Elevated CO\(_2\) decreases soil carbon stability in Tibetan Plateau

Guang Zhao\(^1\), Chao Liang\(^1\), Xiaojuan Feng\(^2,3\), Lingli Liu\(^2,4\), Juntao Zhu\(^1\), Ning Chen\(^1,5\), Yao Chen\(^1,3\), Li Wang\(^6\) and Yangjian Zhang\(^1,4,6\)

\(^1\) Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, 11 Datun Road, Chaoyang District, Beijing 100101, People’s Republic of China

\(^2\) Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, People’s Republic of China

\(^3\) State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Science, Beijing 100101, People’s Republic of China

\(^4\) College of Resources and Environment, University of Chinese Academy of Sciences, Beijing 100101, People’s Republic of China

\(^5\) University of Chinese Academy of Sciences, Beijing 100101, People’s Republic of China

\(^6\) Peking University Shenzhen Graduate School, Shenzhen 518055, People’s Republic of China

E-mail: zhangyj@igsnrr.ac.cn

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Abstract

The lack of ecosystem-scale CO\(_2\) enrichment experiments in alpine regions considerably restricts our ability to predict the feedback of the global carbon (C) cycle to climate change. Here we investigate soil C response in an experiment with 5-year CO\(_2\) enrichment and nitrogen (N) fertilization in a Tibetan meadow (4585 m above the sea level). We found that despite non-significant increase in bulk soil C pool, elevated CO\(_2\) dramatically altered the allocation of C in different soil fractions and soil mineralization potentials. By changing soil microbial composition and enhancing enzyme activities, elevated CO\(_2\) significantly accelerated soil organic matter (SOM) mineralization rates and stimulated the microbial utilization of ‘old C’ relative to that of ‘new C’. Furthermore, N fertilization under elevated CO\(_2\) altered the decomposition process, increased the fungi to bacteria ratio, and decreased the coarse particulate organic matter pool and enzyme activities, indicating that N fertilization counters the CO\(_2\) fertilization effect. Overall, our findings suggest a growing threat of elevated CO\(_2\) in reducing SOM stability, and highlight the key role of N availability in driving soil C turnover under elevated CO\(_2\).

1. Introduction

As a critical part of climate change, elevated CO\(_2\) and subsequent effects on terrestrial ecosystems have been increasingly investigated solely or in combination with other environmental factors (Reich et al 2006, Dietzen et al 2019). Numerous studies suggest that elevated CO\(_2\) can enhance photosynthesis, productivity and, potentially, net ecosystem productivity, suggesting stronger CO\(_2\) uptake by ecosystems (Nowak et al 2004), which could counteract effects of global warming (Carney et al 2007). However, these findings have mostly focused on above-ground processes (Long et al 2004, Kuzyakov et al 2019) with little consideration of belowground ones (van Groenigen et al 2017, Dietzen et al 2019). As a primary carbon (C) pool, small changes of soil organic carbon (SOC) can drive tremendous variations in atmospheric CO\(_2\) concentrations, increasing the uncertainties in forecasting climate change (Lehmann and Kleber 2015). To improve overall prediction accuracies, below-ground processes are urgently entailed to be incorporated into Earth System Models (ESMs) to more effectively predict ecosystem responses to elevated CO\(_2\).

Among the many potential factors of global change, the net effect of elevated CO\(_2\) on soil C pools and turnover remains unclear (Liu et al 2018, Dietzen et al 2019). In addition to variation among various ecosystems, much of this uncertainty may be because the soil C pool is large in size, highly variable in space, and slow in turnover (Bradford et al, 2016, Hungate et al 2009), and thus small changes in soil C under elevated CO\(_2\) may be difficult to assess.

\(^8\) Author to whom any correspondence should be addressed.
(Langley et al 2009). At the same time, the supply of fresh plant-derived C to soil under elevated CO$_2$ could be partly offset by a positive ‘priming effect’ (defined as increases in the decomposition of ‘old C’ due to ‘new C’ inputs), indicating a faster turnover of the native soil C (van Groenigen et al 2017). Importantly, these increases in soil C turnover may have significant consequences for soil C pools by affecting soil organic matter (SOM) stabilization and mineralization (Vestergård et al 2016). Obviously, accurate prediction of soil C pools under CO$_2$ requires in-depth understanding of soil C turnover. However, most previous CO$_2$ studies utilized a single C pool to characterize soil C stocks and turnover, and reached contradictory conclusions in predicting soil C dynamics (Luo et al. 2017). SOM physical fractionation and soil C pool partitioning separate soil C into different pools with varied turnover and stabilization mechanisms, and these methods constitute sensitive and powerful approach to explore mechanisms driving the variation of soil C turnover and dynamics under elevated CO$_2$ (Poeplau et al. 2018, Cotrufo et al. 2019).

Another key uncertainty about soil C dynamics in response to elevated CO$_2$ concerns nitrogen (N) availability. Global ecosystems are highly limited by N (Reich et al. 2006) and this limitation may be more severe under elevated CO$_2$ (Luo et al. 2004, Norby et al. 2010). Thereby, N availability is a key factor dampening the stimulatory effect of CO$_2$ on ecosystem productivity (Hungate et al. 2003, Liang and Balser 2012, Terrer et al. 2018). As soil C and N processes are tightly coupled, N limitation on ecosystems can strongly influence the responses of below-ground C cycles to elevated CO$_2$ and thus affect soil C storage (Janssens et al. 2010, Liang et al. 2016). Theoretically, whether N availability limits soil C sequestration depends on the dynamic balance between N demand and N supply in an ecosystem (Finzi and Schlesinger 2002). However, the degree of the effect of N availability on soil C dynamics under elevated CO$_2$ remains insufficiently described, and conflicting findings from field studies have substantially hampered our capacity to draw general conclusions (Reich et al. 2006). For example, progressively abated N availability under elevated CO$_2$ occurs due to N sequestration in plant, litter, and organic matter (progressive N limitation) (Luo et al. 2004), and this decreased availability constrains soil C accumulation (Norby et al. 2010). Conversely, some studies argue that any additional N demands caused by elevated CO$_2$ could be partially met by strengthened soil N availability (such as the relative amount of inorganic N) via accelerated N mineralization rates, higher efficient N uptake (Finzi and Schlesinger 2002), or by stimulating specific microbial functions such as N$_2$ fixation (van Groenigen et al. 2006, Liang et al. 2016, Trierweiler et al. 2018). In general, more work is required to address how and to what extent N availability could constrain the magnitude of the stimulating effect of enriched CO$_2$ on the soil C pool (Reich and Hobbie 2013, Feng et al. 2015, Terrer et al. 2018).

Cold regions with high elevation and latitude are estimated to contain about 30%–60% of the global soil C pool (Post et al. 1982, Schuur et al. 2015), and these regions are particularly sensitive to ongoing global changes due to their fragile ecosystems and drastic climate changes worldwide (Isbell et al. 2011). As such, determining soil C responses to elevated CO$_2$ in these areas is critical to accurately project global C budgets (Jones et al. 2014). However, few manipulative experiments have been conducted on natural ecosystems in these regions (Jones et al. 2014), and there are insufficient data to adjust and optimize the parameters of ESMs (Handa et al. 2006).

Here, we designed an elevated CO$_2$ experiment in an alpine meadow on the Tibetan Plateau and assessed the response of soil C to elevated CO$_2$ in combination with changed N availability. Because alpine meadows on the Tibetan Plateau are extremely vulnerable to global climate change (Zhang et al. 2013, 2019), the results of study may serve as sentinels for other ecosystems. Specifically, our objectives were to (1) quantify soil C allocation and turnover rates under elevated CO$_2$, and (2) evaluate the effects of soil N availability on soil C cycling under elevated CO$_2$, and its driving mechanisms. We hypothesized that (i) elevated CO$_2$ would cause changes in soil C allocations, and that (ii) N supply would further enhance the size of the soil C pool under elevated CO$_2$.

2. Materials and methods

2.1. Site description

This research was conducted at the Tibetan Plateau Grassland Ecosystem Research Station (Naqu station; 31°38.513′N, 92°0.921′E, 4585 m). The climate is characterized by an annual mean temperature of −1.28 °C and an annual mean precipitation of 430 mm. The growing season normally begins at mid-May and ends in mid-September. The vegetation is dominated by Kobresia pygmaea, and includes Potentilla saundersiana, Potentilla cuneata, Stipa purpurea and Festuca coelestis. The soil at the study site is a turf-bearing soil developed mainly from Cambisols (Chinese Academic Expedition Group 1985, Kaiser et al. 2008).

2.2. Experimental design

Increased CO$_2$ concentration is difficult to implement using a free-air CO$_2$ enrichment (FACE) design owing to gusty winds and low stature vegetation on the plateau. Instead, a semi-FACE design composed of octagonal open-top gas chambers (OTCs) with a length of 1.5 m for each side and a height of 2 m was employed to prevent the rapid dissipation of injected CO$_2$ gas (appendix S1; supplementary figure 1 (https://stacks.iop.org/ERL/15/114002/mmedia)). In
2014, a total of eight octagonal OTCs were constructed with steel frames and glasses. The concentration of CO\(_2\) within the CO\(_2\)-enriched chambers was designed to be 100 ppm higher than ambient levels (ambient CO\(_2\), 380 ppm; elevated CO\(_2\), 480 ppm). The mean soil temperature was 2.0 °C higher in the OTCs than that of the natural environment soil (Zhu et al 2017).

The experiment was planned using a full-factorial split plot design, with eight octagons as eight blocks. Four octagons were exposed to ambient CO\(_2\) concentration and the other four are exposed to elevated CO\(_2\) concentration. Each octagon was divided into four plots with a size of 1.25 m \(\times\) 1.25 m, and one of them was subject to N fertilization. For N fertilization treatment, 5 g N m\(^{-2}\) yr\(^{-1}\) in the form of urea was dissolved in 200 ml water and sprayed in the plots in June of 2014–2018. In August 2018, three soil cores were taken to a depth of 30 cm for every treatment using a standard cylinder auger (7 cm in diameter). Collected three soil cores for each treatment were combined as one composite soil sample and were then homogenized in situ through a 2-mm sieve to remove plant roots, organic debris and rocks. Meanwhile, the root samples were collected from soil cores, and were cleaned and dried for belowground biomass measurements. One randomly placed 0.1 m \(\times\) 0.1 m quadrat in each plot was harvested to determine aboveground biomass. The sieved soil samples were then stored at 4 °C and transported to the lab. Subsamples of the whole soil were oven-dried and thoroughly hand-picked to remove visible roots and organic debris for pH, total C, N, P analysis and density fractionation (appendix S2). Portions of fresh soils were used to measure available nitrogen (NH\(_4^+\)-N and NO\(_3^-\)N), dissolved organic carbon (DOC), microbial biomass carbon and nitrogen (MBC and MBN), phospholipid fatty acids (PLFA) (appendix S3), and soil enzyme activities, as well as for incubation experiments. In total seven soil enzymes activities (βG, β-1,4-glucosidase; NAG, β-1, 4-N-acetylglucosaminidase; AP, Acid phosphatase; LAP, Leucine amino peptidase; BX, β-1, 4-xyllosidase; CB, celllobiohydrolase; αG, α-1,4-glucosidase) were measured by using a 96-well microplate (Saiya-Cork et al 2002). The aboveground and belowground plant tissues were determined after being oven-dried at 65 °C for 48 h to constant, and then the tissues were grinded and analyzed for total C and N by Vario Max CN analyzer (Elementar analysensysteme, Hanau, Germany). Plant aboveground and belowground C and N pool were calculated as aboveground and belowground biomass multiplying corresponding tissue C and N concentrations.

2.3. Soil density fractionation

SOM was physically fractionated according to the previously described method (Cambardella et al 1992). Briefly, the SOM was split into three particle-size classes, encompassing coarse particulate organic matter (cPOM; > 250 mm), fine particulate organic matter (fPOM; 250–53 mm), and mineral-associated organic matter (MAOM; < 53 mm) (appendix S4).

2.4. Soil carbon isotope

Fossil fuel-derived CO\(_2\) that is depleted in \(^{13}\text{C}\), was applied in the CO\(_2\) enrichment treatment. This allows the calculation of the proportions of ‘new’ C from the beginning of CO\(_2\) enrichment treatment and ‘old’ C:

\[
\delta^{13}\text{C}_{\text{soc}} = F_{\text{new}}(\delta^{13}\text{C}_{\text{new}}) + (1 - F_{\text{new}})(\delta^{13}\text{C}_{\text{old}}) \tag{1}
\]

where \(\delta^{13}\text{C}_{\text{soc}}\) and \(\delta^{13}\text{C}_{\text{old}}\) are the average isotope signatures of soil under elevated CO\(_2\) and ambient atmospheric condition, respectively; \(\delta^{13}\text{C}_{\text{new}}\) represents the average isotope signatures of new fixed soil C under elevated CO\(_2\), which is substituted by the \(\delta^{13}\text{C}\) of plant roots under elevated CO\(_2\).

A similar equation derived from equation (1) was used to calculate the portion of new carbon fixed in soil under elevated CO\(_2\) condition as follows:

\[
F_{\text{new}} = \frac{\delta^{13}\text{C}_{\text{soc}} - \delta^{13}\text{C}_{\text{old}}}{\delta^{13}\text{C}_{\text{new}} - \delta^{13}\text{C}_{\text{old}}} \tag{2}
\]

where \(F_{\text{new}}\) is the fraction of new C in the soil C pool under elevated CO\(_2\) environment (\%).

The annual turnover rate (K) of SOM under elevated atmospheric CO\(_2\) was calculated as:

\[
K = -ln (1 - F_{\text{new}}/100)/T \tag{3}
\]

where \(T\) is the duration of elevated CO\(_2\) treatment

Mean residence time (MRT) was calculated as the reciprocal to the turnover rate (K) (Gregorich et al 1996)

\[
MRT = 1/K. \tag{4}
\]

2.5. Soil C and N mineralization incubations

We performed an 80-day laboratory incubation to measure the rates of potential C and N mineralization (\(C_{\text{min}}\) and \(N_{\text{min}}\); appendix S5). The long-term trend in \(C_{\text{min}}\) was modeled by assuming that SOC decomposition occurred in two pools, active and slow, with negligible CO\(_2\) release from the resistant C pool during incubation (Paul et al 2001). The turnover rates and pool sizes for the two pools were estimated using a two-pool exponential decay model as follows (Paul et al 2001):

\[
C_{\text{min}} = \frac{dC}{dt} = - C_a \cdot k_a e^{-k_a t} - C_i \times k_i e^{-k_i t} \tag{5}
\]

where \(C_{\text{min}}\) is the daily C mineralization rate at incubation time \(t\); \(C_a\) is the active C pool size; \(C_i\) is the slow-cycling C pool size; \(k_a\) and \(k_i\) are the decay constants of the soil active C pool and the slow-cycling C pool, respectively. MRT for each pool was calculated as the inverse of the respective decay constant.
Table 1. Plant biomass, C and N concentrations of above and below tissues in response to elevated CO\textsubscript{2} and N fertilization. The values listed in the table were the means with standard deviation in the brackets. CK, control treatment; CO\textsubscript{2}, elevated CO\textsubscript{2}; N, nitrogen fertilization. Bold numbers in the table indicate significance between control and treatments at level of \(P < 0.1\) according to ANOVA analysis. Different lowercase letters indicate significant differences between different treatment according to Tukey’s \(b\) test at \(P < 0.1\).

| Plant     | Treatment | Biomass (g \cdot m\(^{-2}\)) | C Content (%) | C pool (g \cdot m\(^{-2}\)) | N Content (%) | N pool (g \cdot m\(^{-2}\)) | C: N |
|-----------|-----------|-------------------------------|---------------|-----------------------------|---------------|-----------------------------|------|
| Aboveground |          |                               |               |                             |               |                             |      |
|            | CK        | 64.33 (4.04)\(^a\)            | 43.52 (0.78)\(^a\) | 32.79 (7.09)\(^a\)         | 3.03 (0.29)\(^b\) | 2.18 (0.33)\(^b\)         | 14.43 (1.21)\(^b\) |
|            | CO\textsubscript{2} | 140.00 (68.27)\(^ab\)        | 43.10 (0.37)\(^a\) | 56.56 (32.78)\(^ab\)       | 2.73 (0.40)\(^a\) | 3.19 (1.41)\(^b\)         | 17.36 (3.05)\(^b\) |
|            | N         | 135.67 (61.91)\(^ab\)        | 43.94 (0.30)\(^a\) | 88.77 (2.53)\(^ab\)        | 3.28 (0.24)\(^b\) | 6.20 (0.42)\(^c\)         | 13.43 (0.87)\(^a\) |
|            | CO\textsubscript{2} + N | 209.75 (36.34)\(^b\)       | 44.08 (0.90)\(^a\) | 73.52 (26.80)\(^b\)        | 2.72 (0.24)\(^a\) | 4.53 (1.4)\(^b\)          | 16.29 (1.60)\(^b\) |
|            | \(P_{\text{CO2}}\) | 0.018                         | 0.327         | 0.819                       | 0.009         | 0.736                       | 0.045 |
|            | \(P_{N}\) | 0.064                         | 0.023         | 0.145                       | 0.325         | 0.015                       | 0.470 |
|            | \(P_{\text{CO2}} \times N\) | 0.824                     | 0.749         | 0.266                       | 0.435         | 0.112                       | 0.799 |
| Belowground |          |                               |               |                             |               |                             |      |
|            | CK        | 8583.08 (685.21)\(^ab\)      | 35.09 (6.61)\(^a\) | 3059.74 (955.38)\(^b\)     | 0.73 (0.13)\(^a\) | 61.77 (16.62)\(^a\)       | 47.93 (2.74)\(^a\) |
|            | CO\textsubscript{2} | 11242.3 (1423.96)\(^b\)     | 34.06 (3.12)\(^a\) | 3586.60 (728.38)\(^b\)     | 0.74 (0.08)\(^b\) | 80.58 (8.66)\(^b\)        | 46.31 (6.82)\(^b\) |
|            | N         | 8902.64 (1874.86)\(^ab\)     | 37.00 (5.57)\(^a\) | 3257.47 (582.54)\(^b\)     | 0.74 (0.07)\(^a\) | 67.01 (19.37)\(^b\)       | 50.08 (9.96)\(^b\) |
|            | CO\textsubscript{2} + N | 6811.56 (976.88)\(^a\)     | 36.44 (4.62)\(^a\) | 2619.54 (109.38)\(^a\)     | 0.91 (0.08)\(^b\) | 64.90 (2.71)\(^b\)        | 40.08 (2.15)\(^b\) |
|            | \(P_{\text{CO2}}\) | 0.310                         | 0.983         | 0.675                       | 0.091         | 0.327                       | 0.139 |
|            | \(P_{N}\) | 0.065                         | 0.476         | 0.258                       | 0.208         | 0.443                       | 0.620 |
|            | \(P_{\text{CO2}} \times N\) | 0.015                     | 0.732         | 0.086                       | 0.122         | 0.123                       | 0.282 |
Figure 1. Effect of elevated CO$_2$ and N fertilization on soil basic characteristics (A), microorganism compositions (B; phospholipid acid profiles), soil enzymatic activities (C) and their redundancy analysis (RDA). TC, total soil carbon; TN, total soil nitrogen; TP, total soil phosphorus; DOC, dissolved organic carbon; NO$_3^-$, nitrate nitrogen; NH$_4^+$, ammonium nitrogen; MBC, soil microbial biomass carbon; MBN, soil microbial biomass nitrogen. Total, total PLFAs; B, bacteria; F, fungi; Actin, actinomycetes; AMF, arbuscular mycorrhizal fungi; G$^+$, Gram-positive bacteria; G$^-$, Gram-negative bacteria; F/B, the ratio of fungi to bacteria. Boldface in the figure indicates significant difference at level of $P < 0.1$.

2.6. Statistical analysis

Two-factor split-plot ANOVA analysis was used to assess the effect of elevated CO$_2$ and N fertilization with covariate analysis to account for blocks, using the General Linear Model in SPSS (20.0). The experimental plot was treated as a statistical experimental unit. All effects and comparisons were considered significant at level of $P < 0.1$ due to our limited replications, as well as to minimize the chances of failing to detect differences due to Type II error (Hofmockel et al. 2011). Redundancy analysis (RDA) was used to determine the interrelationships between microbial communities, enzymes activities and environmental conditions.

Structural equation modeling (SEM) was applied to identify the pathways that may explain the effects of soil microorganisms and enzyme activities on SOM stability under elevated CO$_2$ and N supply, using AMOS 21.0 (Amos Development Corporation, Chicago, IL, USA). Because these variables are strongly correlated, we first used principal component analysis to create a multivariate index before model analysis. The first principal component including seven enzyme activities explained 55.4% of the total variance, so was used to represent soil enzyme activity (supplementary table 1). To acquire a best-support model, we first designed a prior model (supplementary figure 2), that was optimized by step-wise exclusion of variables with non-significant regression weights according to the modification indices and goodness of fit for the initial model. The fit of the final model was evaluated using the model $\chi^2$ test, comparative fit index (CFI) and the root mean square error of approximation (RMSEA) (Schermelleh-Engel et al. 2003).

3. Results

3.1. Characteristics of plant, soil, microorganisms and enzyme activities

Elevated CO$_2$ significantly increased the aboveground biomass ($P = 0.018$) and had no effect on belowground biomass ($P = 0.310$; table 1). Elevated CO$_2$ also increased C:N ratio of aboveground plant ($P = 0.045$), but had no significant effect on the C:N ratio of belowground plant ($P = 0.139$; table 1). For soil, elevated CO$_2$ had no effect on total C pool (30.19 g$^{-1}$ · kg$^{-1}$—32.52 g$^{-1}$ · kg$^{-1}$; $P = 0.188$), but significantly decreased total N pool from 2.74 g$^{-1}$ · kg$^{-1}$ to 2.50 g$^{-1}$ · kg$^{-1}$ under non-fertilized treatment and from 2.95 g$^{-1}$ · kg$^{-1}$ to 2.55 g$^{-1}$ · kg$^{-1}$ under fertilized treatment ($P = 0.014$; figure 2). Meanwhile, significant
The laboratory incubation-experiment demonstrated that, elevated CO<sub>2</sub> alone significantly increased C<sub>min</sub> by 8.14% and N<sub>min</sub> by 22.72% (P < 0.1) (figures 2(A) and (B)), indicating the enhanced soil mineralization activities increased under elevated CO<sub>2</sub> but decreased with the following N fertilization (figure 1(C); supplementary table 4). Further, except for AP and LAP, the activities of five other enzymes generally showed positive relationships with C<sub>min</sub> and N<sub>min</sub> (supplementary figures 3 and 4). RDA showed that soil microbial community structure was well explained by soil properties, including TN, TC, NO<sub>3</sub><sup>-</sup>, MBC and C: N (figure 1(D)). Soil enzyme activities, such as LAP, αG, βG, CB and BX, had weak correlations with soil properties (figure 1(E)). Furthermore, soil microorganisms had strong relationships with soil enzyme activities (figure 1(F)). Overall, redundancy and correlation analysis indicated that soil enzyme activities were generally more affected by soil microorganisms than by soil properties (figure 1(D), E&F; supplementary table 5).

### 3.2. Potential soil mineralization rates

Increases in C: N, MBC and decreases in total N under elevated CO<sub>2</sub> were observed (P < 0.1) relative to those under ambient CO<sub>2</sub> (figure 1(A); supplementary table 2). Except for MBC, N fertilization showed non-significant effects on other soil properties, i.e. TC, TN, TP; C: N, DOC, NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup> and MBN (figure 1(A); supplementary table 2). Combined elevated CO<sub>2</sub> and N fertilization treatment showed significant interaction effects on DOC, NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, and MBC (P < 0.1) (figure 1(A)).

Overall, elevated CO<sub>2</sub> significantly increased the amounts of total PLFAs, bacteria, fungi, actinomycetes, arbuscular mycorrhizal fungi (AMF), gram positive bacteria (G+), gram negative bacteria (G−), and the fungi to bacteria (F/B) ratio (P < 0.1) (figure 1(B); supplementary table 3). N fertilization caused significant decreases in total PLFAs, actinomycetes, AMF, G+ (P < 0.1) (figure 1(B)). Only bacteria PLFAs were significantly affected by the interaction of CO<sub>2</sub> and N fertilization (P = 0.025), which implies that elevated CO<sub>2</sub> eliminates the negative effect of N fertilization on bacteria. Except for LAP, the hydrolytic enzymes generally showed similar responses to elevated CO<sub>2</sub> and N fertilization, where enzyme...
Table 2. Estimation of the fraction of new C in the soil organic carbon pool (\( F_{\text{new}} \)), the annual turnover rates (\( k \)) and the mean residence time (MRT) for each pool in the alpine meadow under elevated CO\(_2\) treatment. All values in the table were displayed as mean with standard deviation in parentheses. * indicates significance between N treatments at level of \( P < 0.1 \) according to \( t \)-test.

| Parameter          | Unfertilized | Fertilized | Parameter          | Unfertilized | Fertilized | Parameter          | Unfertilized | Fertilized |
|--------------------|--------------|------------|--------------------|--------------|------------|--------------------|--------------|------------|
| \( F_{\text{new}} \) (%) | 32.31 (11.85) | 47.02 (7.49)* | \( k \) (yr\(^{-1}\)) | 0.0805 (0.0369) | 0.1284 (0.02694)* | \( MRT \) (yr) | 14.33 (5.69) | 8.11 (2.05)* |

Figure 3. Structural equation model analysis (SEM) revealing the influences of Nitrogen availability (represented by soil C: N ratio) on enzyme activities, microbial biomass (represented by total PLFAs), microbial composition and the soil stability (the ratio of soil C storage in MAOM against that in cPOM). The black solid lines represent significant positive relationships and the red solid lines represent significant negative relationships (\( p < 0.05 \)), and the grey dashed lines represent non-significant relationships (\( P > 0.05 \)). Arrows represent the directional influence of one variable upon another. The significance level was at level of \( P < 0.05 \) level.

\[ \chi^2 = 2.725, \ P = 0.256, \ RMSEA = 0.016, \ CFI = 0.978 \]

3.3. Soil density fractionation and soil C pool partitioning
Elevated CO\(_2\) alone significantly increased total soil C storage in cPOM from 4.36 g · kg\(^{-1}\) to 6.54 g · kg\(^{-1}\) (\( P < 0.1 \)), and significantly decreased it in MAOM from 15.01 g · kg\(^{-1}\) to 11.52 g · kg\(^{-1}\) (\( P < 0.1 \)) (figures 2(C) and (D); supplementary table 6). Compared to elevated CO\(_2\), combined CO\(_2\) and N fertilization increased the soil C storage in MAOM from 11.52 g · kg\(^{-1}\) to 13.72 g · kg\(^{-1}\) (from 37% to 45% in proportion), but significantly decreased the C storage in cPOM from 6.54 g · kg\(^{-1}\) to 5.35 g · kg\(^{-1}\) (\( P < 0.1 \); from 21% to 17% in proportion; supplementary figure 5). Elevated CO\(_2\) alone significantly enhanced soil N storage in cPOM from 0.33 g · kg\(^{-1}\) potentials. However, C\(_{\text{min}}\) and N\(_{\text{min}}\) declined under combined CO\(_2\) and N fertilization as compared to elevated CO\(_2\) alone.
Table 3. Estimation of mean carbon concentration for each pool and turnover for active ($C_a, k_a$) and slow pools ($C_s, k_s$) in the alpine meadow of the Tibet Plateau under elevated and ambient CO$_2$ concentrations based on a two-pool constrained model. Treatments sharing the same letter are not significantly different according to Tukey's $b$ test at $P < 0.1$.

| Treatment     | $C_a$ (mg · kg$^{-1}$) | $k_a$    | MRT$_a$ (d) | $C_s$ (mg · kg$^{-1}$) | $k_s$    | MRT$_s$ (d) |
|---------------|------------------------|----------|-------------|------------------------|----------|-------------|
| Unfertilized  |                        |          |             |                        |          |             |
| AC            | 0.1585 ± 0.03$^b$      | 0.8030 ± 0.22 | 1.31 ± 0.36  | 14.89 ± 0.87           | 0.00505 ± 0.0063 | 198.47 ± 26.7 |
| EC            | 0.2052 ± 0.01$^a$      | 0.9247 ± 0.09 | 2.02 ± 1.41  | 15.12 ± 1.75           | 0.00568 ± 0.0008 | 177.85 ± 23.9 |
| N Fertilization |                       |          |             |                        |          |             |
| AC            | 0.1473 ± 0.02$^b$      | 0.6798 ± 0.36 | 1.13 ± 0.14  | 14.73 ± 0.54           | 0.00537 ± 0.0002 | 186.09 ± 7.6  |
| EC            | 0.1835 ± 0.03$^a$      | 0.9462 ± 0.33 | 1.17 ± 0.49  | 14.86 ± 2.29           | 0.00530 ± 0.0009 | 192.43 ± 34.9 |
to 0.40 g · kg\(^{-1}\) (P < 0.1; from 12% to 16% in proportion), concurrent with a decrease in MAOM from 1.38 g · kg\(^{-1}\) to 1.09 g · kg\(^{-1}\) (P < 0.1; from 50% to 44% in proportion; supplementary figure 5). Compared with cPOM and MAOM, soil C and N storage in fPOM showed no significant responses to elevated CO\(_2\) and N fertilization (P > 0.1) (figures 2(C) and (D); supplementary table 6). In general, elevated CO\(_2\) caused a non-significant increase in total soil C but significantly decreased total soil N (figures 2(C) and (D); P = 0.014). Soil C isotope signatures showed that the \(\delta^{13}C\) in different fractionations were in the order of MAOM (−21.05‰ ~ −20.57‰) > fPOM (−23.88‰ ~ −23.27‰) > cPOM (−24.79‰ ~ −24.00‰) (supplementary table 7). Elevated CO\(_2\) significantly declined \(\delta^{13}C\) values in cPOM (P = 0.032) from −24.13 ‰ to −24.54 ‰ under non-fertilized treatment and from −24.00 ‰ to −24.79 ‰ under N fertilized treatment. Similarly, elevated CO\(_2\) significantly decreased \(\delta^{13}C\) values in fPOM (P = 0.090) from −23.59 ‰ to −23.88 ‰ under non-fertilized treatment and from −23.27 ‰ to −23.57 ‰ under N addition treatment. In contrast, elevated CO\(_2\) had non-significant effect on \(\delta^{13}C\) values in MAOM (P = 0.321). Based on the obtained \(^{13}C\) signatures of soil and root, soil C turnover characteristics were calculated according to equation (2) and the results were displayed in table 2. 'New C', which were sequestered in soil since the beginning of CO\(_2\) fumigation experiment, accounted for 32.31% in cPOM, 15.75% in fPOM and 3.45% in MAOM. Under elevated CO\(_2\), N fertilization significantly enhanced the 'New C' proportion in cPOM from 32.31% to 47.02% (P < 0.1), but decreased the proportion in fPOM from 15.75% to 12.7%. The MRT ranged from 8.11 yr to 147.25 yr in different density forms, with relative short MRT for POM (8.11 yr to 38.87 yr) and relative long MRT for MAOM (143.56 yr to 147.25 yr; table 2). Under elevated CO\(_2\), N fertilization significantly shortened the MRT of cPOM (P < 0.1) from 14.33 yr to 8.11 yr, but had no effects on fPOM and MAOM (P > 0.1). Collectively, elevated CO\(_2\) and N fertilization had non-significant effects on the soil C turnover rates of MAOM (table 2). For soil C partitioning, elevated CO\(_2\) significantly increased the active C pool from 0.1585 mg · kg\(^{-1}\) to 0.2052 mg · kg\(^{-1}\) with a non-significant increase in MRT (P < 0.1; table 3). Finally, combined elevated CO\(_2\) and N fertilization induced non-significant changes in the slow C pool as well as the MRT.

3.4. Influencing factors driving SOM stability

Given the non-significant effects of elevated CO\(_2\) on soil C pool and strong correlations between soil properties (soil C: N ratio, DOC, NO\(_3\)-, NH\(_4\)+, MBC and MBN), soil microorganisms (PLFAs, Bacteria, Fungi, G+, G−, F/B, and G+/G−) and enzyme activities (\(\beta\)G, NAG, AP, LAP, BX, CB, \(\alpha\)G) and soil C allocation (supplementary figures 6, 7 and 8), SEM analysis was next employed to evaluate the direct and indirect effects of these factors on soil C allocation. This can be used to assess SOM stability (represented by the ratio of soil C in MAOM to soil C in cPOM) (Lavallee et al 2020). The validated SEM yielded a good model fit with a non-significant \(\chi^2\) test (P > 0.05), a high CFI (>0.95), and a low RMSEA (<0.05) (figure 3). The SEM analysis showed that soil C:N ratio was significantly and positively related to microbial biomass (P < 0.05) and F/B ratio (P < 0.05), but was negatively correlated to enzyme activities (P < 0.05). Further, there were significant and negative relationships between enzyme activities, F/B ratio and MAOM to cPOM ratio (P < 0.05), and microbial biomass was positively correlated to MAOM to cPOM ratio (P < 0.05). Microbial biomass significantly correlated with soil enzyme activities (P < 0.05). These results indicate that, through affecting microbial biomass, enzyme activities and F/B ratio, C:N ratio indirectly alters the soil C allocation and thus alters overall SOM stability.

4. Discussion

This study was performed at high-elevation and addressed the consequence of elevated CO\(_2\) worldwide on soil C dynamics. The results of this work help to refine our understanding of factors regulating soil C accumulation and transformation under simultaneous elevated CO\(_2\) and N addition, especially for high-cold regions, where is pivotal for modeling global C cycles but inadequately investigated.

4.1. Elevated CO\(_2\) altered C allocations with no changes in soil C pool

This study revealed that elevated CO\(_2\) significantly stimulated plant growth and increased the accumulation of C in biomass, but caused little impact on soil C pool. The lack of an effect on soil C storage of the strengthened C inputs under elevated CO\(_2\) may be ascribed to the positive ‘priming effect’ (Vestergård et al 2016). By increasing microbial biomass and activities of hydrolytic enzymes, the possible 'priming effect' under elevated CO\(_2\) accelerated SOM decomposition rates and considerably counterbalanced the increased C input to soil. At the same time, elevated CO\(_2\) caused stronger stimulatory effects on N\(_{\min}\) than on C\(_{\min}\). Hypothetically, more soil organic N would decompose and transform to inorganic N to meet increasing N demand under elevated CO\(_2\), which is corroborated by our findings of enhanced inorganic N concentration (NO\(_3\)- and NH\(_4\)+) in soil. One unique feature for alpine meadow on the Tibetan Plateau lies in its slow litter decomposition, which cannot effectively offset the rapid N loss in SOM under elevated CO\(_2\), eventually causing significant reduced soil total N in the study.
Figure 4. Conceptual framework, representing the effect of N fertilization on the soil organic matter cycling under elevated CO$_2$. $C_{min}$, C mineralization rate of soil organic matter; $N_{min}$, N mineralization rate of soil organic matter; AN, available N; CO$_2$, carbon dioxide; cPOM, coarse particle organic matter; fPOM, fine particle organic matter; MAOM, mineral-associated organic matter. The solid lines represent matter transfer between different objects, whereas the dashed lines represent directional influence of one variable upon another. The thickness of the arrows indicates the magnitude of the flux or the changes.

Despite little changes in the total soil C pools, elevated CO$_2$ significantly altered soil C allocation among the three SOM fractions. The C loss from MAOM under elevated CO$_2$ largely offset the increased C in cPOM, leading to a non-significant change in total soil C. As the oldest and most recalcitrant fraction, MAOM possesses higher resistance to degradation than POM (Lavallee et al 2020). However, elevated CO$_2$ preferentially stimulated the degradation of recalcitrant organic matter with century lifetimes (MAOM), and promoted the organic matter accumulation in labile pools (such as cPOM) with decadal turnover time (Vestergård et al 2016). These changes in C allocation should be associated with enhanced N demand in ecosystem under elevated CO$_2$ (Reich et al 2006, Terrer et al 2018). This demand shifted microbial communities to more fungal groups, because fungi biomass has a lower C:N ratio compared to bacteria and is the main decomposers of relatively recalcitrant SOM, such as MAOM (van Groenigen et al 2017). Under CO$_2$-induced N limitation environments, competitions between plants and microbes should favor fungi over bacteria (Bossuyt et al 2001), which then helps to drive the transfer of N from MAOM with high C: N ratio to plant biomass (supplementary figure 9) (Lichter et al 2008). Thus, microbe-driven SOM turnover under elevated CO$_2$ promotes more inorganic N release for each unit of C mineralized.

It indicates that N limitation under elevated CO$_2$ would be compensated not only by accelerating N cycling, but also by exploring the portion of SOM with high N concentration, even though this may acquire N at a higher cost (Luo et al 2004, Langley et al 2009).

In addition to changes in the soil fractions, we observed a significant increase in the size of the active SOC pool and a non-significant increase in the slow SOC pool under elevated CO$_2$. As the active C pool represents newer C in soil that is more easily degraded (Langley et al 2009), an increase in the active C pool supports the density fractionation results of increased C in POM under elevated CO$_2$. These two lines of evidence suggest that elevated CO$_2$ exerts a significant effect on soil C allocation rather than on the soil C pool, which supports our hypothesis (i).

4.2. N availability drives soil C turnover under elevated CO$_2$

N supply caused marginal effects on total soil C after five-year CO$_2$ fumigation, which contradicts our hypothesis (ii). The common viewpoint holds that N supply would increase the soil C stock, and the underlying mechanism lies in that high N availability enhances C input to soil and inhibits the decomposition of slow-cycling, mineral-associated soil C fractions (Hungate et al 2009). However, much uncertainty may arise from our limited understanding
on the degree by which N supply constrains soil C dynamics in combination with elevated CO₂. Previous studies (van Groenigen et al 2006, de Graaff et al 2006) reported that elevated CO₂ stimulates soil C sequestration only above a certain threshold of exogenous N, because enhanced C input caused by elevated CO₂ would be greatly counterbalanced by microbial respirations at low N addition (de Graaff et al 2006). Compared to plants, soil microorganisms are more C-limited than N-limited (Treseder 2008). It may be that when N is sufficient for plant and microbial growth, SOM decomposition rate may decrease, and more C could be sequestered in soil under elevated CO₂ (Reich et al 2006, Hungate et al 2009, Kuzyanov et al 2019). In this study, the responses of plant tissue N concentration and soil N pool to elevated CO₂ indicate that added N may not completely alleviate the CO₂-induced N limitation. Importantly, the alpine meadow ecosystem in Tibetan Plateau is considered extremely N limited, which strictly constrains its potential for C storage under continuously elevated CO₂.

It is noteworthy that, despite non-significant effects on the soil C pool, N supply dramatically altered the SOM decomposition process, which generally plays an antagonistic role against elevated CO₂ effect. Specifically, N supply dampened the effect of elevated CO₂ that promoting MAOM decomposition, and meanwhile decreased the C proportion in POM as well as in the active C pool. The 1³C signatures provided direct evidence that under elevated CO₂, N supply enhanced the proportion of new C and accelerated SOM turnover for cPOM, but reduced C turnover rates in MOAM. This linking of soil C allocation with C isotope characteristics led to a clearer picture of soil C accumulation in resistant C pool (such as MAOM) and reduction in the labile C pool (such as POM and active C pool) under N supply. The mechanisms responsible for these changes in C allocation involve N-induced variations in microbial community and enzyme activities (Carney et al 2007). For instance, decreases in microbial biomass, F/B ratio and enzyme activities indicated that the N supply may partly meet the CO₂-induced N demand in the ecosystem. Under this condition, there is no intense need for microbes to explore extra N in MAOM at a high cost (Hofmockel et al 2011) and labile C is preferentially utilized under N supply.

Through SEM analysis, we found that N availability, which is partially represented by soil C: N stoichiometry, plays a crucial role in modulating SOM stability by altering soil C allocations under elevated CO₂. The soil C: N ratio mostly works on soil processes indirectly, i.e. its effects on soil C turnover are triggered by changed soil microbial compositions and enzyme activities (Kuzyanov et al 2019). That is, Changes in N availability reshape the decomposer community and enzyme activities, indirectly affecting the SOM mineralization process. Notably, these indirect effects caused non-significant changes in total soil C levels, but also altered the C allocation in MAOM versus cPOM, which further hints that the effects of N availability on turnover are capitalized by changing the soil C stabilization and decomposition under elevated CO₂. The results also revealed that the ratio between MAOM and cPOM can indicate the response of SOM stability to elevated CO₂, with higher sensitivity than the decay model to predict SOM dynamics.

4.3. Ecological implications

We sketched a conceptual model to illustrate the pivotal role of N availability in regulating soil C turnover responses to elevated CO₂ (figure 4). Specifically, when N availability is low, increased soil C: N resulting from enhanced litter input and changed litter chemistry under elevated CO₂ would promote microbial growth and enzyme activities to accelerate soil N turnover (figure 4). The increased F/B ratio provides access for microbes to more N in MAOM, with an accumulation of ‘new C’ in POM. These two interacting processes increasingly stimulate inorganic N release through heterotrophic respiration to meet the increasing N demand under elevated CO₂, with the release of more CO₂ back to the atmosphere. Consequently, increased C input may primarily be counterbalanced by intensified SOM decomposition, which largely limits soil C storage under elevated CO₂. For ecosystems with high N availability, the N demand of elevated CO₂ conditions is satisfied, which indirectly restricts microbial growth and enzyme activities, as well as decreases the F/B ratio and mineralization rates (figure 4). Thus, cPOM is preferentially degraded compared to MAOM with liberation of few inorganic N and CO₂ from SOM. With greater C input to soil under elevated CO₂ and high N availability, C input would suppress C release, eventually strengthening the overall soil C pool.

Notably, CO₂-induced shift of soil C from slow- to fast-cycling pool is not conductive to long-term SOM stabilization in this alpine meadow ecosystem. Although high N availability can suppress this shift in C allocation and stabilize the SOM, natural N deposition may be insufficient to meet future N demand under elevated CO₂. Therefore, elevated CO₂ may gradually lower SOM stability and aggravate its vulnerability to disturbance, which may create extreme risks for soil C release when subjected to other global change drivers, such as warming.

5. Conclusions

Our study illustrates that elevated CO₂ caused a non-significant increase in the soil C pool, but apparently altered C allocation among different soil fractions. Elevated CO₂ increased the relative abundance of fungi in the microbial community and increased
enzyme activities, which stimulated the decomposition of ‘old C’ over that of ‘new C’, and further reduced SOM stability. Further, N supply under elevated CO$_2$ decelerated the decomposition process in SOM by increasing the F/B ratio, shrinking the cPOM pool, and reducing enzyme activities. Overall, elevated CO$_2$ has a significant N availability-driven effect on soil C allocation but not the soil C pool. For this alpine meadow in Tibetan Plateau where plant growth is extremely limited by N availability, the soil may have a low potential for C storage under continuously rising CO$_2$, with the rising potential to decrease SOM stability.

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Data availability statement

The data that support the findings of this study are available upon reasonable request from the authors.

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Author contributions

YJZ and JTZ designed the experiment and built the facility; GZ, NC, YC and LW collected the experiment data; GZ and YJZ analysed the experimental data, and GZ wrote the first draft of the manuscript. CL, XJF, LLL contributed substantially to revisions.

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