



# More labile carbon inputs lessen the positive effects of nitrogen enrichment on soil carbon storage in a temperate grassland

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## Abstract

**Background and aims** Grassland ecosystems play a vital role in soil carbon (C) sequestration and experience human disturbances that influence soil C storage. Nitrogen (N) enrichment elevates C assimilation and C return, and pervasive interactions between N and C inputs occur but have seldom been addressed simultaneously. This study evaluated the effects of N addition on the soil C pool under labile C input (glucose) and the underlying mechanisms.

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**Methods** In a full factorial designed temperate grassland field experiment treated with five different levels of N addition (0, 2.5, 5, 10 and 20 g N m<sup>-2</sup> yr<sup>-1</sup>) and C addition (0, 25 and 50 g C m<sup>-2</sup> yr<sup>-1</sup>) we tracked C pools in plant, litter and soil for 5 years. **Results** The soil C pool significantly increased with time, and was enhanced by increased N addition rates. Additionally, N enrichment (0–20 g N m<sup>-2</sup> yr<sup>-1</sup>) caused a significant increase in C pools of fresh plants and litter. The positive response of the litter C pool to increasing N addition rates increased with time. The structural equation model revealed a trade-off between the fresh plant and soil C pools and a direct contribution of the litter C pool to the increase in the soil C pool under N input over the 5 years. **Conclusions** The intermediate rates of labile C input favored soil C storage by stimulating C transformation from plant to soil, but more labile C lessened the positive effects of continuous N addition in temperate grassland ecosystems.

**Keywords** N enrichment · Labile C addition · Soil C pool · Plant C pool · Grassland

## Introduction

Grassland ecosystems cover approximately 40% of the terrestrial area on Earth and store one-third of the global carbon (C) (Bai and Cotrufo 2022; White et al. 2000). Globally, grasslands store considerably more C in soils than vegetation by 2.5–8.2 times, accounting

for 579 Gg C in the upper 100 cm of the soil (White et al. 2000). Moreover, grasslands are highly vulnerable to human activities, e.g., a total of 150 Tg yr<sup>-1</sup> anthropogenic nitrogen (N) is applied to the land surface due to increased fertilization and the production of N oxides from industry and vehicles (Bardgett et al. 2021; Schlesinger 2009). The input of N has been shown to significantly increase soil C storage in grasslands, which might strengthen the importance of this system as a C sink under increasing N input (Bai and Cotrufo 2022; Yue et al. 2016).

Enhanced plant N uptake caused by N addition increases leaf N contents and plant photosynthesis and ultimately stimulates plant growth in N-limited grasslands (Beier et al. 2009; Yue et al. 2016). Consequently, more C is assimilated into plant biomass and then increases labile C inputs, such as plant litter and roots exudates to soils (Fleischer et al. 2019; Xu et al. 2021). Several meta-analyses reported that the fresh plant C pool, litter C pool, and soil C pool significantly increased under N addition in temperate grassland ecosystems, especially at higher N addition rates (Hou et al. 2021; Song et al. 2019; Yue et al. 2016). However, opposite results have often been reported. For example, N addition simultaneously increased the plant C pool and recalcitrant plant C compounds, thereby slowing litter turnover without significant effects on soil C (Yang et al. 2019a). Likewise, a long-term field experiment found that N addition increased total plant inputs, yet this increase did not translate to greater soil C storage (Keller et al. 2022). In addition, responses of C pools such as soil C and litter C to N addition have been reported to differ with the experimental duration, in which the soil C pool weakly increased with an increasing duration, while the addition of N only accelerated early-stage decomposition of litter (Gill et al. 2022; Yue et al. 2016).

Since C and N cycles are highly coupled in terrestrial ecosystems, C pools will indeed exhibit positive or negative feedback to N enrichment and in return differentially regulate the effects of N enrichment on C storage (Hyvönen et al. 2007; Yue et al. 2016). For example, labile C input may be used as a source or energy to promote decomposition of litter C and thus increase soil C capture and storage (Fleischer et al. 2019; Yue et al. 2016). Alternatively, inputs of labile C may stimulate the mineralization of soil C, causing greater C loss in soils (Hicks et al. 2019; Yue et al. 2016). Therefore, the balances among plant C, litter C and soil C will determine whether and how C sequestration

varies in response to the increasing N addition rates. However, due to limited literature, how soil C will be influenced by a future increase in plant-derived C combined with N enrichment is still poorly understood.

The main objective of this study was to investigate and quantify the effects of labile C and N addition on the soil C pool and the underlying mechanisms. Specifically, the following questions are addressed: (1) what is the contribution of N enrichment to plant C, litter C and soil C pools among years in grasslands? (2) does labile C input influence the effects of N enrichment on C pools in grasslands, and if so, how? Nitrogen addition increases plant productivity in N-limited ecosystems (Zhang et al. 2015), and some studies suggested that critical thresholds of plant productivity occur in response to increasing N inputs, including the area of this study (Goffman et al. 2006; Yang et al. 2023). Thus, we first hypothesized that continuous N addition could increase both fresh plant C and litter C pools and consequently soil C pool, sometimes with a threshold. In addition, labile C addition stimulates N immobilization under N treatments (Ning et al. 2021), and thus our second hypothesis is labile C inputs could lessen the positive effects of continuous N addition on C pools. To answer these questions, an experiment with five levels of N addition and three levels of labile C addition was conducted in a temperate grassland. Fresh plants, litter, and soils were sampled annually for five consecutive years, and the C pools, N pools and C:N ratios were examined. Variations in these indices and correlations between them and varying rates of N and C addition over five consecutive years were quantified, further providing insights into the causes and consequences of soil C pool changes in response to N enrichment in temperate grasslands.

## Materials and methods

### Study sites and experimental design

The trial was initiated in May 2014 at the Erguna Forest-Grassland Ecotone Ecosystem Research Station (50°12'19" N, 119°30'28" E) in Inner Mongolia, China. The experimental field was a native grassland that large grazers were excluded for several years (Luo et al. 2023). We conducted the study in a disturbance-suppressed grassland (no fire, no cattle, and no mowing). The region is characterized by a meadow steppe and chernozems, with a mean temperature of

-2 °C and a mean annual precipitation of 336 mm. The initial values of soil C, soil N, and bulk density were  $30.45 \pm 0.28 \text{ g kg}^{-1}$ ,  $2.65 \pm 0.02 \text{ g kg}^{-1}$  and  $1.12 \pm 0.03 \text{ g cm}^{-3}$ , respectively. Weather data in the study area in 2014–2018, including the average maximum temperature, the average minimum temperature, and the annual precipitation, are shown in Table 1.

The split-plot experimental design consisted of 30 plots (each 10 m × 10 m in size) with five N concentrations in a randomized block design with six replicates per treatment (Fig. S1). To simulate N enrichment, N addition rates were 0, 2.5, 5.0, 10.0 and 20.0 g N  $\text{m}^{-2} \text{ year}^{-1}$  and denoted as N0, N2.5, N5, N10 and N20, respectively. Within each plot, three subplots (3 m × 3 m) were arranged with C addition rates of 0, 25 and 50 g C  $\text{m}^{-2} \text{ year}^{-1}$  and denoted as C0, C25 and C50, respectively (Fig. S1). Consequently, a total of 90 subplots were set up. N addition was applied as granular urea in early May annually. Moreover, glucose (used for C addition) was weighed, mixed with water, and sprinkled evenly on the soil surface using a sprayer. To provide a clear schedule of the experiment, a research flowchart is shown in Fig. S2.

#### Vegetation sampling and measurements

For each subplot, aboveground plant parts (by cutting all plants) and litter were collected within two 25 cm × 25 cm grids in August from 2014 to 2018 (denoted as D1–D5, respectively, to show the experimental durations). The plant species and litter were separately put into a paper bag, dried for 48 h at 80 °C and weighed to determine the biomass. The dried plants and litter were then ground and the concentrations of plant total C and plant total N were measured using a Vario MACRO cube analyzer (Elementar Analysensysteme Vario MACRO cube, Germany). Then, the C or N pool in plant/litter ( $\text{g m}^{-2}$ ) was calculated as follows: C or N concentration ( $\text{g kg}^{-1}$ ) × plant/litter biomass ( $\text{kg m}^{-2}$ ).

**Table 1** Weather data in the study area in 2014–2018

	2014	2015	2016	2017	2018
Annual precipitation (mm)	343	259	278	275	336
Daily maximum temperature (°C)	5.3	5.9	3.8	5.4	4.9
Daily minimum temperature (°C)	-7.6	-6.4	-8.4	-7.2	-7.6

#### Soil sampling and measurements

Soil samples were collected in August from 2014 to 2018. In each subplot, five undisturbed topsoil samples (0–10 cm) were collected using a soil core (3-cm diameter) and composited into a single sample. After removal of plant residues and stones, each soil sample was homogenized, passed through a 2-mm sieve, and then air-dried to measure the concentrations of soil C and soil N using a Vario MACRO cube analyzer (Elementar Analysensysteme Vario MACRO cube, Germany). The C and N pools (0–10 cm) were calculated based on the following equation: C or N pool in soils ( $\text{g m}^{-2}$ ) = C or N concentration ( $\text{g kg}^{-1}$ ) × soil bulk density ( $1,120 \text{ kg m}^{-3}$ ) × soil depth (0.1 m).

#### Statistical analyses

A three-way ANOVA was carried out to analyze the effects of different factors (C addition rate, N addition rate, and duration) and their interactions on the C/N pool of plants and soil. The results are shown in Table 2, in which the interactions without any significant effect were removed. Regression with the least squares method was used to determine the responses of plant and soil properties to the N addition rate and duration. Pearson correlations between different C pools were calculated. All analyses described above were performed and visualized in R (version 3.6.2).

To estimate the responses of C pools (including fresh plant C pool, litter C pool, and soil C pool) to N addition rates and their correlations under three levels of C addition over five successive years, structural equation models were established using AMOS (version 21.0.0) and SPSS 16.0 software. The maximum likelihood  $\chi^2$  test, comparative fit index (CFI), and root mean square error of approximation (RMSEA) were used to assess the model fitness.

## Results

#### Effects of N addition rate, duration, and C addition rate on fresh plant C and N pools

The fresh plant C pool was significantly influenced by the N addition rate, duration, C addition

**Table 2** Significance levels (three-way ANOVA) of the effects of different factors (N addition rate, duration and C addition rate) and their interactions on the C/N pool of plants and soil (interactions without any significant effect are not shown)

	N addition rate (n=90)		Duration (n=90)		C addition rate (n=150)		N addition rate × duration (n=18)		C addition rate × duration (n=30)	
	<i>F</i> value	<i>P</i> value	<i>F</i> value	<i>P</i> value	<i>F</i> value	<i>P</i> value	<i>F</i> value	<i>P</i> value	<i>F</i> value	<i>P</i> value
Plant C pool	91.8	<2e-16	20.1	9.5e-06	7.8	0.0005	6.0	0.01	3.5*	0.03
Plant N pool	216	<2e-16	96.1	<2e-16	7.0	0.001	58.4***	1.4e-13	2.4	0.09
Plant C:N ratio	169	<2e-16	84.7	<2e-16	2.1	0.1	8.2**	0.004	0.9	0.4
Litter C pool	174	<2e-16	412	<2e-16	4.5	0.01	114***	<2e-16	0.9	0.4
Litter N pool	271	<2e-16	373	<2e-16	2.9	0.056	202***	<2e-16	0.3	0.7
Litter C:N ratio	146	<2e-16	0.2	0.7	3.1	0.05	18.2***	2.4e-05	3.6*	0.03
Soil C pool	14.6	0.0001	272	<2e-16	8.5	0.0002	4.4*	0.04	0.1	0.9
Soil N pool	34.5	8.3e-09	80.0	<2e-16	9.7	7.8e-05	4.7*	0.03	0.2	0.8
Soil C:N ratio	20.8	6.7e-06	217	<2e-16	0.4	0.7	0.5	0.8	0.2	0.9

\*, \*\*, and \*\*\* indicate the significances at  $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.001$ , respectively

rate and the interaction between the N addition rate and duration as well as the interaction between the C addition rate and duration (Table 2). The plant C pool significantly increased with increasing N addition rates whether labile C was added or not (Fig. 1a), and the slopes of the linear models between the plant C pool and N addition rates were not significantly correlated with duration (Fig. 1a insert). In addition, the plant C pool was significantly decreased with the input of C at both addition rates (Fig. 1b insert) and only significantly increased with duration under the input of labile C (Fig. 1b). The plant N pool showed a similar trend to that of the plant C pool (Table 2 and Fig. S3).

The fresh plant C:N ratio was only significantly influenced by the N addition rate, duration, and the interaction between the N addition rate and duration (Table 2). In addition, the fresh plant C:N ratio significantly decreased with increasing N addition rates, which could be enhanced by duration (Fig. 1c), while C addition had little effect on the plant C:N ratio (Fig. 1d).

Effects of N addition rate, duration, and C addition rate on litter C and N pools

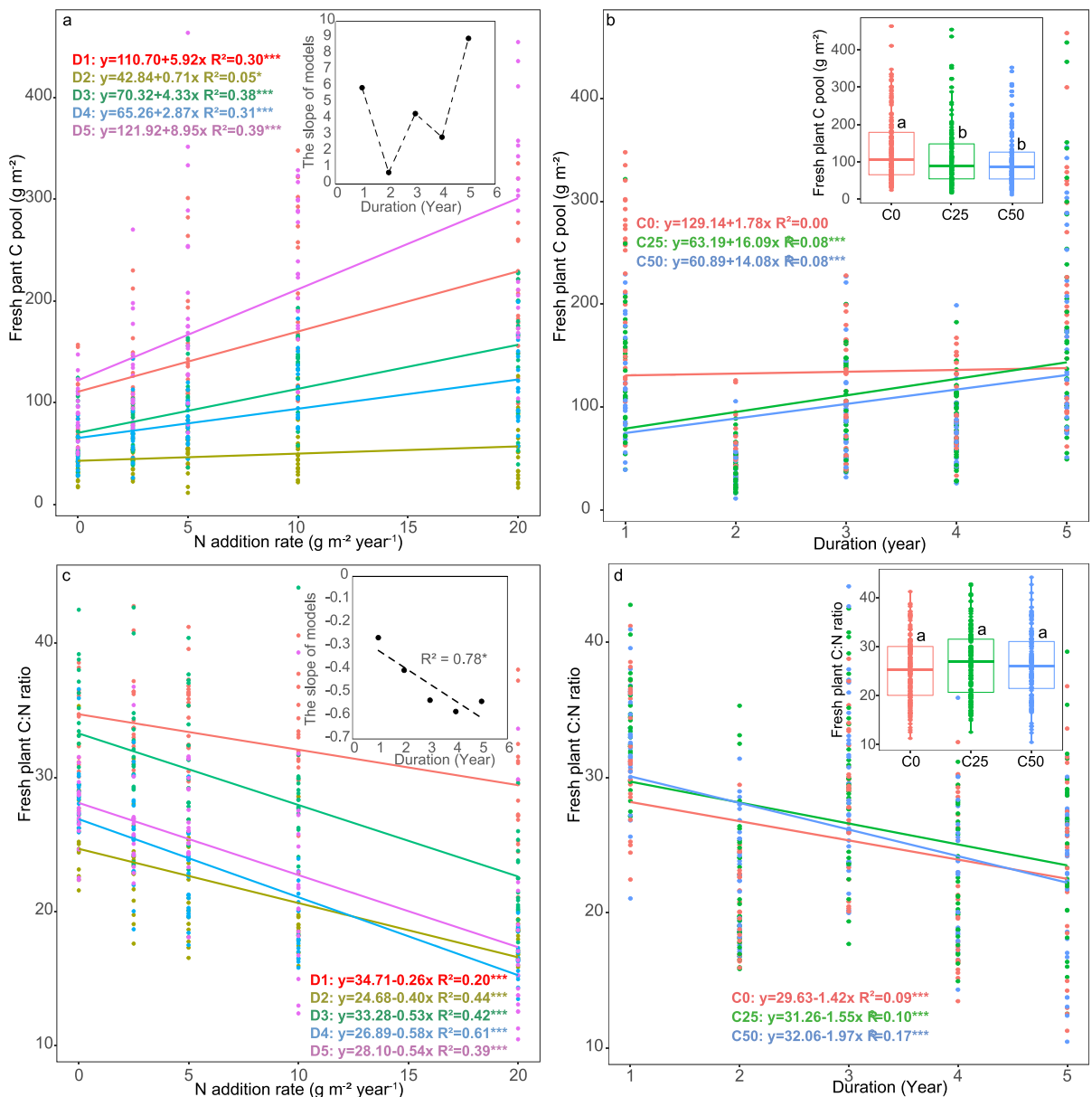
Likewise, the litter C pool was significantly influenced by the N addition rate, duration, C addition rate and the interaction between the N addition rate

and duration (Table 2). Specifically, the litter C pool significantly increased with increasing N addition rates and duration at all rates of C addition (Fig. 2a and b). The slopes of the linear models between the litter C pool and N addition rate significantly increased with duration (Fig. 2a insert). In addition, although a downward trend in the litter C pool with C input was detected, there was no significant difference between different rates of C addition (Fig. 2b). The litter N pool showed a similar trend to that of the litter C pool (Table 2 and Fig. S4).

The litter C:N ratio significantly decreased with increasing N addition rates but not with duration (Fig. 2c and d), and the slopes of the linear models between the litter C:N ratio and N addition rate were significantly correlated with duration in a quadratic regression model (Fig. 2c inserted plot). In addition, the litter C:N ratio increased with the addition of labile C, increasing significantly at the higher addition rate (Fig. 2d inserted plot).

Effects of N addition rate, duration, and C addition rate on soil C and N pools

Duration, C addition rate and the interaction between N addition rate and duration significantly influenced the soil C and N pools (Table 2). Specifically, the soil C pool significantly increased with increasing



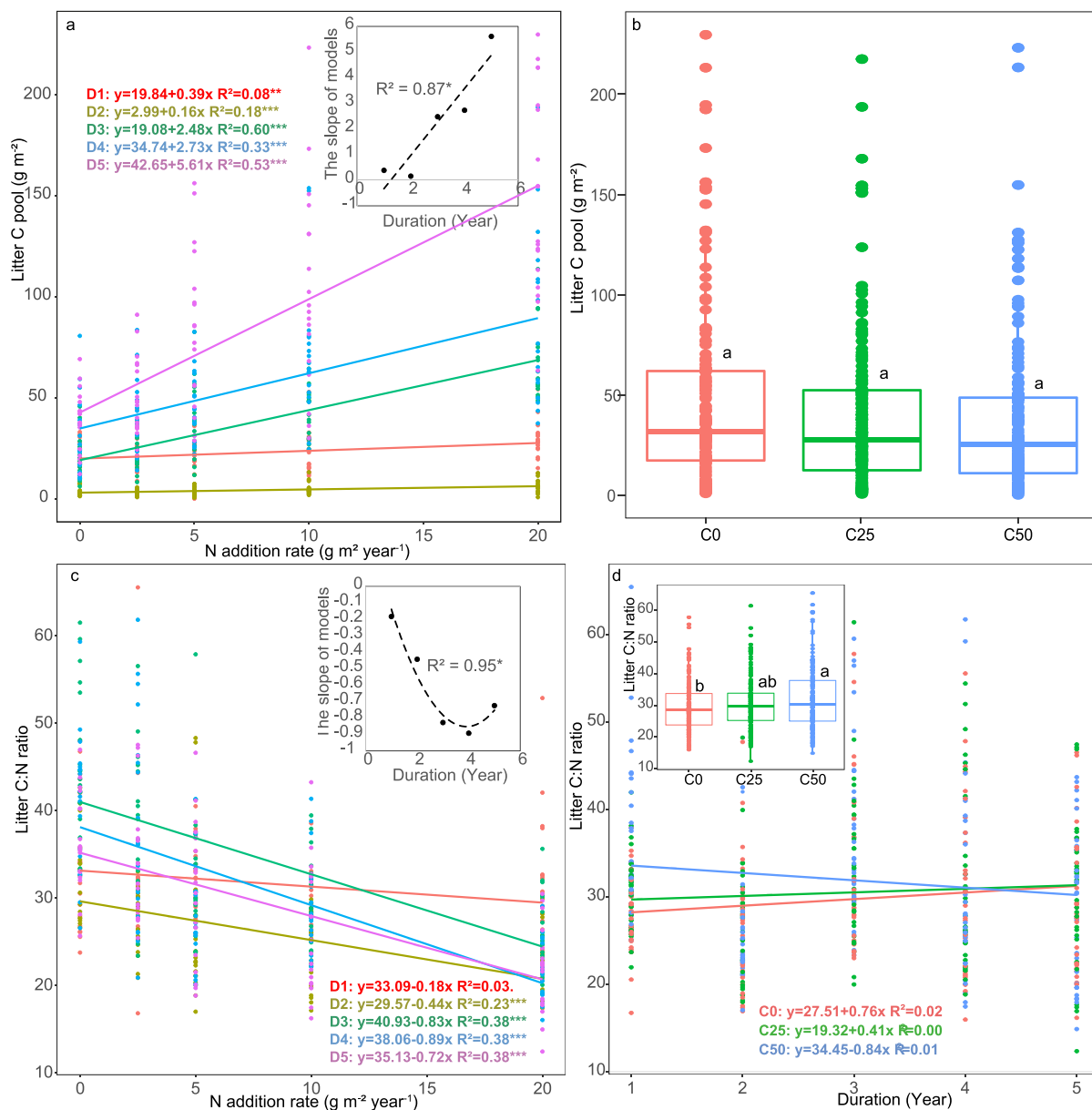
**Fig. 1** Relationship between plant C pools and plant C:N ratio and N addition rates (**a**, **c**) and duration (**b**, **d**). Linear regression equations are labeled. The correlations between the slope of the linear models and duration are inserted in (**a**) and (**c**). D1-D5 indicate a duration of 1–5 years of N input, respectively. The comparison of the plant C pool and plant C:N ratio between different C addition rates is shown as inserted box-

N addition rates at D1-D5 (Fig. 3a), and the slopes of these linear models were significantly correlated with N addition rates in a quadratic regression model (Fig. 3a inserted plot). The soil C pool was significantly the highest with the input of C at a lower

plots (Duncan's test,  $P < 0.05$ ) in (**b**) and (**d**), respectively. C0, C25, and C50 indicate inputs of 0, 25 and 50 g C m<sup>-2</sup> year<sup>-1</sup>, and are labeled in red, green, and blue, respectively. The lower-case letters indicate significant differences between different C addition rates at the  $P < 0.05$  level. \*, \*\* and \*\*\* indicate the significance at  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$ , respectively

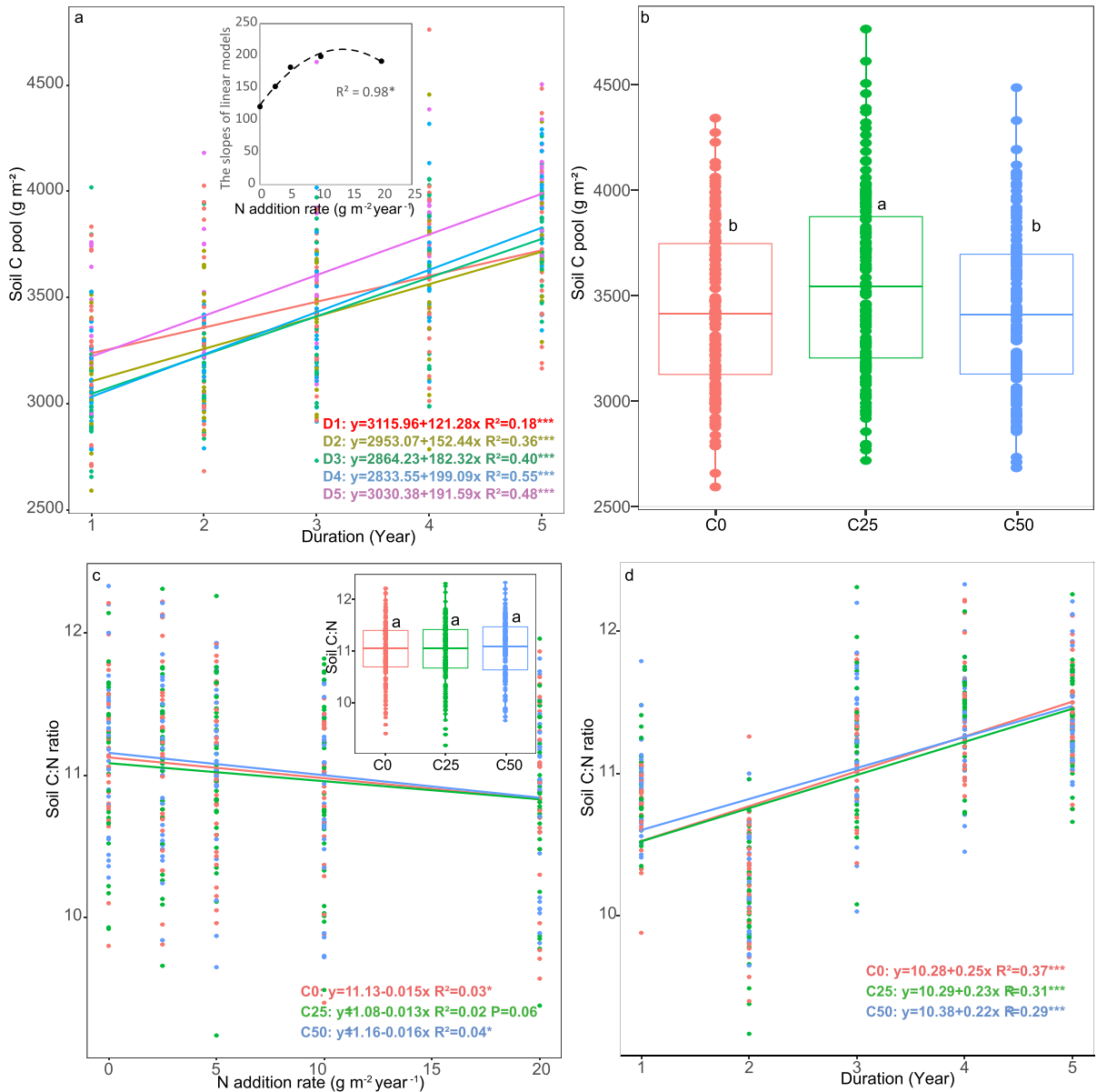
addition rate (Fig. 3b). Similar results were detected in the soil N pool (Fig. S5).

However, the soil C:N ratio was not significantly influenced by C addition (Fig. 3c inserted plot), but was significantly decreased with increasing N



**Fig. 2** Litter C pool (**a**, **b**) and C:N ratio (**c**, **d**) across the samples. (**a**) Relationship between litter C pools and N addition rates. (**b**) The comparison of the litter C pool between different C addition rates is shown as boxplots (Duncan's test,  $P < 0.05$ ). The relationship between litter C:N ratio and N addition rate and duration is shown in (**c**) and (**d**), respectively. Linear regression models and equations are labeled. The correlations between the slope of the linear models and duration are inserted in (**a**) and (**c**). The comparison of the litter C:N ratio

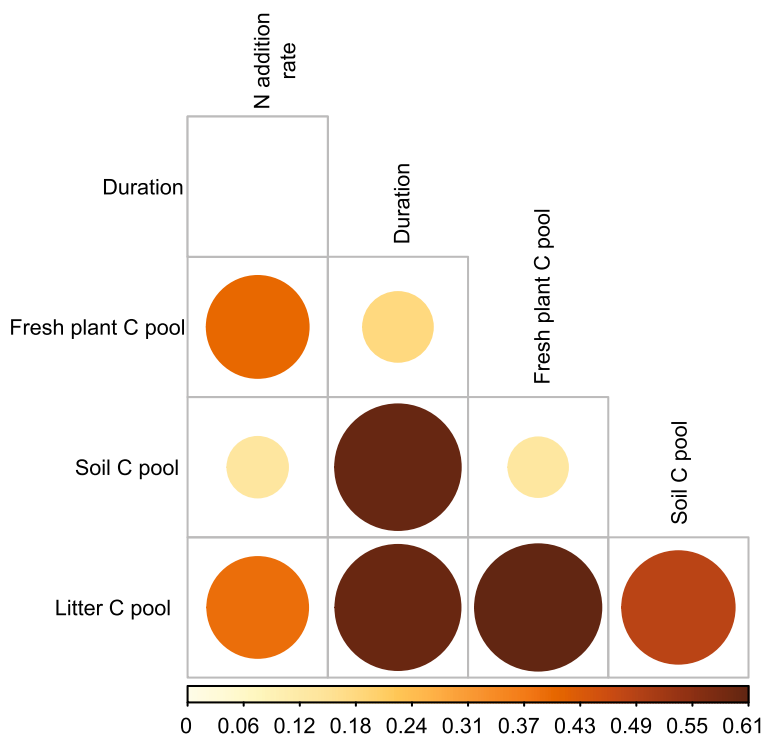
between different C addition rates is shown as inserted boxplots (Duncan's test,  $P < 0.05$ ) in (**d**). D1-D5 indicate a duration of 1–5 years of N addition, respectively. C0, C25, and C50 indicate inputs of 0, 25 and  $50 \text{ g C m}^{-2} \text{ year}^{-1}$ , and are labeled in red, green, and blue, respectively. The lowercase letters indicate significant differences between different C addition rates at the  $P < 0.05$  level. \*, \*\* and \*\*\* indicate the significance at  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$ , respectively



**Fig. 3** Soil C pool (**a** and **b**) and C:N ratio (**c** and **d**) across the samples. **(a)** Relationship between soil C pools and duration. Linear regression models and equations are labeled. The correlations between the slope of the linear models and duration are inserted. **(b)** The comparison between different C addition rates is shown as boxplots (Duncan's test,  $P < 0.05$ ). The relationship between soil C:N ratio and N addition rates and duration is shown in **(c)** and **(d)**, respectively. Linear regression models and equations are labeled. The comparison of the

soil C:N ratio between different C addition rates is shown as inserted boxplots (Duncan's test,  $P < 0.05$ ) in **(c)**. C0, C25, and C50 indicate inputs of 0, 25 and  $50 \text{ g C m}^{-2} \text{ year}^{-1}$ , and are labeled in red, green, and blue, respectively. D1-D5 indicate a duration of 1–5 years of N addition, respectively. The lower-case letters indicate significant differences between different C addition rates at the  $P < 0.05$  level. \*, \*\* and \*\*\* indicate the significance at  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$ , respectively

**Fig. 4** The spearman's correlations between N addition rates, duration and C pools of fresh plants, litter, and soil. Only significant correlations are showed ( $P < 0.05$ ). The sizes and colors were sorted by the  $P$  values and correlations, respectively

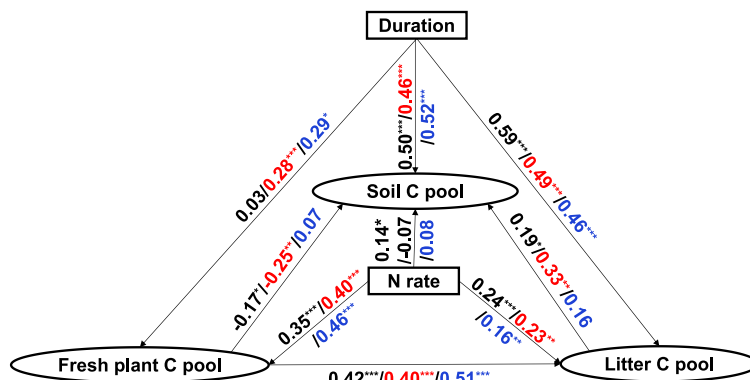


addition rates (Fig. 3c) and significantly increased with duration (Fig. 3d).

#### Structural equation model

Significant correlations were detected between the pools of fresh plant, litter, and soil (Fig. 4). The structural equation model showed that N addition rates had

significant and positive direct effects on both the fresh plant C pool and litter C pool at all C addition rates, but only significantly influenced the soil C pool in samples without labile C addition (Fig. 5). Labile C addition, especially at the higher addition rate (C50), enhanced and decreased the fresh plant C pool and litter C pool, respectively (Fig. 5). Duration had significant and positive direct effects on litter C and soil



**Fig. 5** Structural equation model for the effects of the N addition rate on C pools under three C addition rates. Goodness-of-fit statistics: chi-square=0.0,  $df=3$ ,  $P=1.0$ , comparative fit index (CFI)=1.0, root mean square error of approximation (RMSEA)=0.0. Numbers between variables are standardized

partial regression coefficients. Forward slashes separate numbers under three C addition rates, including 0 (black), 25 (red), and 50 (blue)  $\text{g m}^{-2} \text{ year}^{-1}$ . \*, \*\* and \*\*\* indicate the significance at  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$ , respectively



C at all rates of C input, but significantly and directly influenced fresh plant C only when labile C was added (Fig. 5).

In addition, the fresh plant C pool had a significant and positive direct effect on the litter C pool at all C addition rates, which further significantly and positively increased the soil C pool at the C0 and C25 rates but not at the C50 rate. Likewise, the fresh plant C pool had significant and negative direct effects on the soil C pool at the C0 and C25 rates (Fig. 5).

## Discussion

Fresh plant and litter C pools increased with N enrichment, but responded differently with duration

In this study, first, partially as expected, experimental N addition caused a significant increase in the size of the C pool in both fresh plants and litter by 1.1–3.0 times and 1.1–3.9 times, respectively, when the effects of the experimental duration were not considered (Figs. 1a and 2a). N addition has been reported to promote plant growth and significantly increase plant biomass in temperate grasslands, which consequently contributes to enhanced plant C pools (Yang et al. 2022b; Yue et al. 2016). The size of plant C pools was further significantly accelerated by an increasing N addition rate, which is also common in most available studies (Lu et al. 2021; Yang et al. 2022b). However, neither plant C pool nor litter C pool had a threshold in response to the increasing N rates, which we believed it was, at least partially, contributed to the experimental duration. A linear relationship between the plant productivity and N addition rates was reported at the initial years of the experiment (Zhang et al. 2015), and the responses of plant productivity to the N addition rates always changed among different years, even after a few years of experimentation (Isbell et al. 2013; Wang et al. 2019; Yang et al. 2023). Thus we supposed that the thresholds of plant and litter C pools under N addition may occur after a period in addition to be influenced by climate. Moreover, the duration of N addition did not have a positive relationship with the effects of N addition rates on the C pool in fresh plants, although it substantially changed the linear relationships between them (Fig. 1a inserted plot). More surprisingly, the fresh plant C pool was substantially lower

in D2–D4 than in D1 and D5. Plant biomass is highly dependent on precipitation in temperate grasslands (Hao et al. 2018; Hovenden et al. 2019). In our study, the annual precipitation was indeed lower in D2–D4, and we inferred that lower precipitation could, at least partially, attenuate the effects of N addition rates on plant biomass accumulation, and then on fresh plant biomass C. However, in contrast, the duration of N addition significantly enhanced the upward trends of the litter C pool with increasing N addition rates. The cumulative effect is likely because the grassland had never been mowed before our experiment, and changes in plant biomass in one year had little influence on the whole C pool in litter. Therefore, under consecutive years of N addition, the C pool in fresh plants may be a mirror of nutrient assimilation for the current year, while the C pool in litter may reflect the potential for nutrient release to soils.

Soil C pool sequestered over time in response to N enrichment

Regarding the soil C pool, a significant increase over time was observed in this study, independent of the N treatments (Fig. 3a). Temporal trends in soil C accumulation have been reported in aggrading grasslands where plants turn over annually (Pastore et al. 2021; Yang et al. 2019b). Likewise, N addition has often been reported to increase terrestrial soil C pools globally (Deng et al. 2020; Fleischer et al. 2019). However, unlike our first hypothesis, the soil C pool only significantly increased when the highest rate of N was added in the fifth year (Fig. 3a), suggesting that the effects of N addition on the soil C pool could not be achieved in a short-term treatment, even with a relatively high addition rate. Nevertheless, our results found that increasing N addition rates significantly enhanced the accumulation of the soil C pool over time, with a point of inflection (Fig. 3a inserted plot). For example, it took four years to significantly increase soil C by 10.6% in treatments without N addition, while a significant increase in soil C had occurred at 3 years of N input at any rate (Fig. 3a). Alternatively, increases in the soil C pool over time in the fifth year peaked at 24.4% with  $10.0 \text{ m}^{-2} \text{ yr}^{-1}$  N addition, and then decreased to 23.1% when the N addition rate was  $20.0 \text{ m}^{-2} \text{ yr}^{-1}$  (Fig. 3a). In this case, N addition indeed had a positive effect on soil C sequestration, as previously reported (Fleischer

et al. 2019). However, it should be pointed out that we only examined the soil C pool at the surface layer, considering applications of C and N without tillage. Movements of nutrients and their effects on the soil C pool are also important and needed to be investigated in the future. In addition, because N pools increased more in fresh plant and litter in response to N addition rates, downward trends in the C:N ratio of both fresh plant and litter with increasing N addition rates were found, which were enhanced by the experimental duration (Figs. 1c, 2c, S3a, and S4a). Plants with a lower plant C:N ratio may decompose more easily and release more nutrients back to soils (Fleischer et al. 2019; Pastore et al. 2021; Yang et al. 2022b). Unlike plants, the soil C:N ratio significantly decreased with N addition rates but increased with duration (Fig. 3c-d). Litter decomposition increases as soil C:N ratio decreases (Blanco et al. 2023), further strengthens the importance of plant C pools under N addition in grasslands. Overall, the soil C pool accumulated more than the soil N pool over time in response to N enrichment.

#### Plant-soil C pool relationship and its underlying mechanisms in response to N enrichment under C addition

N addition rates had only a weak direct effect on the soil C pool in the structural equation model. Then, we explored the relationships between the C pools of plants and soil in response to consecutive years of different levels of N addition. In addition to a positive effect of the litter C pool on the soil C pool, there was a trade-off between the fresh plant C pool and the soil C pool in response to N addition rates. A trade-off between plant and soil C was also reported under elevated CO<sub>2</sub>, although positive responses of both were observed (Terrer et al. 2021). This trade-off seemed to be attributed to plant nutrient acquisition, in which plants mine the soil to increase their biomass when plant growth is severely limited by other nutrients (Jiang et al. 2020; Terrer et al. 2021). The study highlighted that the trade-off was a key point but always overlooked (Terrer et al. 2021). The same applies in our study, and the final fate of soil C also depends on whether litter C could make up for the consumption of soil C.

More importantly, the annual input of 25 g labile C m<sup>-2</sup> significantly enhanced the soil C pool by 0.12

kg C m<sup>-2</sup> (at a depth of 10 cm) across the samples (Fig. 3b), indicating that labile C may also benefit to soil C sequestration in response to N addition. Since N input stimulates C return driven by plant C pools (Fleischer et al. 2019; Huang et al. 2020) as discussed above, further investigations on the effects of labile C on the response of C pools of both fresh plants and litter were also conducted. First, a lower rate of labile C addition increased the direct and positive effects of N addition rates and duration on the fresh plant C pool, while those on the litter C pool showed the opposite pattern. The scenarios were contrary to our second hypothesis and suggested that a lower input of labile C, like plant-derived C return, would enhance the positive effects of continuous N addition on the fresh plant C pool on the one hand. Then, the trade-off of C pools between fresh plants and soil increased. On the other hand, the input of labile C stimulates the decomposition of litter C, thereby promoting the coupling between the C pools of soil and litter, i.e., transforming C from litter to soil. The input of labile C has often been reported to increase microbial growth and activity in soils, which could compete with plants for nutrients as well as decompose litter (Mehnaz et al. 2018; Ning et al. 2021). Our results suggested that the litter C pool occupied the central stage after calculating the sum of effects in the structural equation model, which was enhanced by a lower rate of labile C addition.

When the addition rate of labile C increased, the positive effect on the fresh plant C pool weakened, but the stimulation of litter decomposition remained. The results suggested that the importance of litter decomposition became stronger with a high rate of labile C addition, which could also be confirmed by the significantly changed litter C:N ratio at a higher C addition rate, although the litter C pool was not changed in this study. In this case, the litter C pool may be more active. However, there was no corresponding increase in the soil C pool, indicating that native soil C was also decomposed more with a high rate of C addition (Kundu et al. 2007). Furthermore, the contributions of the C pools of fresh plants and litter to the soil C pool disappeared, indicating that the relationships between them were uncoupled when more labile C was added. Although the root C pool was not examined in this study, meta-analyses demonstrated that additional N input significantly decreased root biomass in N-limited grasslands (Bai et al. 2021;

Peng et al. 2017). In this case, plants should not invest more C to roots to acquire N when experimental N was added (Li et al. 2011; Peng et al. 2017; Yang et al. 2022a). Therefore, although N input maintains a positive effect on C sequestration in grassland (Fleischer et al. 2019), the outlook of soil carbon sequestration may be grim due to more C released by plants caused by continuous N input. At present, C loss in gaseous forms cannot be ignored and needs to be given more attention in the future.

## Conclusion

The soil C pool significantly increased with duration, which was enhanced by N enrichment up to a limit. Likewise, the size of C pools in fresh plants and litter significantly increased under experimental N addition, both of which were enhanced by increasing N addition rates, but only the latter was enhanced by duration. There was a trade-off between the fresh plant C pool and soil C pool, and the litter C pool contributed to the increase in the soil C pool in response to the five-year continuous addition of different levels of N under labile C input. Moreover, an intermediate rate of labile C input favored soil C storage by stimulating the transformation of plant C to soil. However, when more labile C is added, the positive effects of continuous N addition on C pools are weakened in temperate grassland ecosystems.

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**Data availability** The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Competing interests** The authors have no relevant financial or non-financial interests to disclose.

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