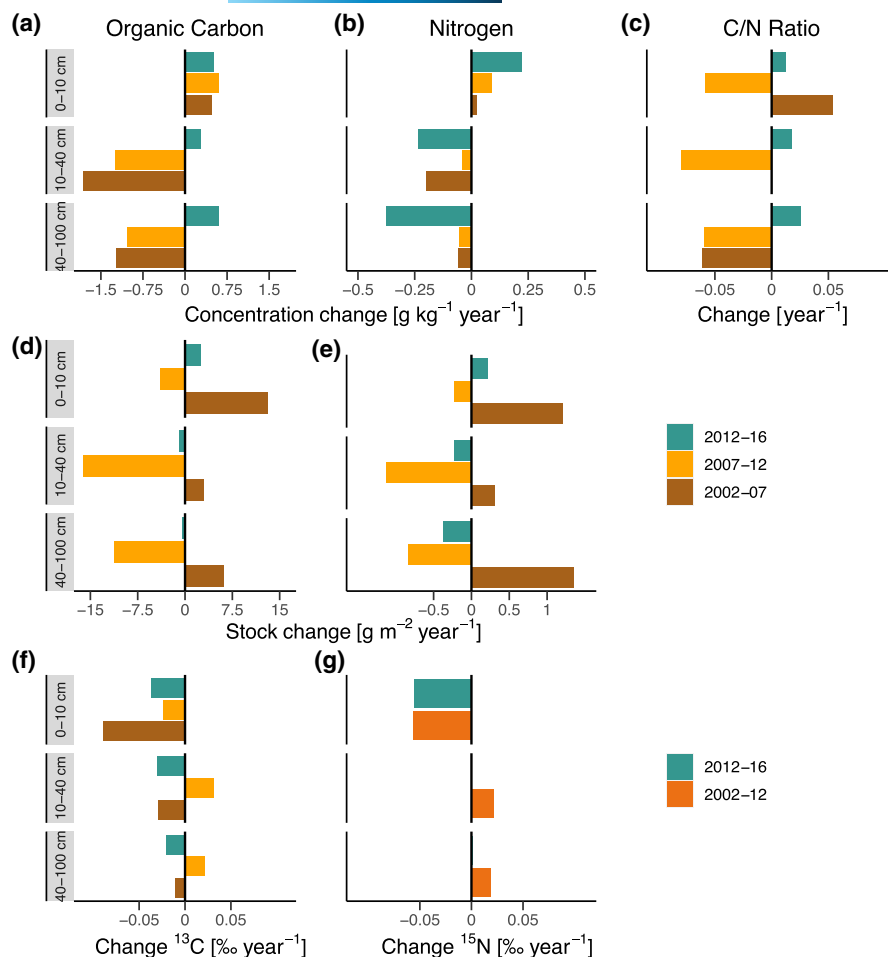
FIGURE 2 Changes of soil (a) organic carbon and (b) soil nitrogen concentrations, (d, e) their stocks, their stable isotope ratios (f) $\delta^{13}C$ and (g) $\delta^{15}N$ values, as well as the (c) carbon-to-nitrogen ratio (C/N ratio) at different soil depth compartments (0–10 cm, 10–40 cm, 40–100 cm) during three soil sampling periods. Concentrations of the 5 cm soil increments were averaged per soil depth compartments and stocks were summed up per compartment. Please note that there are no data on $\delta^{15}N$ values available in 2007.

Besides some transient significant effects of individual plant functional groups on the changes in C concentration, tall herbs showed consistent effects. Surprisingly, C concentrations were reduced in the presence of tall herbs during the first 5-year period in the top 20 cm of the soil (without tall herbs: 0.0 ± 2.3 g kg^{-1}, with tall herbs: -0.1 ± 2.3 g kg^{-1}). Moreover, the presence of tall herbs even increased C losses in the subsoil between 60 and 80 cm until the second 5-year period (without tall herbs: -0.6 ± 1.2 g kg^{-1}, with tall herbs: -1.1 ± 1.4 g kg^{-1}). This loss of C, induced by the presence of tall herbs, was accompanied with a depletion in $\delta^{13}C$ values. However, while the negative effects of the tall herbs on C concentrations decreased toward the end of the study period, the depletion in $\delta^{13}C$ values became stronger (without tall herbs: 0.1 ± 0.5‰, with tall herbs: -0.3 ± 0.4‰). The impacts of tall herbs on the soil N concentration and its $\delta^{15}N$ values were similar to its negative effects on the C concentration and the $\delta^{13}C$ values but not so strong and were only significant at few individual soil depth increments. The presence of small herbs led to reduced depletion in $\delta^{13}C$ values at almost the entire soil column considered, while C concentrations were not significantly affected. These changes occurred mainly in the first 5-year period (without small herbs: -0.5 ± 0.4‰, with small herbs: 0.1 ± 0.9‰) and attenuated with time (difference from 2002 to 2016 without small herbs: -0.2 ± 0.3‰, with small herbs: 0.0 ± 0.5‰, Figure 3). Moreover, the presence of grasses depleted

the $\delta^{13}C$ values in the first period; but this effect declined and finally vanished over time (Figure 3). The presence of legumes did not change $\delta^{13}C$ values. In contrast, the N concentrations increased in the presence of legumes at the top 5 cm, and smaller losses were found below the former ploughing horizon. In the last study period, the positive effects of legume presence were only present in the depth between 70 and 80 cm (without legumes: -2.1 ± 2.1 g kg^{-1}, with legumes: -1.0 ± 2.1 g kg^{-1}). The presence of legumes was not related to changes in $\delta^{15}N$ values, indicating an overall small impact on the $\delta^{15}N$ values of the existing soil N pool.

C/N ratios increased strongly in the top 5 cm of the soil and decreased in the lower soil layers. However, plant diversity and plant functional composition affected changes in C/N ratios only sporadically and not consistently within the soil profile.

4 | DISCUSSION

Our study represents the first long-term study that elucidates the mechanisms underlying the positive plant diversity effects on soil C and N storage as well as their changes over time within a soil profile to 1 m depth. Using stable isotope analyses, we were able to show how the positive plant diversity effects on soil organic matter storage strengthen over time and extend to subsoil.

FIGURE 3 Explained variance (R^2) of changes in soil organic matter parameters (organic carbon and nitrogen concentrations, stocks, δ^{13}C and δ^{15}N values, and carbon to nitrogen ratio) by plant diversity and functional composition. Effects were estimated after periods of 5, 10, and 14 years. The size of the circles represents the R^2 of significant plant effects (plant species richness, functional group richness, and the presence of grasses, legumes, small herbs, and tall herbs; $\alpha = .05$) on the respective soil parameter. Positive effects are displayed in blue, and negative effects in yellow. Please note that data on δ^{15}N values were not available in 2007.

Explained variance of soil organic matter change
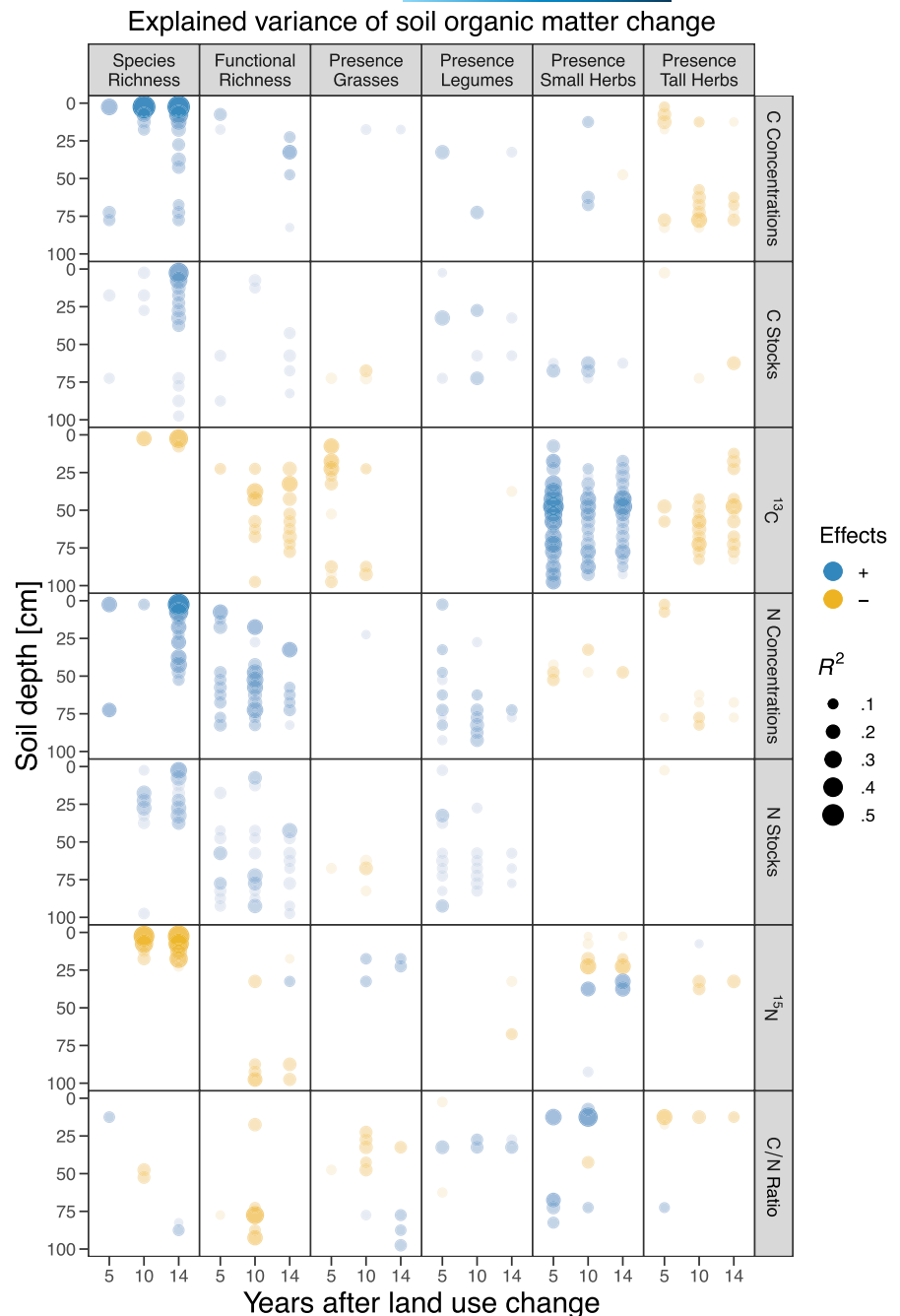
4.1 | Soil organic matter changes following the land-use change

This study was conducted as part of the Jena Experiment on a field site that was formerly used as arable land (Roscher et al., 2004). Thus, before establishing the experiment, the soil profile of the field site showed the typical distribution of soil organic matter concentrations for agricultural fields: soil C and N concentrations were homogeneously distributed within the plough horizon and stocks increased ongoingly toward the deeper layers of the plough horizon. Both C and N concentrations and stocks strongly decline in depth segments below the plough horizon (Angers et al., 1997). The conversion from cropland to a non-fertilized experimental grassland resulted in the well-known exponential decline of C and N concentrations and stocks with soil depth (e.g., Angers & Eriksen-Hamel, 2008; Guo & Gifford, 2002; Poeplau et al., 2011; Post & Kwon, 2000). Specifically, the increase in C and N concentrations and stocks in the top 10 cm of the soil and their decline in the lower parts of the plough horizon result from missing vertical mixing that caused higher biomass input to the deeper layers of the plough horizon and higher root-derived organic matter inputs to the top 10 cm of the soil (Ravenek et al., 2014). Moreover, although changes in C and N concentrations were highly correlated in all soil layers ($R^2 = .82$, Figure S1), the C/N ratios of soil organic matter decreased below the uppermost centimeters toward the C/N ratio that is typical for microbial biomass (Cleveland & Liptzin, 2007). This suggests that in addition to the fresh root-derived inputs

(Eisenhauer et al., 2017), other soil and ecosystem parameters, like the composition and activity of micro-, meso-, and macro-decomposers (Lange et al., 2015; Morriën et al., 2017; Spehn et al., 2000; Stephan et al., 2000) contribute to C and N cycling and storage. Moreover, the decline of C and N concentrations and stocks in the lower plough horizon and below was relatively small in most depth segments. However, in low-diversity plant communities, these losses were larger than the gains in the upper 10 cm of the soil (Table S2). This indicates that land-use changes from croplands to low-diversity meadows might take considerably longer compared with high-diversity meadows until C stocks are as high as before the land-use changes (Poeplau et al., 2011).

4.2 | Plant diversity and community composition effects on C and N

Generally, concentrations of C and N reacted more sensitively to plant diversity effects and the plant effects were observed earlier than in C and N stocks. This might be due to the additional variability caused by physical soil effects, such as bulk density. This is in line with earlier findings reporting that concentration was the most important determinant of stock variance in grassland sites while bulk densities were more important in croplands (Schrumpf et al., 2011).

The increase of both C and N concentrations and stocks within the upper 10 cm of the soil profile was mainly driven by higher plant species richness, confirming results from earlier studies (Cong et al., 2014; Fornara & Tilman, 2008; Lange et al., 2015). This positive plant species richness effect in the top 10 cm of the soil is most likely mediated by the increased allocation of plant material to the soil (Fornara & Tilman, 2008; Lange et al., 2015) in more diverse plant communities (Eisenhauer et al., 2017; Ravenek et al., 2014), as indicated by the strongly depleted $\delta^{13}C$ and $\delta^{15}N$ values. This diversity effect was independent of the soil texture variations across the plots (Table S3). Below the upper 10 cm of the soil, higher plant species richness decreased losses related to the land-use change (see discussion above). These reduced losses are most likely driven by increased inputs under higher plant species. For topsoil, plant diversity has been reported to increase rhizosphere C inputs into the microbial community (Eisenhauer et al., 2010; Lange et al., 2015). This results in both increased microbial activity and C storage as more plant-derived C is converted into forms that persist in soil (Lange et al., 2015). This positive relationship between plant species richness and soil microbial activity became negative in subsoil layers (Table S4). This switch indicates that the role of soil microorganisms in soil organic matter dynamics changes with soil depth. Namely, in the topsoil layer, with its ample supply of plant-derived C and energy, soil microorganisms may contribute to the accumulation of soil organic matter (Gleixner et al., 2002; Lange et al., 2015; Liang et al., 2017), while plant inputs strongly decrease with soil depth (Jobbagy & Jackson, 2000), microbial communities increasingly uses soil organic matter as energy and nutrient source. Thus, the role of microbial communities may shift toward decomposition of already stored soil organic matter (Fontaine et al., 2007) within the soil profile.

Similar effects of plant species richness on C and N concentrations were reported earlier (Fornara & Tilman, 2008). However, the present study for the first time shows that changes in N concentrations and $\delta^{15}N$ values are caused by different mechanisms than those driving changes in C concentrations and $\delta^{13}C$ values. While increasing plant diversity typically results in higher amounts of fresh plant-derived C inputs to the soil (Eisenhauer et al., 2017), only legumes are able to symbiotically fix atmospheric N_2. However, in our study, the presence of legumes did not explain the increases in soil N concentrations or the shifts in the $\delta^{15}N$ values in the first 30 cm of the soil. This is in line with earlier findings, reporting increased N storage in an experimental grassland without the presence of legumes (Cong et al., 2014). However, in our study, the presence of legumes resulted in reduced losses of N in subsoil, while $\delta^{15}N$ values were not significantly affected. This was in contrast to our expectations, as symbiotically fixed N_2 is less discriminated in ^{15}N than inorganic N taken up by plants, for example, as nitrate (Högberg, 1997). Together with the positive effect of plant species richness on N storage within the plough horizon, we suspect that plant communities with higher diversity are more effective to reallocate N from deeper soil layers into upper layers by their root transport. In particular during the period from 2012 to 2016, after the shift from a fertilized arable field to an unfertilized meadow (Lange et al., 2019; Oelmann et al., 2011) the increasing N concentrations in the top 10 cm of the soil and the decreasing below the top 10 cm (Figure 2b) together indicate a stronger N exploitation in deeper soil layers and support the assumption of N reallocation.

Generally, we observed a shift over time of the main drivers of soil C and N dynamics from plant species richness to plant functional richness or functional composition. This indicates that specific functional traits related to plant functional groups impact subsoil processes. Specifically, the presence of tall herbs resulted in losses of C between 60 and 80 cm soil depth, but simultaneously $\delta^{13}C$ values were more depleted. This is suggestive of positive priming, a process in which decomposition of existing organic C is stimulated when new C enters the system through increased microbial activity (Kuzyakov, 2010). Tall herbs are considered as deep-rooting plants (Ebeling et al., 2014). Therefore, we assume that fresh C from deep roots in the subsoil will eventually lead to the decomposition of already stored C (Fontaine et al., 2007).

Small herbs did not significantly affect soil C concentrations, but in their presence, $\delta^{13}C$ values were less depleted in almost all soil layers below 20 cm soil depth. Due to the growth of small herbs close to the soil surface, an uptake of isotopically more depleted CO_2 derived from soil respiration compared with air is likely (Roscher et al., 2011). As more depleted plant inputs would lead to a depletion of the soil $\delta^{13}C$ values, the observed enrichment in $\delta^{13}C$ in the presence of small herbs indicates enhanced respiration and recycling of the already stored organic matter. The enrichment in the presence of small herbs was only observed in the first years after land-use change, indicating that this enrichment in ^{13}C was related to the land-use change.

The positive effect of plant species richness on soil C has been reported for short-term experiments and observations (De Deyn et al., 2009; Steinbeiss et al., 2008; Xu et al., 2020). Our study shows for the first time a strengthening of the positive BEF relationship for soil organic matter storage, generalizing an increasing plant diversity effect over time which has mostly been reported for plant biomass (e.g., Cardinale et al., 2007; Guerrero-Ramírez et al., 2017; Reich et al., 2012), but see Eisenhauer et al. (2010). However, before plant species richness became the dominating driver, changes in the N concentrations were more strongly driven by the richness of plant functional groups, indicating complementarity between plant functional groups (Fornara & Tilman, 2008). Moreover, over time, plant diversity became more important for subsoil processes, while the effects of individual plant functional groups decreased in their importance. Together, this indicates that specific plant traits, such as rooting depth or the ability for symbiotic fixation of N_2, drive subsoil organic matter dynamics in the shorter term. In contrast, in the long-term plant species richness may become more and more important as a driver of subsoil organic matter dynamics. This assumption is in line with Hobley et al. (2017) who proposed that organic matter storage in soils is input-driven down the whole profile; a process likely driven by plant diversity over time.

On a global scale, the vertical distribution of root biomass is highly related to the vertical distribution of soil organic C (Jobbagy & Jackson, 2000), which led to the assumption that the root depth distribution drives the soil organic matter within the soil profile. In our study, however, the root biomass sampled in 2014 (i.e., the latest timepoint after the land-use change in 2002) as well as the root production of the years 2015 and 2016 was most strongly correlated to changes in C and N concentrations during the entire period at different soil depth segments (Tables S5 and S6) but less to the specific period of sampling. Moreover, the root biomasses of earlier years were even less strongly correlated with changes in C and N concentrations in the respective time periods (Table S5). This, together with the fact that the relationship between species richness and root biomass strengthens with time in the Jena Experiment (Ravenek et al., 2014), raises the possibility that the root development follows the soil changes after the land-use change.

Moreover, the positive effect of plant species richness on subsoil C and N together with the negative effect of deep-rooting tall herbs on subsoil C indicate that the positive plant diversity effects are not solely driven by the allocation of fresh plant-derived organic matter to deeper soil layers. Instead, the results of our study suggest that plant diversity effects on soil organic matter storage start at the topsoil and extend over time to deeper soil layers. This effect is most probably mediated by the transport of already stored soil organic C to deeper soil layers, for example, through bioturbation by deep-burrowing earthworms, such as *Lumbricus terrestris* (Eisenhauer et al., 2009; Fischer et al., 2014). We further propose that the plant diversity effect on C storage in subsoil is co-determined by the leaching of soil organic matter from the topsoil and transporting it to deeper soil layers as dissolved organic matter (Kaiser & Kalbitz, 2012). Although, only sporadic correlations of dissolved

organic matter concentrations with soil C changes were observed (Table S7), dissolved organic matter concentrations themselves are highly correlated to the concentrations of overlying soil C, particularly in the subsoil (Table S8). This continuous transport of small amounts of organic material to deeper soil layers is likely to contribute to the formation of soil organic matter (Neff & Asner, 2001). Indeed, in the Jena Experiment, higher concentrations of dissolved organic matter were found with increasing plant diversity (Lange et al., 2019). And although dissolved organic matter undergoes strong transformation and degradation during its soil passage (Roth et al., 2019), increased concentrations of dissolved and less microbially transformed organic matter were shown to reach deeper soil layers at high plant diversity (Lange et al., 2021). Thus, the spatial extension of the positive plant diversity effect on subsoil organic matter highlights how grassland biodiversity can contribute to soil C sequestration (Bai & Cotrufo, 2022; Yang et al., 2019) and thereby potentially mitigate the anthropogenic increase of atmospheric carbon dioxide concentrations (Balesdent et al., 2018; Paustian et al., 2016).

The present study demonstrates that subsoil organic matter storage is significantly related to plant-derived C inputs and their microbial conversion to soil organic matter, in particular, over longer time periods. Managing plant diversity may, thus, have significant implications for subsoil C storage and other critical ecosystem services. Moreover, the strengthening of the positive plant diversity effects on organic matter storage in the topsoil and the incipient effects in subsoil indicate that new input pathways of organic matter to the surface take some time to establish. After the land use changed from arable land to a grassland system regular ploughing was no longer applied, and, thus, the accompanied transfer of organic matter from the surface and topsoil to deeper soil layers stopped. However, the pathways of organic matter input to the subsoil in the grassland system required first the build-up of a large topsoil pool, which can then be transferred to the subsoil via leaching of dissolved organic matter and bioturbation.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Edmond, the Open Research Data Repository of the Max Planck Society, under https://doi.org/10.17617/3.N1GTKL.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.