

Discrepant responses of soil organic carbon dynamics to nitrogen addition in different layers: a case study in an agroecosystem

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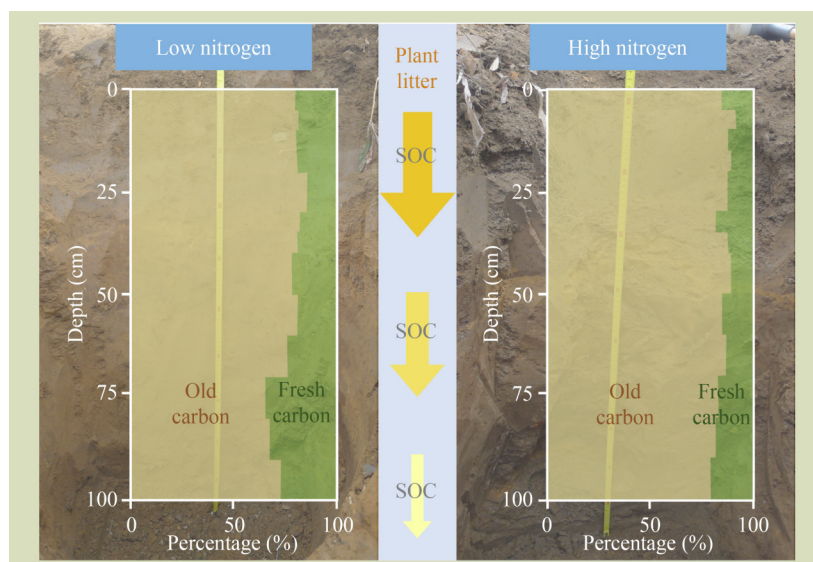
KEYWORDS

¹³C natural abundance, nitrogen addition, soil depth, organic carbon turnover

HIGHLIGHTS

- ¹³C isotope analysis was used to estimate the contribution of new and old carbon to SOC.
- The maize plot with high N rate improved SOC fixation than the maize plot with low N rate.
- The maize plot with high N rate transferred organic matter to a deeper soil layer.
- There are remarkable differences in turnover time of SOC under different N rates.

GRAPHICAL ABSTRACT



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ABSTRACT

Empirical research indicates that heightened soil nitrogen availability can potentially diminish microbial decomposition of soil organic carbon (SOC). Nevertheless, the relationship between SOC turnover response to N addition and soil depth remains unclear. In this study, soils under varying N fertilizer application rates were sampled up to 100 cm deep to examine the contribution of both new and old carbon to SOC across different soil depths, using a coupled carbon and nitrogen isotopic approach. The SOC turnover time for the plot receiving low N addition ($250 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1} \text{ N}$) was about 20–40 years. Conversely, the plot receiving high N ($450 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1} \text{ N}$) had a longer SOC turnover time than the low N plot, reaching about 100 years in the upper 10–20 cm layer. The rise in SOC over the entire profile with low N addition primarily resulted from an increase in the upper soil (0–40 cm) whereas with high N addition, the increase was mainly from greater SOC in the

deeper soil (40–100 cm). Throughout the entire soil layer, the proportion of new organic carbon derived from maize C₄ plant sources was higher in plots treated with a low N rate than those treated with a high N rate. This implies that, in contrast to low N addition agricultural practices, high N addition predominantly enhances the soil potential for fixing SOC by transporting organic matter from surface soils to deeper layers characterized by more stable properties. This research offers a unique insight into the dynamics of deep carbon under increased N deposition, thereby aiding in the formulation of policies for soil carbon management.

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1 Introduction

Soil is the largest reservoir of organic carbon in terrestrial ecosystems. Approximately 1500 Gt of carbon in the world is stored in the first meter of soil as organic matter, and this value is more than twice the carbon pool of terrestrial plants and the atmosphere^[1]. Accordingly, a slight change in soil organic carbon (SOC) pool can markedly alter the atmospheric carbon dioxide (CO₂) concentration, thereby affecting climate change^[2,3]. The change in SOC pool is affected by many natural environmental factors, such as inputs from plant litter, temperature, precipitation, soil physical and chemical properties, and soil texture^[4]. Many anthropic factors, such as land use change, grazing, tillage and fertilizer application, also affect the size of SOC pool by changing plant growth and microbial activity^[3]. Generally, the size of SOC pool is determined by two dynamic processes, namely, carbon input and carbon output. When the carbon output is greater than the carbon input, soil is unsuitable for functioning as a carbon sink, which can lead to the additional carbon to release into atmosphere and thus cause global environmental problems such as the enhanced atmospheric greenhouse effect.

Microbial decomposition is not only the main output process of SOC but also an important part of soil carbon cycle in terrestrial ecosystem^[5]. The amount of CO₂ released by microbial respiration is an important indicator of SOC quality and nutrient cycling speed^[6]. Microbial respiration is closely related to microbial activity, quantity, community composition and structure^[7]. Also, microbial respiration is affected by many abiotic factors, such as temperature, moisture, active organic carbon components and soil texture^[8]. Microbial respiration is also extremely sensitive to human disturbances, such as tillage and fertilizer application, which can considerably influence the microbial respiration rate and production^[9].

With increasing human activity, the increase in atmospheric nitrogen deposition has changed the input of exogenous N in

terrestrial ecosystems^[10], consequently affecting the carbon input of aboveground plants. Clearly, N turnover is tightly coupled to the carbon cycle^[11]. Although the application of N fertilizer exerts great practical importance on improving the soil nutrient status of grassland and promoting the productivity of terrestrial ecosystem^[12], it changes the composition and structure of community species and reduces the richness of species^[13,14]. It should be noted that there is no consistent conclusion on the effect of N fertilizer application on SOC pools, with some studies reporting positive effects and others reporting negative effects, and some indicating no significant impact^[15,16]. Nevertheless, this effect operates through two primary mechanisms. One involves influencing the input of SOC by enhancing plant productivity or altering root deposition^[16,17], while the other involves modifying the output of SOC by altering microbial decomposition processes^[15]. Nevertheless, the effect is determined through two main ways. One way is to affect the input of SOC by increasing the productivity of plants or changing the root deposition^[16–18], the other is to affect the output of SOC by changing the microbial decomposition processes^[15].

Although there has been extensive research on the relationship between the decomposition of SOC and the application of N fertilizer^[19–22], the majority of studies have focused on surface soil, with a notable absence of systematic research on deep soils. The regulatory mechanism of deep SOC is different from that of surface SOC. The biomass and turnover rate of belowground biomass are more influential in the accumulation of SOC than the input of aboveground biomass^[23–25]. Previous research has indicated that organic carbon in deep soils primarily originates from belowground biomass, which governs the circulation and distribution of organic carbon within deep soil layers^[26,27]. Globally, the vertical distribution pattern of organic carbon in soil profiles is more closely associated with vegetation than climate^[28,29]. However, the overall quantity of SOC exhibits a stronger correlation with climate than with vegetation^[30]. Generally, total SOC levels

increase with higher precipitation and clay content, but decrease with rising temperatures. This trend is likely due to the accumulation of organic carbon in deeper soil layers, where climate exerts greater influence on surface organic carbon changes, while clay content regulates deeper organic carbon alterations^[31]. An earlier investigation identified variances in both the biochemical attributes and mineralization rates of SOC between deep and surface soil layers, along with fluctuations in its susceptibility to disturbances^[4]. In combination, it is reasonable to infer that the regulatory mechanisms governing organic carbon in deep soils might differ from those in surface soils under conditions of N deposition.

Information regarding incoming fluxes stemming from root mortality and exudation by living roots remains obscure in the absence of tracers. Additionally, quantifying the outflow from the organic reservoir via microbial heterotrophic respiration *in situ* poses considerable challenges^[32]. Consequently, isotopic methods offer a fitting solution for tracking deep carbon dynamics. The radiocarbon age of deep carbon serves as an indicator of its sluggish turnover^[33,34]. However, relying solely on radiocarbon dating, which furnishes mean ages, fails to provide precise estimations of the proportions of active and stable carbon^[35]. Alternatively, a viable approach involves stable-isotope-based observations to discern the actual depth distribution of soil carbon ages. This method hinges on sites characterized by natural shifts in the $^{13}\text{C}/^{12}\text{C}$ ratio of vegetation at known dates. Essentially, this mirrors the continuous *in situ* labeling of atmospheric carbon atoms, which integrate into soil organic matter over a specified duration eventually replacing preexisting organic carbon, and are retrievable at the time of sampling^[22].

In this study, soil samples were collected from field plots that had been treated with varying rates of N fertilizers. Through employing stable carbon isotope analysis, we examined the contributions of both new and old carbon to SOC, aiming to elucidate the role of soil depth. Also, we investigated the response mechanism of SOC dynamics to N fertilizer application rates in deeper soil layers by integrating the physical and chemical properties of soil with the vertical distribution of organic carbon and N.

2 Materials and methods

2.1 Study area and field experiment

The research site was situated east of Xinlitun Village, within the Haidian District of Beijing, China (39°56' N, 116°24' E).

This region has a typical warm temperate semi-humid continental monsoon climate, characterized by hot and rainy summers and cold, dry winters. The average annual temperature ranges between 10 and 12 °C, with January typically has temperatures between −7 and 4 °C, while July has temperatures between 25 and 26 °C. The temperatures extremes recorded are −27.4 and 41.6 °C. Annual rainfall is about 640 mm, with a noticeable seasonal pattern. Over 70% of precipitation occurs during the summer months of June through August. The soil composition comprises tidal cinnamon soil and sandy loam soil, with pH ranging from 6.8 to 7.1 in the study area.

At the study site, nine 50 m × 30 m plots were subjected to three treatments (three replicate plots for each treatment: 0, 250, and 450 kg·ha^{−1}·yr^{−1} N. To investigate the differential effects of these N application levels on SOC turnover, the low and high rates were based on the commonly used rate of 300 kg·ha^{−1}·yr^{−1} N applied by local farmers^[36]. The vegetation at the site was changed from C₃ (mixed species) to C₄ (maize) in 2006. Planting starts from April to May, and maize crops were harvested from August to September every year. The basal fertilizer represented 38.8% of the N applied. Topdressing fertilizer was applied at the six-leaf, 10-leaf and silking stages, representing 11.2%, 37.6% and 12.4%, respectively, of total N applied. Prior to vegetation change, the aboveground C₃ vegetation was mainly composed of annual weeds, such as *Digitaria sanguinalis*, *Eleusine indica*, *Portulaca oleracea*, *Salsola collina*, *Thlaspi caerulescens*, and *Xanthium sibiricum*.

2.2 Collection and pretreatment of soil samples

The field experiment started with vegetation change in 2006 and ended with the sampling in 2011. Soil profile samples in the nine plots assigned to the three treatments were collected in November 2011. Two 1-m profiles were collected on each plot about 5 m apart. The sampling intervals for 0–40 and 40–100 cm were 5 and 10 cm, respectively. A total of 14 samples were collected for each profile. When these were collected, two parallel samples were obtained by the ring-knife method and mixed them into one sample for soil bulk density measurement. Subsequently, about 1 kg of the same layer sample was collected with a soil shovel for the determination of other soil physical and chemical properties.

Soil samples was froze, crushed, and sieved (2 mm). For the proportion of the sample > 2 mm, the any soil aggregates were weighed after the removal of plant residues and used for the calculation of bulk density. For the proportion of sample < 2 mm, the visible plant residues was removed before bagging

and sealing for subsequent analysis.

2.3 Measurement of basic physical and chemical properties of soil

Soil bulk density was measured by the ring knife method, and each sample was measured in parallel twice. SOC density (D_{SOC} , $\text{kg}\cdot\text{m}^{-2}$) and soil N density (D_{SN} , $\text{kg}\cdot\text{m}^{-2}$) were obtained as:

$$D_{SOC} = \sum_i^n SOC \times \gamma_i \times H_i \times \frac{\left(1 - \frac{\delta_{2mm}}{100}\right)_i}{100} \quad (1)$$

$$D_{SN} = \sum_i^n SN \times \gamma_i \times H_i \times \frac{\left(1 - \frac{\delta_{2mm}}{100}\right)_i}{100} \quad (2)$$

where, SOC is the soil organic carbon content ($\text{g}\cdot\text{kg}^{-1}$), γ_i is the soil bulk density ($\text{g}\cdot\text{cm}^{-3}$), H_i is the soil layer thickness (cm) of the i th layer in the soil profile, n is the number of soil layers in the soil profile, and δ_{2mm} is the proportion of particles with a particle size of > 2 mm (%) and SN is the soil N content ($\text{g}\cdot\text{kg}^{-1}$).

Total organic carbon and total N in soil were determined with an element analyzer (Vario EL Cube, Elementar, Germany) after treatment with HCl ($1 \text{ mol}\cdot\text{L}^{-1}$) to remove inorganic carbon (e.g., CaCO_3). $\delta^{13}\text{C}_{org}$ and $\delta^{15}\text{N}$ were examined using a mass spectrometer (Thermo-Finnigan MAT253) coupled with a COSTECH elemental analyzer. The standard deviations were less than 0.05% for organic carbon and total N and 0.15‰ for $\delta^{13}\text{C}_{org}$ and $\delta^{15}\text{N}$, respectively. $\delta^{13}\text{C}_{org}$ and $\delta^{15}\text{N}$ were expressed by the following formulas:

$$\delta^{13}\text{C}_{org} = \frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}} - (^{13}\text{C}/^{12}\text{C})_{\text{standard}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} \times 1000/\text{‰} \quad (3)$$

$$\delta^{15}\text{N} = \frac{(^{15}\text{N}/^{14}\text{N})_{\text{sample}} - (^{15}\text{N}/^{14}\text{N})_{\text{standard}}}{(^{15}\text{N}/^{14}\text{N})_{\text{standard}}} \times 1000/\text{‰} \quad (4)$$

The standard substance used for the analysis of stable carbon isotopes was Pee Dee Belemnite and for stable N isotope analysis it was atmospheric N_2 .

Soil clay ($< 2 \mu\text{m}$) content was determined by using the laser diffraction method^[37]. Specifically, freeze-dried sample of 0.25–0.35 g added to 200 mL beakers with 5 mL 10% H_2O_2 to remove organic matter. The addition of H_2O_2 was repeated 3–4 times. After the reaction was completed, water was added and boiled to hydrolyze excess H_2O_2 . A sufficient amount of $3 \text{ mol}\cdot\text{L}^{-1}$ HCl was then added and boiled. After the carbonate was removed, the sample was washed with about 150 mL of deionized water. Then, 5 mL of $0.05 \text{ mol}\cdot\text{L}^{-1}$ sodium hexametaphosphate was added and boiled for 5 min to disperse

the sample fully. The resulting suspension was cooled, and particle size was measured with a laser particle size analyzer (MasterSizer 2000, Malvern Panalytical, UK). Background values were deducted from the measurement results.

2.4 Proportion of new incorporated carbon and turnover time of SOC

The natural isotopic ($\delta^{13}\text{C}_{org}$) difference due to different photosynthetic pathways allows for the proportion of new incorporated carbon that is derived from maize residues (C_4 plant) to be calculated using a two-compartment mixing-model^[38]:

$$f_1 = (\delta_X - \delta_I) \times D_{SOC-X} / \Delta\delta_{C_4-C_3} \times 100 \quad (5)$$

$$f_2 = (1 - f_1) \times 100 \quad (6)$$

where, f_1 is the fraction of new carbon derived from maize residues (C_4 plant) in 2011, f_2 is the fraction of initial carbon derived from C_3 plant residues, δ_X is the $\delta^{13}\text{C}$ of SOC in 2011, δ_I is the $\delta^{13}\text{C}$ of the corresponding SOC under the initial C_3 plants in 2006, D_{SOC-X} is the SOC in 2011 and $\Delta\delta_{C_4-C_3}$ is the difference in vegetation $\delta^{13}\text{C}$ between the new C_4 plant and initial C_3 plant and was determined from plant litter samples. δ_X and δ_I were obtained from the soils collected at the same depth when estimating f_1 in a soil layer.

The turnover time of SOC was calculated as:

$$T_{SOC} = -\Delta t / (\ln(1 - f_1)) \quad (7)$$

where, T_{SOC} is the turnover time of SOC, Δt is the period between vegetation switch and soil sampling.

2.5 Data analysis and statistics

Data were represented as arithmetic mean \pm standard deviation calculated from replicates. All statistical tests were performed using SPSS 18.0 software (SPSS Inc., USA). D_{SOC} and D_{SN} between treatments for each of the experiments were compared using one-way ANOVA, followed by Duncan tests at $P < 0.05$. All data are subject to homogeneity test before ANOVA to ensure they are in a normal distribution. The OriginPro 2021 software were used to visualize data.

3 Results

3.1 Effects of N addition on SOC and total N in soils

Compared to control, both SOC and total N increased

markedly in the treatments with added N (Fig. 1). However, the N addition rate had varying effects on soil organic matter. Over the whole profile, the SOC with low with high N addition were about 5% and 50% higher than the control, respectively, and total N was about 7% and 35% higher, respectively.

The differences in SOC and total N between N addition and the control varied depending on soil depth (Fig. 2(a,b)). In the 0–20 cm soil layer, no marked difference in SOC and total N was found between treatments. In the 20–100 cm layer, both SOC and total N were significantly higher with the high N rate than in the control. For the low N rate, the SOC at 20–40 cm and above 70 cm was higher than in the control but was the reverse at 40–70 cm (Fig. 2(a)), and the total N at 20–40 and 60–100 cm were higher than in the control but the reverse at 40–60 cm layer (Fig. 2(b)).

With low N addition, the SOC and total N respectively increased by 6% and 13% relative to the control at 0–40 cm and by 2% and 10% at 40–100 cm layer, respectively (Fig. 2(c,d)). With high N addition, the SOC and total N increased by 18% and 13% at 0–40 cm relative to the control and by 115% and 67% at 40–100 cm, respectively. In other words, the increase in the organic matter over the whole profile with low N addition was mainly due to an increase in organic matter in the upper soil (0–40 cm) whereas high N addition it was mainly due to an increase in organic matter in the deeper soil (40–100 cm).

The median point for dividing the SOC and total N over the whole profile into half varied between different treatments (Fig. 2(c,d)). For SOC, the median point of the control and low N treatment both were at about 25–30 cm whereas, for the high N treatment, it was at about 40 cm. For total N, the median

point for the control and low N treatment were about at about 30–35 cm whereas, for the high N treatment, it was at about 40 cm. Overall, the distribution of SOC and total N in the profile did not markedly change with low N addition relative to the control. However, for high N addition, the SOC and total N had a significant shift down the profile, indicating that deeper soil in this treatment was more conducive for the fixation and accumulation of soil organic matter than shallow soil.

3.2 Vertical distribution characteristics of soil clay content

There were no evident changes of soil clay content found in surface soils (0–35 cm layer) relative to the control (Fig. 3). Soil clay content peaked at about 80% to 90% at 35–60 cm and decreased gradually to depth. For low N treatment, soil clay content did not markedly change in surface soil (0–35 cm layer) but fluctuated to depth with the lower values at 35–40, 60–70, and 90–100 cm. Soil clay content with high N addition had a slight decreasing trend.

Compared with the control plot, the clay content over the whole profile decreased markedly with both low and high N addition. At 0–35 cm, no significant difference in soil clay content was found between plots with low and high N addition. However, at 35–100 cm, the soil clay content with low N addition was generally lower than the high N treatment (Fig. 3).

3.3 Vertical distribution characteristics of SOC $\delta^{13}\text{C}$ and total N $\delta^{15}\text{N}$

The $\delta^{13}\text{C}$ of SOC in the soil surface (0–5 cm) in the control plot

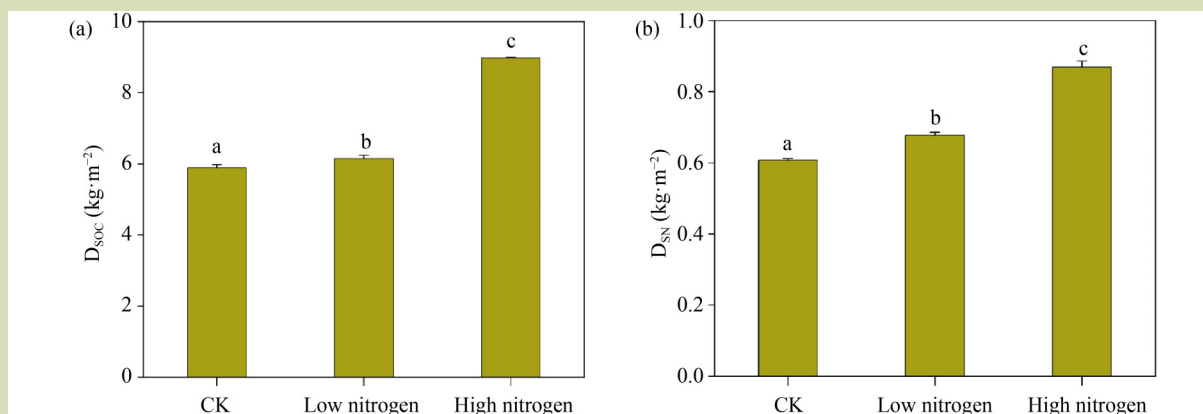


Fig. 1 Soil organic carbon density (D_{SOC}) (a) and total soil nitrogen density (D_{N}) (b) over the whole soil profile (1 m) for three N application rates: 0, 250, and 450 kg·ha⁻¹·yr⁻¹ N (CK, low and high). Bars with the different letter are significantly different (Duncan-test, $P < 0.05$).

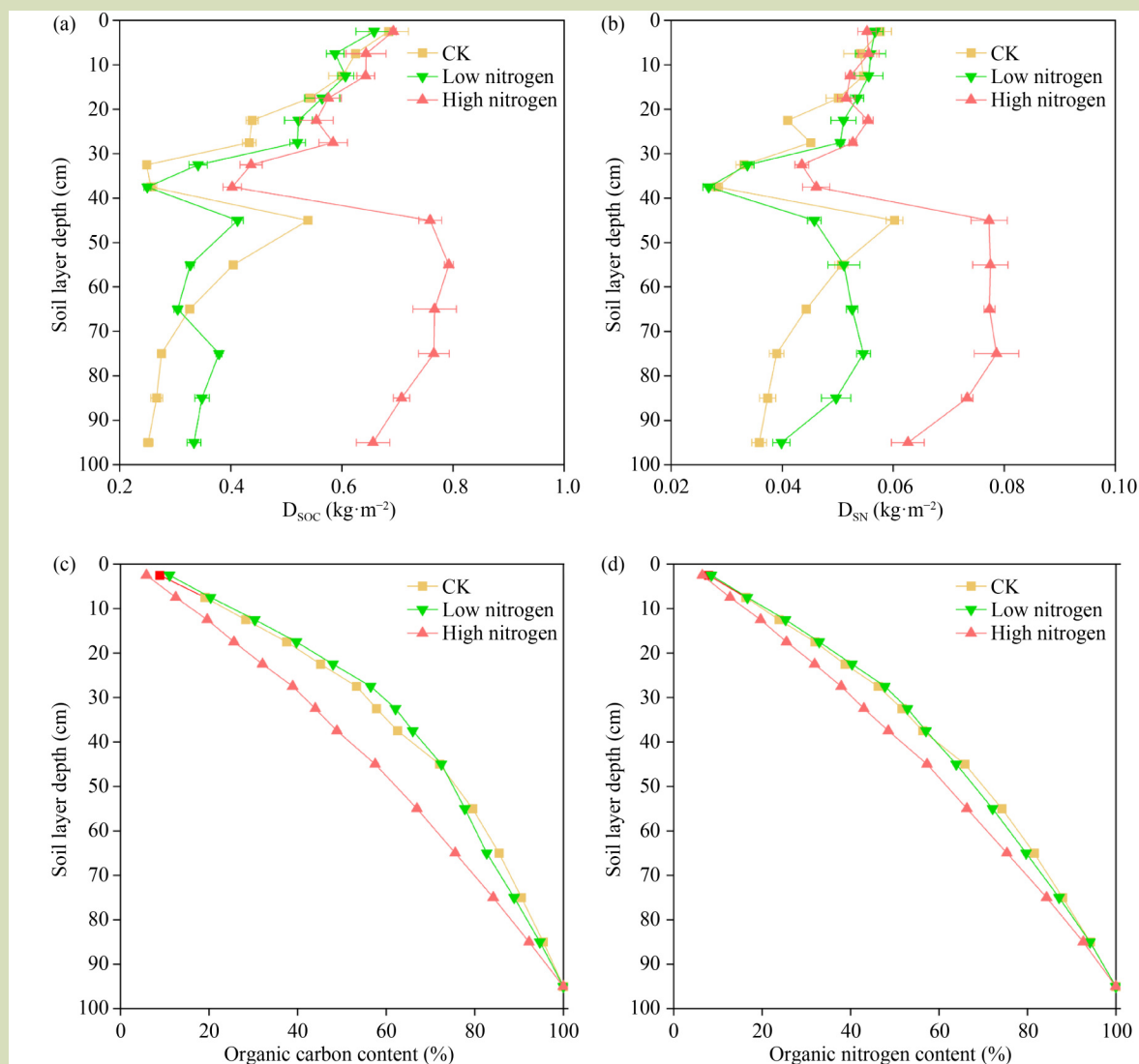


Fig. 2 Soil organic carbon density (D_{SOC}) (a), total nitrogen density (D_{SN}) (b), organic carbon content (c), and organic nitrogen content (d) across the soil profile for three N application rates: 0, 250, and 450 $\text{kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ N (CK, low, and high). The organic carbon (or nitrogen) content refers to the percentage of accumulated D_{SOC} (or D_{SN}) from the surface layer to a certain layer in the whole soil profile (0–100 cm).

was about -25‰ (Fig. 4(a)), indicating that the vegetation cover was mainly C_3 plants. With maize grown with N addition, the $\delta^{13}\text{C}$ value of SOC in the surface layer (0–5 cm) increased significantly, and the increase was greater with low N addition.

The vertical distribution of SOC markedly changed after vegetation conversion. The variation in $\delta^{13}\text{C}$ of SOC in the whole soil profile was -24.6‰ to -20.6‰ , -22.3‰ to -17.4‰ , and -22.9‰ to -18.2‰ for the control, and low and high N treatments, respectively (Fig. 4(a)). With increasing depth, $\delta^{13}\text{C}$ of SOC had an increasing trend across the three treatments. The variation in $\delta^{15}\text{N}$ of total N in the whole soil profile was

4.0‰ to 7.4‰ , 4.6‰ to 6.4‰ , and 4.5‰ to 6.8‰ for the control, and low and high N treatments, respectively (Fig. 4(b)). With increasing depth, $\delta^{15}\text{N}$ between treatments was similar and had a gradual increasing trend (Fig. 4(b)), that is, the soil $\delta^{15}\text{N}$ of total N gradually was enriched to depth.

The difference ($\Delta^{15}\text{N}$) in $\delta^{15}\text{N}$ between each soil layer and the soil surface layer (0–5 cm) was used to indicate the enrichment of $\delta^{15}\text{N}$ of total N to depth. The degree of enrichment of $\Delta^{15}\text{N}$ in different soil layers was control, high and low N treatments, in that order (Fig. 5). Overall, the gradually enriching trend of $\delta^{15}\text{N}$ of total N to depth was markedly weaker in the treatments with N applied compared to the control.

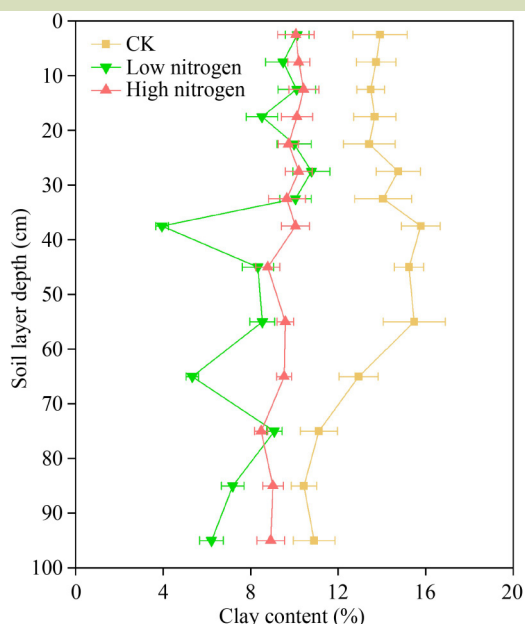


Fig. 3 Distribution of soil clay content across the soil profile for three N application rates: 0, 250, and 450 kg·ha⁻¹·yr⁻¹ N (CK, low and high).

3.4 Estimation of new and old organic carbon pools and turnover time of organic carbon in soils

The $\delta^{13}\text{C}$ of SOC in the soil of control plot were considered to

represent the value (δ_1) of the $\delta^{13}\text{C}$ of the original C₃ plant cover in each soil layer (Table S1). The $\delta^{13}\text{C}$ of maize litter in this study was -13.8‰ . If the isotope fractionation value in the process of converting the maize litter into surface SOC was estimated to be 1.5‰ , the $\delta^{13}\text{C}$ value of surface SOC should be -12.3‰ . We further assumed that the variation of $\delta^{13}\text{C}$ value of SOC in the soil in treatments with added N was consistent with the control, and the $\delta^{13}\text{C}$ end value of the maize C₄ plant source (δ_2) in the corresponding layers could be calculated (Table S1). The percentage of new organic carbon input from maize C₄ plant source in the treatments low N addition was greater than with high N addition, but both were not more than 40% (Fig. 6). Across the whole soil profile, the percentage of new organic carbon with low N addition was between 13% and 34%, with the highest value at 70–90 cm. N addition had a significant impact on the organic carbon turnover time. The organic carbon turnover time at 0–20 cm with low N addition was between 20 and 30 years whereas with high N addition it was about 35 years at 0–5 cm but reached about 100 years at 5–20 cm (Fig. 7).

4 Discussion

Vegetation conversion is a land use change that exerts influence on SOC dynamics through two primary mechanisms. Firstly, it alters the quantity of plant residues returned to the

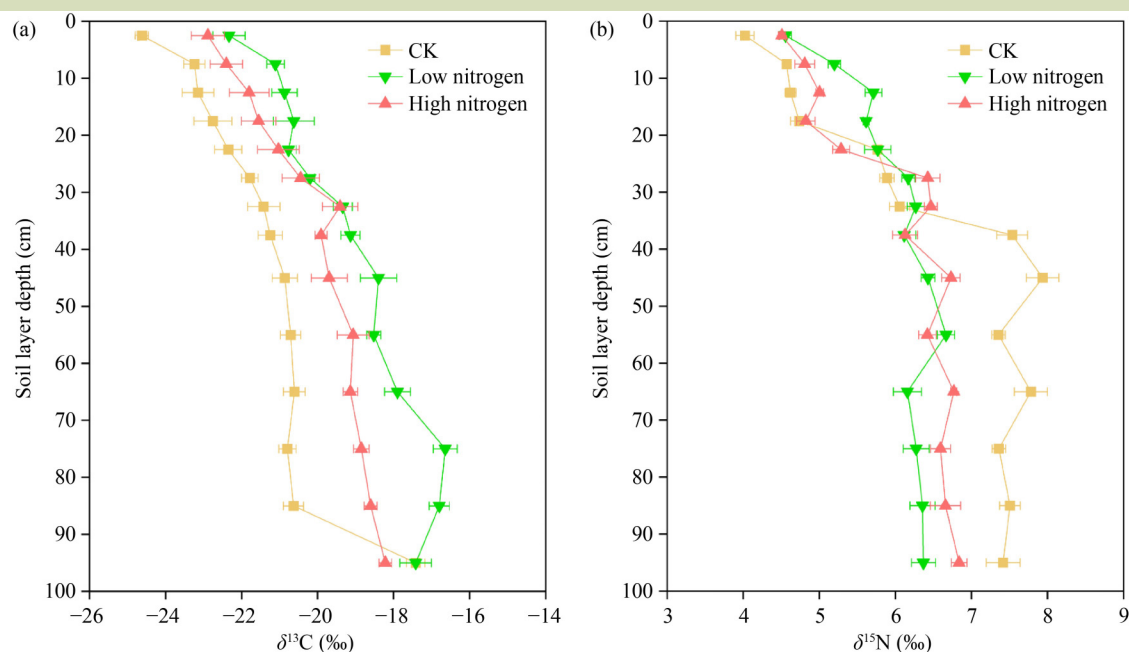


Fig. 4 Variations in soil organic carbon $\delta^{13}\text{C}$ (a) and total nitrogen $\delta^{15}\text{N}$ (b) cross the whole soil profile for three N application rates: 0, 250, and 450 kg·ha⁻¹·yr⁻¹ N (CK, low and high).

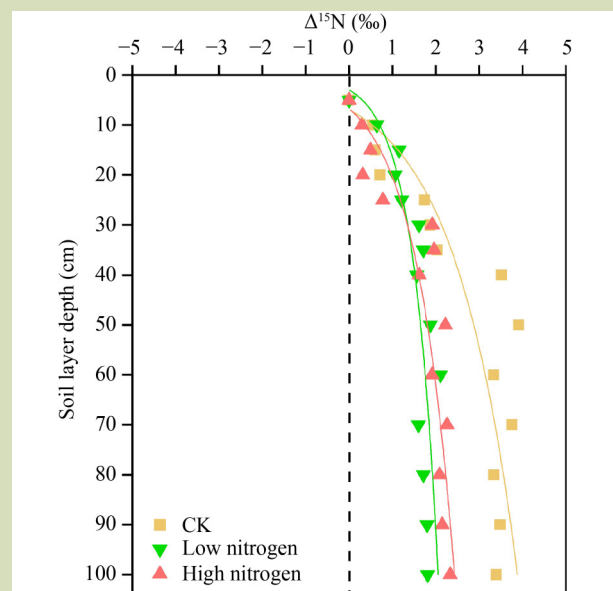


Fig. 5 Variations of $\Delta^{15}\text{N}$ of total N across the soil layer. $\Delta^{15}\text{N}$ indicates the difference between the $\delta^{15}\text{N}$ values of each soil layer and the soil surface layer (0–5 cm) for three N application rates: 0, 250, and 450 $\text{kg}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ N (CK, low and high).

soil by impacting plant growth. Secondly, it modifies the decomposition rate of SOC by influencing soil conditions, thereby affecting the amount of SOC released. Soil tillage has dual effects. It directly reduces soil aggregates, leading to the loss of physical protection for soil organic matter and its exposure. Simultaneously, it enhances soil properties such as aeration, moisture and temperature, thereby promoting microbial activity. These processes collectively accelerate SOC decomposition to varying degrees^[39,40], consequently resulting

in SOC loss^[41,42].

Generally, SOC is considered to be positively correlated with clay content, mainly because clay can combine with SOC to form a stable organic-inorganic complex. Soil clay content is influenced by several factors including vegetation, time and management practices^[43]. Vegetation can influence clay content through its impact on organic matter input and root activity, which can affect soil structure and aggregation. Time is a significant factor in soil clay content as soil formation processes gradually alter the mineral composition over time. Human activities, such as agriculture and land use changes, can also profoundly impact soil clay content through practices like tillage and soil erosion. In combination, these factors might have contributed to our results that the clay content in the maize soil was markedly lower than that in the control soil (Fig. 3). Our results indicate that the decomposition of SOC can accelerate after the transformation of grassland soil to farmland, which is consistent with the conclusion that soil tillage can promote the decomposition of SOC. However, our results indicated that SOC had an increasing trend, especially in the plot added with high N rate (Fig. 1). This result could be attributed to reduced tillage and the simultaneous return of crop straw to the field. Compared with standard tillage practices, the plots in the present study had a greatly reduced the frequency of tillage, which reduced damage to the soil structure. This might slow the increase in SOC accumulation after addition of N, thereby reducing the output flux of SOC. Straw return is an important way to improve potential of farmland soil as a carbon store^[44]. It not only increases the input flux of organic carbon into the soil but also reduces soil erosion. Therefore, straw return to the plots in the present study were also an important reason for the marked increasing

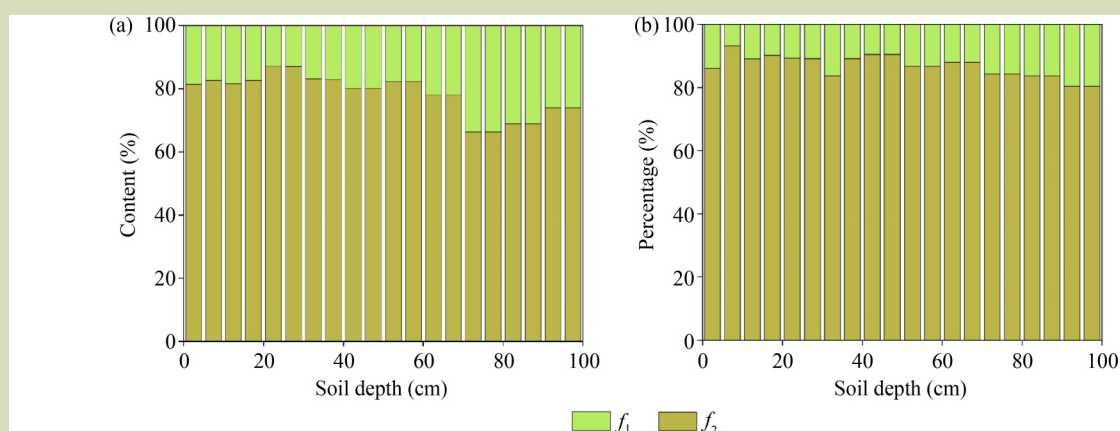


Fig. 6 Content of soil organic carbon from different sources across the soil profile. f_1 and f_2 indicate the organic carbon from the recent maize C_4 plant source and from original C_3 plant source, respectively, for two N application rates: (a) 250 and (b) 450 $\text{kg}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ N.

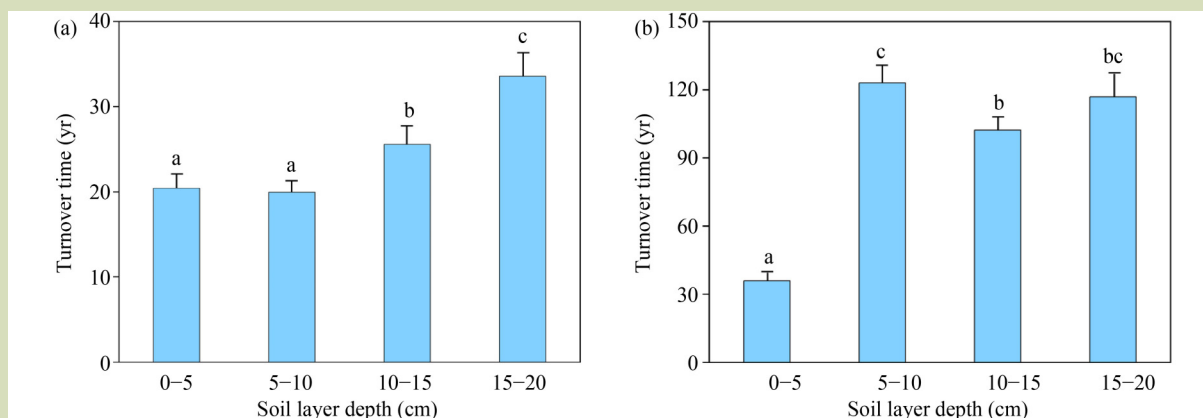


Fig. 7 Turnover time of soil organic carbon in the plots for two N application rates: (a) 250 and (b) 450 kg·ha⁻¹·yr⁻¹ N. Bars with the same letter are not significantly different (Duncan-test, $P < 0.05$).

SOC. Reduced tillage combined with straw return was conducive to the improvement of SOC in the study site.

The present study found that the potential of low N fertilizer application to improve SOC was markedly smaller than that of high N rate (Fig. 1), which was consistent with previous research results^[45–47]. The effect of increased N application promoting SOC accumulation was mainly related to three factors. Firstly, N addition increased the amount of crop stubble returned and SOC content^[48]. Secondly, because of the increased SOC content, soil quality and soil productivity improved, thereby promoting soil carbon fixation^[49]. Thirdly, the increased soil N and microbial biomass decreased the respiration of microorganisms^[50], that is, the decomposition of soil organic matter was slowed. This interpretation is consistent with the finding that high N treatment resulted slower SOC turnover than low N rate treatment (Fig. 7).

SOC is influenced not only by the content of organic carbon in the soil but also by soil bulk density^[51]. Changes in soil density are closely related to soil management practices, including soil moisture and fertilizer application^[52]. In the present study, the changes in soil bulk density at different layers could be the reason for the inconsistencies in direction of change in organic carbon and nitrogen between the control versus the low N treatment (Fig. 2(a,b)). In the high N treatment, deeper soils had more carbon sequestration than the shallower soil (Fig. 2), which could be due to the change in soil organic matter migration down the profile. Recently there has been considerable research on the observation and modeling of belowground carbon pool dynamics^[53], but the results of such studies are not always consistent, and have delivered some contrasting findings.

Soil organic matter decomposition involves isotope fractionation, which can be used to monitor the dynamics of belowground carbon pools by monitoring changes in carbon isotopes during organic matter decomposition. A model was introduced for SOC isotopes, which estimates soil carbon dynamics and variations in carbon isotope abundance between soil layers^[54]. Compared with single soil carbon dynamic model, this optimizing model can better determine some parameters involving in belowground ecological processes, thus improving the accuracy of model prediction. This optimizing model showed that the downward migration rate of soil organic matter is key to the change in soil carbon isotope in soil. It is commonly observed that as the downward migration rate increases, the enrichment of organic carbon isotopes in the upper soil tends to diminish. The dynamics of N decomposition and the fluctuations in N isotopes exhibit similarities to those of carbon dynamics and carbon isotopes within the soil^[55,56]. Therefore, the effect of downward migration rate of soil organic matter on the N isotope in soil was the same as that of soil carbon isotope. In the present study when N was applied, the proportion of $\delta^{15}\text{N}$ in the soil was markedly decreased (Fig. 5), which indicates that the rate of downward migration of soil organic matter had increased. The greatest downward migration rate was recorded in the low N treatment followed by the high N treatment. Thus, the low N treatment had the highest proportion of new organic carbon (Fig. 6(a)). Compared to the control, the low N treatment had a greater rate of downward migration of organic carbon. However, SOC in deep soils did not markedly increase, which might have been due caused by the higher SOC decomposition rate in deep soils. The rate of organic carbon migration was slower in the high N treatment than low N treatment. This is likely due to a slower decomposition of organic carbon with

high N addition, resulting in the accumulation of SOC in the deeper layers. Accordingly, following the conversion of grassland to cropping with high N addition, it was observed that the migration of SOC from surface layers to deeper, more stable soils became the primary mechanism for accumulation of SOC.

5 Conclusions

SOC increased markedly with N addition compared to the control plot, but the increase range varied with the amount of N applied. High N addition gave a better potential to

improvement SOC fixation than low N addition, which was mostly due to reduced tillage and return of crop straw to the field. Marked differences in turnover rate of SOC under the two N rates was observed. The SOC turnover time with low N was about 20–40 years at 0–20 cm whereas with high N was higher at 100 years at 10–20 cm. The high N addition mainly improved SOC fixation by transferring organic matter from the surface down to a more stable deeper soil. Since the majority of the organic matter in the deeper soils with low N addition was derived from the contribution of the new organic carbon to the surface soil, and because its turnover rate was higher, the SOC sequestration capacity of this treatment was not as much as with high N addition.

Supplementary materials

The online version of this article at <https://doi.org/10.15302/J-FASE-2024565> contains supplementary materials (Table S1).

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Compliance with ethics guidelines

Hualing Hu, Liang Zhao, Wenbing Tan, Guoan Wang, and Beidou Xi declare that they have no conflicts of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors.

REFERENCES

1. Lal R, Monger C, Nave L, Smith P. The role of soil in regulation of climate. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 2021, **376**(1834): 20210084
2. Davidson E A, Janssens I A. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, 2006, **440**(7081): 165–173
3. Bhattacharya S S, Kim K H, Das S, Uchimiya M, Jeon B H, Kwon E, Szulejko J E. A review on the role of organic inputs in maintaining the soil carbon pool of the terrestrial ecosystem. *Journal of Environmental Management*, 2016, **167**: 214–227
4. Salomé C, Nunan N, Pouteau V, Lerch T Z, Chenu C. Carbon dynamics in topsoil and in subsoil may be controlled by different regulatory mechanisms. *Global Change Biology*, 2010, **16**(1): 416–426
5. Chapin F S, McFarland J, McGuire A D, Euskirchen E S, Ruess R W, Kielland K. The changing global carbon cycle: linking plant-soil carbon dynamics to global consequences. *Journal of Ecology*, 2009, **97**(5): 840–850
6. Wang X G, Zhu B, Li C S, Gao M R, Wang Y Q, Zhou Z X, Yuan H Y. Dissecting soil CO₂ fluxes from a subtropical forest in China by integrating field measurements with a modeling approach. *Geoderma*, 2011, **161**(1–2): 88–94
7. Blagodatsky S, Blagodatskaya E, Yuyukina T, Kuzyakov Y. Model of apparent and real priming effects: linking microbial activity with soil organic matter decomposition. *Soil Biology & Biochemistry*, 2010, **42**(8): 1275–1283
8. Jindaluang W, Kheoruenromne I, Suddhiprakarn A, Singh B P, Singh B. Influence of soil texture and mineralogy on organic matter content and composition in physically separated fractions soils of Thailand. *Geoderma*, 2013, **195–196**: 207–219
9. Ding W X, Yu H Y, Cai Z C, Han F X, Xu Z H. Responses of soil respiration to N fertilization in a loamy soil under maize cultivation. *Geoderma*, 2010, **155**(3–4): 381–389
10. Liu E, Yan C, Mei X, Zhang Y, Fan T. Long-term effect of manure and fertilizer on soil organic carbon pools in dryland farming in northwest China. *PLoS One*, 2013, **8**(2): e56536
11. Rumpel C, Crème A, Ngo P T, Velásquez G, Mora M L, Chabbi A. The impact of grassland management on biogeochemical cycles involving carbon, nitrogen and phosphorus. *Journal of Soil Science and Plant Nutrition*, 2015, **15**(2): 353–371
12. Jankju M. Role of nurse shrubs in restoration of an arid rangeland: effects of microclimate on grass establishment.

- Journal of Arid Environments*, 2013, **89**: 103–109
13. Isbell F, Tilman D, Polasky S, Binder S, Hawthorne P. Low biodiversity state persists two decades after cessation of nutrient enrichment. *Ecology Letters*, 2013, **16**(4): 454–460
 14. Borer E T, Grace J B, Harpole W S, MacDougall A S, Seabloom E W. A decade of insights into grassland ecosystem responses to global environmental change. *Nature Ecology & Evolution*, 2017, **1**(5): 0118
 15. Mack M C, Schuur E A G, Bret-Harte M S, Shaver G R, Chapin F S III. Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization. *Nature*, 2004, **431**(7007): 440–443
 16. Fornara D A, Tilman D. Soil carbon sequestration in prairie grasslands increased by chronic nitrogen addition. *Ecology*, 2012, **93**(9): 2030–2036
 17. Clark C M, Tilman D. Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. *Nature*, 2008, **451**(7179): 712–715
 18. Li J H, Zhang J, Li W J, Xu D H, Knops J M H, Du G Z. Plant functional groups, grasses versus forbs, differ in their impact on soil carbon dynamics with nitrogen fertilization. *European Journal of Soil Biology*, 2016, **75**: 79–87
 19. Craine J M, Morrow C, Fierer N. Microbial nitrogen limitation increases decomposition. *Ecology*, 2007, **88**(8): 2105–2113
 20. Manzoni S, Taylor P, Richter A, Porporato A, Ågren G I. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytologist*, 2012, **196**(1): 79–91
 21. Kirkby C A, Richardson A E, Wade L J, Passioura J B, Batten G D, Blanchard C, Kirkegaard J A. Nutrient availability limits carbon sequestration in arable soils. *Soil Biology & Biochemistry*, 2014, **68**: 402–409
 22. Tan W, Wang G, Huang C, Gao R, Xi B, Zhu B. Physico-chemical protection, rather than biochemical composition, governs the responses of soil organic carbon decomposition to nitrogen addition in a temperate agroecosystem. *Science of the Total Environment*, 2017, **598**: 282–288
 23. Rasse D P, Rumpel C, Dignac M F. Is soil carbon mostly root carbon? Mechanisms for a specific stabilization. *Plant and Soil*, 2005, **269**(1–2): 341–356
 24. Filley T R, Boutton T W, Liao J D, Jastrow J D, Gamblin D E. Chemical changes to nonaggregated particulate soil organic matter following grassland-to-woodland transition in a subtropical savanna. *Journal of Geophysical Research. Biogeosciences*, 2008, **113**(G3): G03009
 25. Mambelli S, Bird J A, Gleixner G, Dawson T E, Torn M S. Relative contribution of foliar and fine root pine litter to the molecular composition of soil organic matter after *in situ* degradation. *Organic Geochemistry*, 2011, **42**(9): 1099–1108
 26. Nepstad D C, de Carvalho C R, Davidson E A, Jipp P H, Lefebvre P A, Negreiros G H, da Silva E D, Stone T A, Trumbore S E, Vieira S. The role of deep roots in the hydrological and carbon cycles of Amazonian forests and pastures. *Nature*, 1994, **372**(6507): 666–669
 27. Trumbore S E, Davidson E A, Barbosa de Camargo P, Nepstad D C, Martinelli L A. Belowground cycling of carbon in forests and pastures of Eastern Amazonia. *Global Biogeochemical Cycles*, 1995, **9**(4): 515–528
 28. Canadell J, Jackson R B, Ehleringer J B, Mooney H A, Sala O E, Schulze E D. Maximum rooting depth of vegetation types at the global scale. *Oecologia*, 1996, **108**(4): 583–595
 29. Jackson R B, Canadell J, Ehleringer J R, Mooney H A, Sala O E, Schulze E D. A global analysis of root distributions for terrestrial biomes. *Oecologia*, 1996, **108**(3): 389–411
 30. Trumbore S. Radiocarbon and soil carbon dynamics. *Annual Review of Earth and Planetary Sciences*, 2009, **37**(1): 47–66
 31. Jobbágy E G, Jackson R B. The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications*, 2000, **10**(2): 423–436
 32. Chen G, Yang Y, Robinson D. Allometric constraints on, and trade-offs in, belowground carbon allocation and their control of soil respiration across global forest ecosystems. *Global Change Biology*, 2014, **20**(5): 1674–1684
 33. Mathieu J A, Hatté C, Balesdent J, Parent É. Deep soil carbon dynamics are driven more by soil type than by climate: a worldwide meta-analysis of radiocarbon profiles. *Global Change Biology*, 2015, **21**(11): 4278–4292
 34. He Y, Trumbore S E, Torn M S, Harden J W, Vaughn L J S, Allison S D, Randerson J T. Radiocarbon constraints imply reduced carbon uptake by soils during the 21st century. *Science*, 2016, **353**(6306): 1419–1424
 35. Ahrens B, Reichstein M, Borken W, Muhr J, Trumbore S E, Wutzler T. Bayesian calibration of a soil organic carbon model using $\Delta^{14}\text{C}$ measurements of soil organic carbon and heterotrophic respiration as joint constraints. *Biogeosciences*, 2014, **11**(8): 2147–2168
 36. Chen X, Cui Z, Fan M, Vitousek P, Zhao M, Ma W, Wang Z, Zhang W, Yan X, Yang J, Deng X, Gao Q, Zhang Q, Guo S, Ren J, Li S, Ye Y, Wang Z, Huang J, Tang Q, Sun Y, Peng X, Zhang J, He M, Zhu Y, Xue J, Wang G, Wu L, An N, Wu L, Ma L, Zhang W, Zhang F. Producing more grain with lower environmental costs. *Nature*, 2014, **514**(7523): 486–489
 37. Bieganski A, Ryzak M, Sochan A, Barna G, Hernádi H, Beczek M, Polakowski C, Makó A. Laser diffractometry in the measurements of soil and sediment particle size distribution. *Advances in Agronomy*, 2018, **151**: 215–279
 38. Balesdent J, Mariotti A. Measurement of soil organic matter turnover using ^{13}C natural abundance. In: Boutton T W, Yamasaki S, eds. *Mass Spectrometry of Soils*. New York: Marcel Dekker, 1996, 83–112
 39. Denef K, Six J, Merckx R, Paustian K. Carbon sequestration in microaggregates of no-tillage soils with different clay mineralogy. *Soil Science Society of America Journal*, 2004, **68**(6): 1935–1944
 40. Razafimbelo T M, Albrecht A, Oliver R, Chevallier T, Chapuis-Lardy L, Feller C. Aggregate associated-C and physical protection in a tropical clayey soil under Malagasy conventional and no-tillage systems. *Soil & Tillage Research*,

- 2008, **98**(2): 140–149
41. Boyer J N, Groffman P M. Bioavailability of water extractable organic carbon fractions in forest and agricultural soil profiles. *Soil Biology & Biochemistry*, 1996, **28**(6): 783–790
 42. Six J, Elliott E T, Paustian K. Aggregate and soil organic matter dynamics under conventional and no-tillage systems. *Soil Science Society of America Journal*, 1999, **63**(5): 1350–1358
 43. Zhang X, Zhang W C, Wu W, Liu H B. Horizontal and vertical variation of soil clay content and its controlling factors in China. *Science of the Total Environment*, 2023, **864**: 161141
 44. Lal R. Soil carbon sequestration impacts on global climate change and food security. *Science*, 2004, **304**(5677): 1623–1627
 45. Ramirez K S, Craine J M, Fierer N. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Global Change Biology*, 2012, **18**(6): 1918–1927
 46. Zhou L, Zhou X, Zhang B, Lu M, Luo Y, Liu L, Li B. Different responses of soil respiration and its components to nitrogen addition among biomes: a meta-analysis. *Global Change Biology*, 2014, **20**(7): 2332–2343
 47. Riggs C E, Hobbie S E, Bach E M, Hofmockel K S, Kazanski C E. Nitrogen addition changes grassland soil organic matter decomposition. *Biogeochemistry*, 2015, **125**(2): 203–219
 48. Halvorson A D, Reule C A, Follett R F. Nitrogen fertilization effects on soil carbon and nitrogen in a dryland cropping system. *Soil Science Society of America Journal*, 1999, **63**(4): 912–917
 49. Gregorich E G, Liang B C, Ellert B H, Drury C F. Fertilization effects on soil organic matter turnover and corn residue C storage. *Soil Science Society of America Journal*, 1996, **60**(2): 472–476
 50. Liu L, Greaver T L. A global perspective on belowground carbon dynamics under nitrogen enrichment. *Ecology Letters*, 2010, **13**(7): 819–828
 51. Xiao L, Leng M, Greenwood P, Zhao R, Xie Z, You Z, Liu J. Temporal and vertical dynamics of carbon accumulation potential under grazing-excluded grasslands in China: The role of soil bulk density. *Journal of Environmental Management*, 2024, **351**: 119696
 52. Wang Y K, Zhang Z B, Guo Z C, Chen Y M, Yang J S, Peng X H. *In-situ* measuring and predicting dynamics of soil bulk density in a non-rigid soil as affected by tillage practices: Effects of soil subsidence and shrinkage. *Soil & Tillage Research*, 2023, **234**: 105818
 53. Cox P M, Betts R A, Jones C D, Spall S A, Totterdell I J. Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. *Nature*, 2000, **408**(6809): 184–187
 54. Poage M A, Feng X H. A theoretical analysis of steady state $\delta^{13}\text{C}$ profiles of soil organic matter. *Global Biogeochemical Cycles*, 2004, **18**(2): 2003GB002195
 55. Bosatta E, Ågren G I. Theoretical analyses of carbon and nutrient dynamics in soil profiles. *Soil Biology & Biochemistry*, 1996, **28**(10-11): 1523–1531
 56. Baisden W T, Amundson R, Brenner D L, Cook A C, Kendall C, Harden J W. A multiisotope C and N modeling analysis of soil organic matter turnover and transport as a function of soil depth in a California annual grassland soil chronosequence. *Global Biogeochemical Cycles*, 2002, **16**(4): 82–1-82–26