



Decoupling of plant carbon and nitrogen under elevated CO₂ and nitrogen addition in a typical alpine ecosystem

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Abstract

Aims Vegetation in high-altitude regions is hypothesized to be more responsive to increasing atmospheric CO₂ concentrations due to low CO₂ partial pressure. However, the underlying mechanisms driving this response at an ecosystem scale are poorly understood. We aimed to explore the plant carbon (C) and nitrogen (N) relationships and biomass allocation in

response to elevated CO₂ and N addition in a Tibetan meadow.

Methods A 5-year manipulation experiment was conducted in an alpine meadow (4585 m above sea level) to explore the responses of plant carbon (C), nitrogen (N), and biomass dynamics, as well as their allocation schemes, to elevated CO₂ (from 380 ppm to 480 ppm) and N fertilization.

Results Elevated CO₂ alone significantly enhanced aboveground plant biomass by 98%, exhibiting a stronger CO₂ fertilization effect than the global average level (20%) for grasslands. Elevated CO₂ favored N accumulation in aboveground parts despite the declined concentration. Nitrogen fertilization alleviated the N constraints on CO₂ fertilization effects, which strengthened C sequestration capacity for the aboveground plant tissues. Moreover, our results indicate a decoupling between C and N cycles in alpine ecosystems under elevated CO₂, especially in the N-enrichment environments.

Conclusions Overall, this study shows a high sensitivity of aboveground plant biomass and decoupled C-N relationships under elevated CO₂ and N fertilization for high-elevation alpine ecosystems, highlighting the need to incorporate altitude effects into Earth System Models in predicting C cycle feedbacks to climate changes.

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Introduction

From molecular metabolism to ecosystem nutrient cycling, carbon (C) and nitrogen (N) are biologically coupled because of the conserved elemental stoichiometry of plants and microorganisms (Finzi et al. 2011). Carbon and N are crucial to many aspects of plant physiological function and microbial metabolism, through their biochemical controls on primary production, respiration, and decomposition in terrestrial ecosystems (Evans and Burke 2013). However, global changes, such as elevated CO₂ and N deposition, have drastically altered the biogeochemical cycles of C and N in Earth's ecosystems. These disturbances are suggested to decouple C and N cycles (eliminate the interrelationship between them), owing to the different degrees of controls by biological and geochemical processes (Peñuelas et al. 2012; Delgado-Baquerizo et al. 2013).

Human activities have raised CO₂ concentrations in the atmosphere more than 40% above their pre-industrial levels, and this rising trend is projected to continue for the coming decades (Terrer et al. 2018). Elevated CO₂ can stimulate the rate of CO₂ assimilation by plants (Leakey et al. 2009; Franks et al. 2013), and potentially reinforce the C sequestration abilities of terrestrial ecosystems (Ainsworth and Long 2005), which may counterbalance the increasing CO₂ in the atmosphere released by human activities (Schimel et al. 2015). This CO₂ fertilization effect on the terrestrial C sink is supposed to be largely hampered by N availability (Luo et al. 2004; Reich and Hobbie 2013; Langley and Magonigal 2010). Decades of increased atmospheric N deposition across various ecosystems may partially lessen the N constraints on CO₂-induced enhancement of terrestrial productivity (Eastman et al. 2021). This human-induced C and N fertilization may cause the asynchrony in C and N supply and the biogeochemical cycles of C and N could be uncoupled, with subsequent consequence on terrestrial ecosystem C storage and cycling (Asner et al. 1997; Evans and Burke 2013). For example, many studies have reported an increase C:N ratio of plant tissue as a result of elevated CO₂, and N deposition generally decreases the C:N ratio (Wang et al. 2021). Both litter decomposition and herbivory are shown to be at least in part a function of tissue C:N ratios in many studies (Knops et al. 2007; Burghardt et al. 2018). Therefore, if an

unbalanced C and N supply and demand decouple the C and N cycles, and further affect tissue and litter chemistry, elevated CO₂ and N deposition may accelerate or decelerate litter decomposition rates and ecosystem C cycles (Knops et al. 2007; Park et al. 2020).

The altered C and N cycles under environmental disturbance are found to affect plant C, N, and biomass allocation between the above- and belowground portions (Valentine and Mäkelä 2012), which reflects evolutionary strategies for resource acquisition and adaptation to environments (Dietze et al. 2014; Dickman et al. 2015). The above- and belowground plant structures are exposed to dramatically different temperatures and moisture conditions, largely determining the decomposition of plant litter and thus the permanence of sequestered C in the ecosystem (Wang and Taub 2010; Schmidt et al. 2011). Consequently, the tradeoff of plant C, N, and biomass between short-lived organs (aboveground parts) and long-lived organs (belowground parts) can produce significant effects on the pathway for ecosystem C reentering the atmosphere (Friedlingstein et al. 1999). Current empirical and theoretical methods have demonstrated distinct strategies of plant C, N and biomass allocation, which varies with environments and plant species (Peichl et al. 2012; Poorter et al. 2012). To optimize growth and survival, plants conventionally respond to global changes by altering the ratio of C, N and biomass allocation to shoots or roots (Poorter et al. 2012; Bachofen et al. 2019; Zhou et al. 2020). For example, some ecosystems increased N and biomass allocation to roots to alleviate CO₂-induced N limitation in soils (Suter et al. 2002; Iversen et al. 2008; Leuzinger and Hättenschwiler 2013). Conversely, some forests decrease belowground components in response to elevated CO₂, likely because elevated CO₂ can mitigate water limitation by reducing plant water consumption (Franks et al. 2013). Nitrogen fertilization increases biomass allocation to aboveground parts more strongly than to belowground parts (Li et al. 2020). Furthermore, a growing body of reports from long-term observations and manipulative experiments have demonstrated that elevated CO₂ and N deposition are not occurring separately, and their combined effects will likely be more complex (Reich et al. 2006b; Sillen and Dieleman 2012; Zhao et al. 2020). Despite these advances, we know little about the response of C and N cycles

associated with biomass allocation to the interaction of elevated CO₂ and N deposition for alpine ecosystems.

Because of the non-uniform environmental stresses worldwide, elevated CO₂ and N deposition may have regional, but not global, effects on the plant C and N cycles associated with their allocation (Wang and Taub 2010), emphasizing the high uncertainty in projecting future global C budgets. Alpine ecosystems at high elevation, with an area of approximately 2,847,868 km², are extremely vulnerable to global changes (Elser et al. 2020). High-elevation plants are supposed to be more responsive to increasing CO₂ concentrations in the atmosphere due to their low CO₂ partial pressure (Hättenschwiler et al. 2002). Although some experiments in alpine regions have been designed to test the co-regulations of elevated CO₂ and N addition on plant C and N dynamics and allocation, inconsistent conclusions from these studies constrain the incorporation of altitude effects of CO₂ fertilization into Earth System Models (Schäppi and Körner 1997; Dawes et al. 2011; Inauen et al. 2012). Further field experiments are needed to complement our knowledge in those under-represented ecosystems (Curtis and Wang 1998; Zhang et al. 2014; Bachofen et al. 2019).

As the highest plateau in the world, the Tibetan Plateau hosts the largest alpine grassland ecosystem worldwide (Zhang et al. 2019). It is an ideal region to investigate the responses of plants to global changes. The Tibetan Plateau is currently experiencing significant anthropogenic environmental change, including increased N deposition. Understanding how these two primary global change factors (e.g., rising CO₂ and N deposition) affect plant C and N dynamics and the biomass allocation are critical to understanding ecosystem C cycling in facing the accelerated global changes on the plateau. In this study, we explored the responses of plant C and N dynamics, and the biomass allocation to treatments in an elevated CO₂ and N deposition study (simulated by N addition) in a Tibetan meadow over five years. We aimed to test the two hypotheses: (i) alpine plants may exhibit stronger responses to elevated CO₂ as they grow at lower CO₂ partial pressure, and (ii) altered CO₂ and N availability may decouple the C:N relationships in plant tissues due to the imbalanced nutrient supply.

Materials and methods

Study site

The study was conducted in a typical alpine meadow ecosystem in the North Tibetan Plateau, China (31°38.513'N, 92°0.921'E, 4585 m a.s.l.). This study constitutes the highest-elevation CO₂ enrichment experiment conducted on grassland worldwide (Zhu et al. 2020). The climate is characterized by a mean annual temperature of approximately −1.16 °C and a mean annual precipitation of ~430 mm, mainly falling during the summer season from June to September (Zhu et al. 2017). Moreover, the area has gale-force winds lasting nearly a quarter of each year. The vegetation community is dominated by *Kobresia pygmaea*, with companion species including *Potentilla saundersiana*, *Potentilla cuneata*, *Youngia simulatrix* and *Saussurea stoliczkae*. The growing season normally starts in mid-May and ends in mid-September.

Experimental design

Due to the gusty winds and low stature vegetation on the Tibetan Plateau, CO₂ enrichment could not be implemented using a free-air CO₂ enrichment (FACE) design. Instead, eight semi-FACE designed open-top chambers (OTCs) were constructed with steel frames and glass in 2013 (Fig. 1). Each octagonal OTC measures 1.5 m in length for each side and has a height of 2 m, covering a surface area of 10.86 m². The CO₂ concentration within the CO₂-enriched chambers (480 ppm) was designed to be 100 ppm higher than the ambient levels (380 ppm) (Fig. 2). From 2014 to 2018, enriched CO₂ was injected into the OTCs to artificially increase CO₂ concentrations during the growing season of each year.

The experiment follows a full-factorial split-plot design, with elevated CO₂ as the primary factor and N addition as the secondary factor. Among them, four octagons were exposed to ambient CO₂ concentration (380 ppm), and the other four were exposed to rising CO₂ concentrations (480 ppm). Each octagon was divided into four plots with a size of 1.25 m×1.25 m, with one supplemented with N and one without N (the remaining two plots had no N fertilizer and were not considered in the study). For each N deposition treatment (simulated by N fertilization), 5 g N m⁻² year⁻¹ in the form of urea was

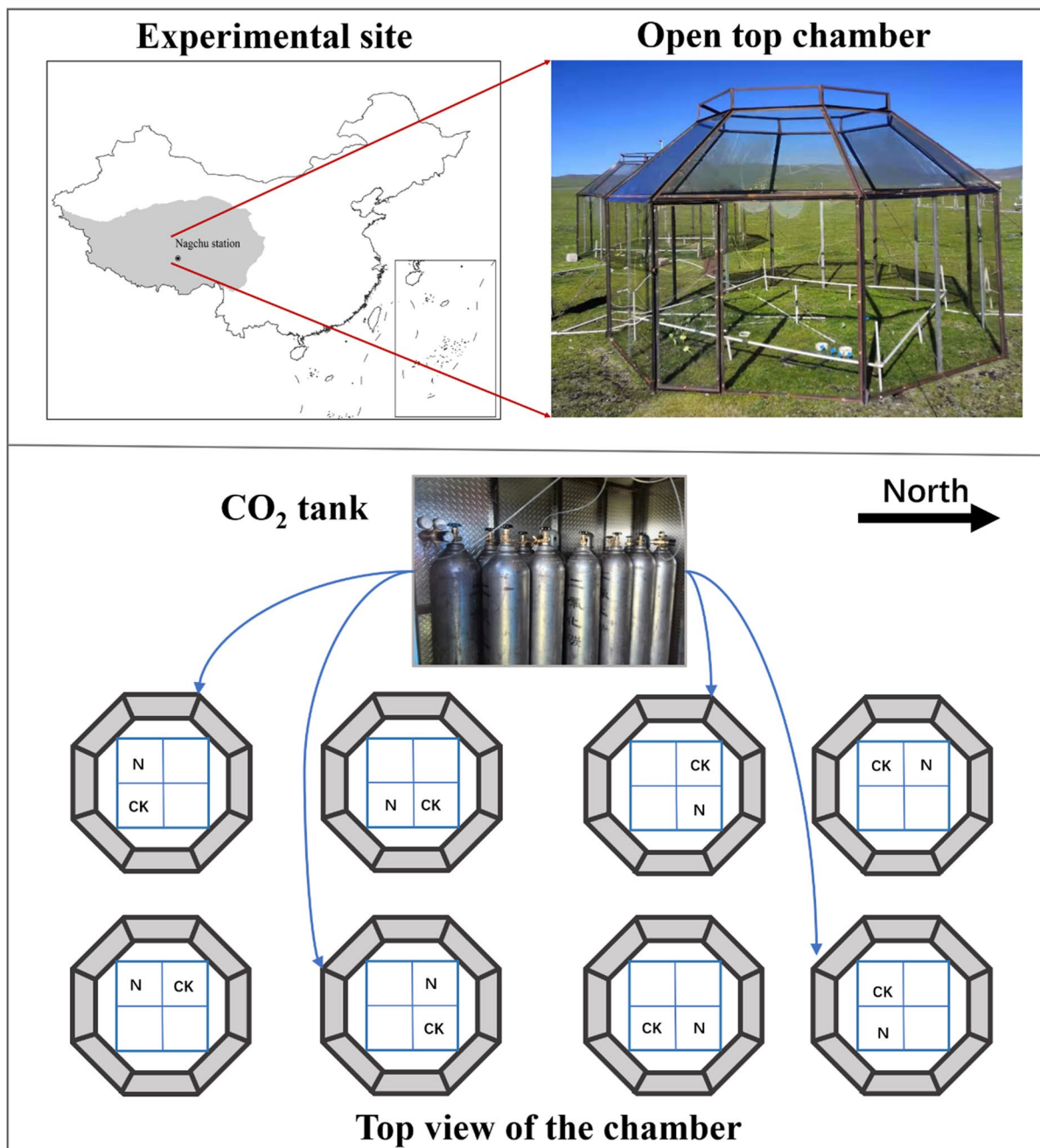


Fig. 1 Open-top chamber (OTC) designed for enriching CO_2 in the Tibetan Plateau (4585 m above sea level). N, nitrogen addition; CK, control check

dissolved in 200 ml water and sprayed on the plots during June from 2014 to 2018. To prevent possible active N transfer along the soil profile between adjacent plots, a stainless-steel plate was inserted to the depth of 30 cm soil in 2013.

In OTCs with CO_2 enrichment, polyvinyl chloride (PVC) pipes had 1 mm pinholes drilled every 0.1 m along the pipe and were framed 0.3 m aboveground, to transport pressured CO_2 from tanks to chambers. The CO_2 flux from the tank was controlled by a flow

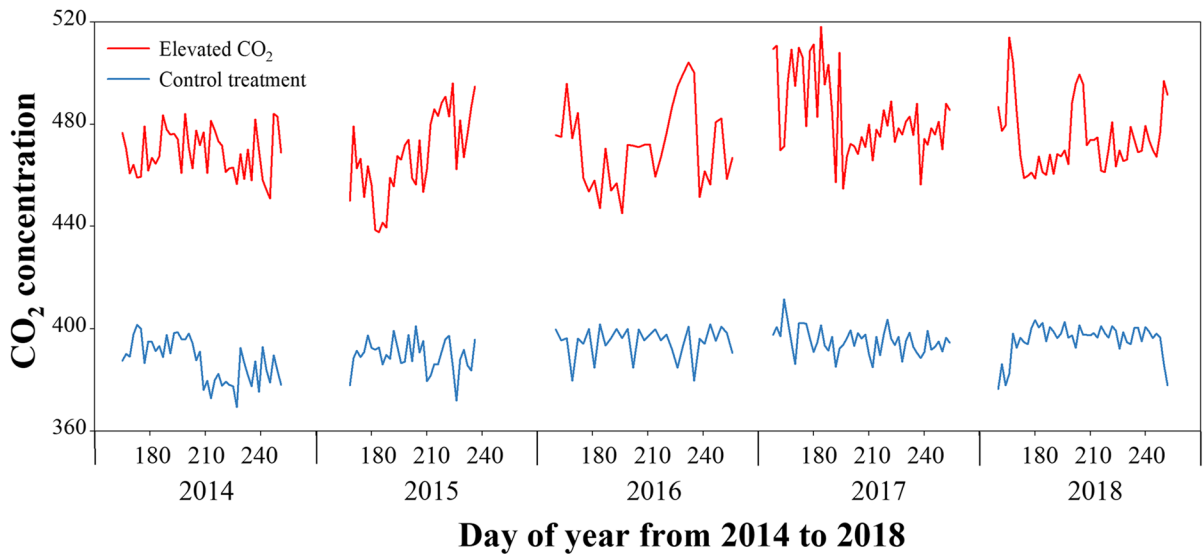


Fig. 2 CO₂ concentrations in atmosphere for open-top chambers (OTC) with or without CO₂ enrichment treatment, respectively, from 2014 to 2018

meter carrying the Vaisala GMP222 sensor (Vaisala, Helsinki, Finland), which monitored CO₂ concentrations in the middle of chambers every 15 min. To account for shading effects, the same pipes were installed for the ambient CO₂ chambers. Rainfall shielded by the OTCs was supplemented by spraying equal amounts of water in the chambers.

Plant and soil sampling

In the middle of each growing season (late August) from 2015 to 2018, a 10 cm×10 cm quadrat was randomly selected in each plot, and all aboveground plants within the selected quadrat were harvested. After collecting the aboveground biomass, a cylinder auger (7 cm in diameter) was used to take a soil core to a depth of 30 cm. Collected soil cores for each treatment were passed through a 2-mm sieve to remove roots, organic debris, and rocks. Root samples were then collected from soil cores, which were cleaned and dried for belowground biomass measurements. To reduce the influence of soil sampling on plot, the cores were filled with local soil each time after sampling. All above- and belowground plant samples were oven-dried at 65 °C for 48 h to a constant weight. Then, soil and plant samples were ground and analyzed for C and N concentration using the elemental analyzer (Vario MAX CN analyzer,

Germany). Above- and belowground plant C and N storage were calculated as the product of biomass and corresponding tissue C and N concentrations.

Statistical analysis

In assessing responses of plant biomass and C:N stoichiometry to elevated CO₂ and N fertilization in different tissues, the “relative change” index was applied and calculated as:

$$\text{Relative change(\%)} = \frac{(X_e - X_a)}{X_a} \times 100\%$$

X_a represents the average value of a specific variable in the control group, and X_e represents the average values of a specific variable in each experimental group.

Repeated measures analysis of variance was applied to evaluate the effect of elevated CO₂, N deposition, and year on plant biomass from 2015 to 2018. A split-plot analysis of variance was used to assess the effects of elevated CO₂ and N fertilization on plant and soil C and N concentrations using statistical software 20.0 (SPSS Inc., Chicago, IL, USA). Regression analysis and Pearson’s correlation were used to evaluate the relationship between C and N concentrations as well as the relationship of the biomass allocation

and the C:N ratio. All the differences and effects were considered statistically significant at $P < 0.05$.

Results

Biomass, C and N allocation in plant above- and belowground components

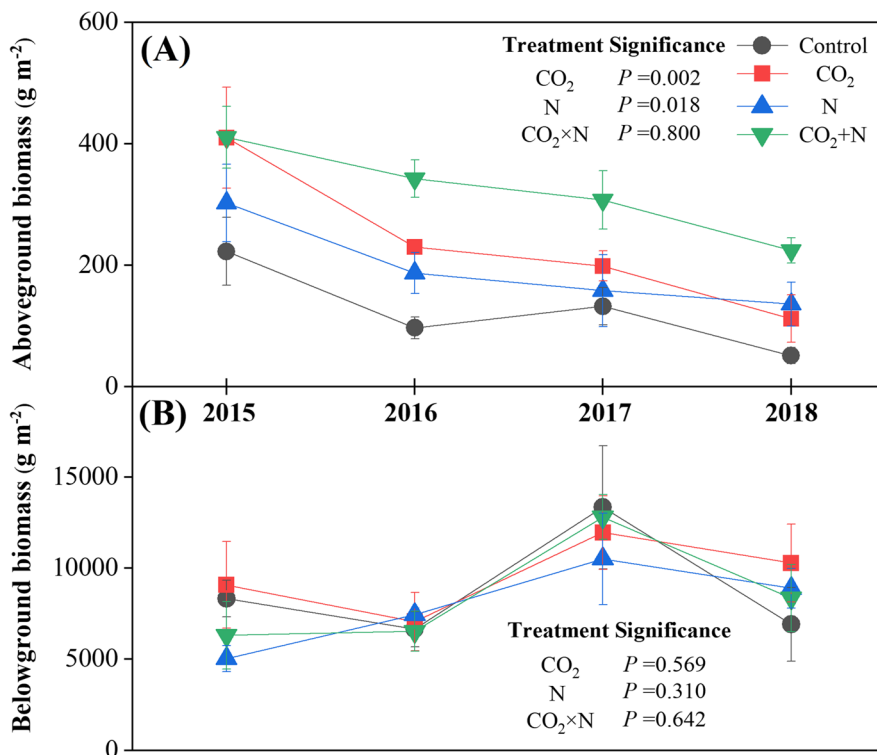
For this alpine meadow, the above- and belowground biomass range during 2015–2018 was 51–410 g m⁻², and 6649–13,340 g m⁻², respectively (Fig. 3). In general, plant belowground biomass accounted for 94–99% of the community total. Elevated CO₂ significantly increased aboveground biomass ($P < 0.05$) but showed no significant effects on belowground parts (Fig. 3; $P > 0.05$). Nitrogen fertilization alone significantly increased aboveground biomass by 36–166% ($P = 0.018$), with no significant effect on belowground biomass ($P = 0.310$). Combined elevated CO₂ and N fertilization significantly enhanced aboveground plant biomass by 84–340% ($P < 0.05$).

Plant above- and belowground biomass presented a significant relationship under control treatment, but the correlations were nonsignificant under elevated

CO₂ or N addition treatment (Fig. 4). The ratios of belowground biomass (BGB) to aboveground biomass (AGB) varied between 31 and 74. Elevated CO₂ alone altered the biomass allocation between above- and belowground plant components ($P = 0.001$), causing a significant decrease (40%) in the ratio of BGB to AGB. N fertilization alone significantly lowered the ratio of BGB to AGB by 35% ($P = 0.006$). Combined elevated CO₂ and N fertilization significantly decreased the ratio of BGB to AGB by 58% ($P < 0.05$), while there were no significant interaction effects of elevated CO₂ and N fertilization on the biomass allocation ($P = 0.337$). For the biomass allocation strategy (Fig. 4), the relative changes in the ratios of BGB to AGB were caused more by the changes in AGB (percentage changes between -0.47% and 3.90%) rather than the changes in BGB (percentage between -0.47% and 0.69%).

The plant C and N storage range varied from 2819 g m⁻² to 3479 g m⁻², and from 66 g m⁻² to 80 g m⁻², respectively (Fig. 5). Overall, elevated CO₂ and N fertilization caused no significant effects on belowground plant C and N storage ($P > 0.05$). On the contrary, elevated CO₂ and N fertilization significantly

Fig. 3 Effects of elevated CO₂ and N addition on above- (A) and belowground (B) plant biomass for 4 years, from 2015 to 2018. Error bars indicate standard error. The inserted text is the results of the repeated measures ANOVA analysis. Control, control treatment; CO₂, elevated CO₂; N, nitrogen addition; AGB, aboveground biomass; BGB, belowground biomass



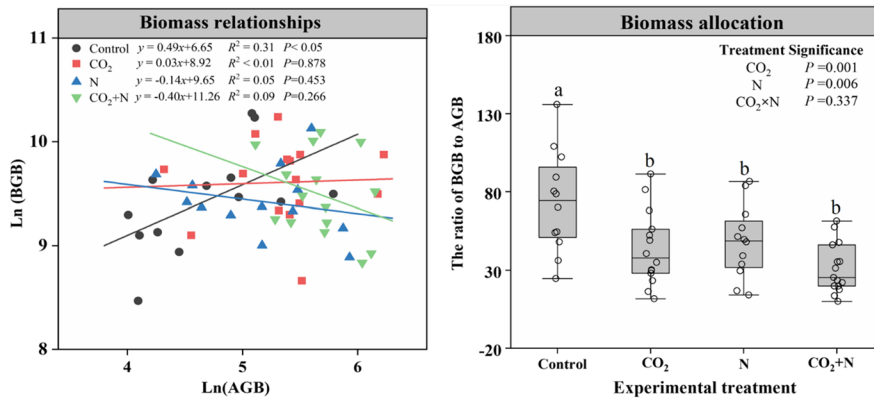


Fig. 4 Effects of elevated CO₂ and N addition on relationships between above- and belowground biomass, and biomass allocation (right). For the right figure, solid line in the box marks the median of each dataset, with the upper and lower ends of boxes representing the 0.25 and 0.75 percentiles, respectively. The upper and lower whisker caps denote the maximum and minimum values, respectively. The inserted text is the results

increased aboveground plant C storage by 100% and 70%, respectively. Combined CO₂ and N fertilization significantly enhanced aboveground plant C storage by 180% ($P < 0.05$). Meanwhile, elevated CO₂ and N fertilization significantly increased aboveground plant N storage by 87% and 102% ($P < 0.05$), respectively. For plant C and N allocation, both elevated CO₂ and N fertilization, as well as their combined effects, significantly lowered the ratio of BGB to AGB for C and N storage ($P < 0.05$).

Dynamics of C:N stoichiometry in the alpine ecosystem

The total soil C concentrations varied from 42.1 mg g⁻¹ to 51.0 mg g⁻¹, and the total soil N concentrations varied from 3.8 mg g⁻¹ to 4.2 mg g⁻¹ (Table 1). Elevated CO₂ alone significantly increased soil C concentrations from 42.1 mg g⁻¹ to 51.0 mg g⁻¹ ($P < 0.05$) and increased soil C:N ratio from 11.16 to 12.07 ($P < 0.05$). However, nitrogen fertilization had no significant effects on soil total C and N, and the associated C:N ratio ($P > 0.05$; Table 1). For plants, elevated CO₂ significantly increased C concentration ($P = 0.013$) and the associated C:N ratio ($P < 0.01$) in the aboveground plant portions, and significantly decreased the N concentration in aboveground tissues ($P = 0.002$; Table 2). Nitrogen fertilization

of the two-way ANOVA analysis. Different lowercase letters in the right figure indicate a significant difference of C, N and C:N among treatments according to Turkey's b test at $P < 0.05$. Control, control treatment; CO₂, elevated CO₂; N, nitrogen addition; AGB, aboveground biomass; BGB, belowground biomass

alone significantly increased N concentrations and decreased C:N of aboveground tissues but had no significant effects on C concentrations. Combined elevated CO₂ and N fertilization significantly increased C concentrations in aboveground tissues from 421.6 mg g⁻¹ to 432.8 mg g⁻¹ but had no significant effect on N concentration and C:N ratio ($P > 0.05$). Elevated CO₂ and N fertilization showed no significant effects on C and N concentrations, and the C:N ratio of the belowground tissues ($P > 0.05$).

Response of plant C and N relationships to elevated CO₂ and N fertilization

Under natural conditions, C and N concentrations in both above- and belowground plant tissues were significantly and positively correlated ($P < 0.01$) with R^2 values of 0.49 and 0.46, respectively (Fig. 6). However, elevated CO₂ decoupled this relationship in aboveground tissues ($P = 0.20$) and lowered the C and N correlation coefficients from 0.49 to 0.27. Under CO₂ enrichment environments, N fertilization further weakened the C and N correlation coefficients from 0.27 to 0.24 and from 0.12 to 0.07 for above- and belowground tissues, respectively (Fig. 6).

Given that the biomass allocation is largely regulated by signals involving plant C and N dynamics, the relationship between the C:N ratio and plant

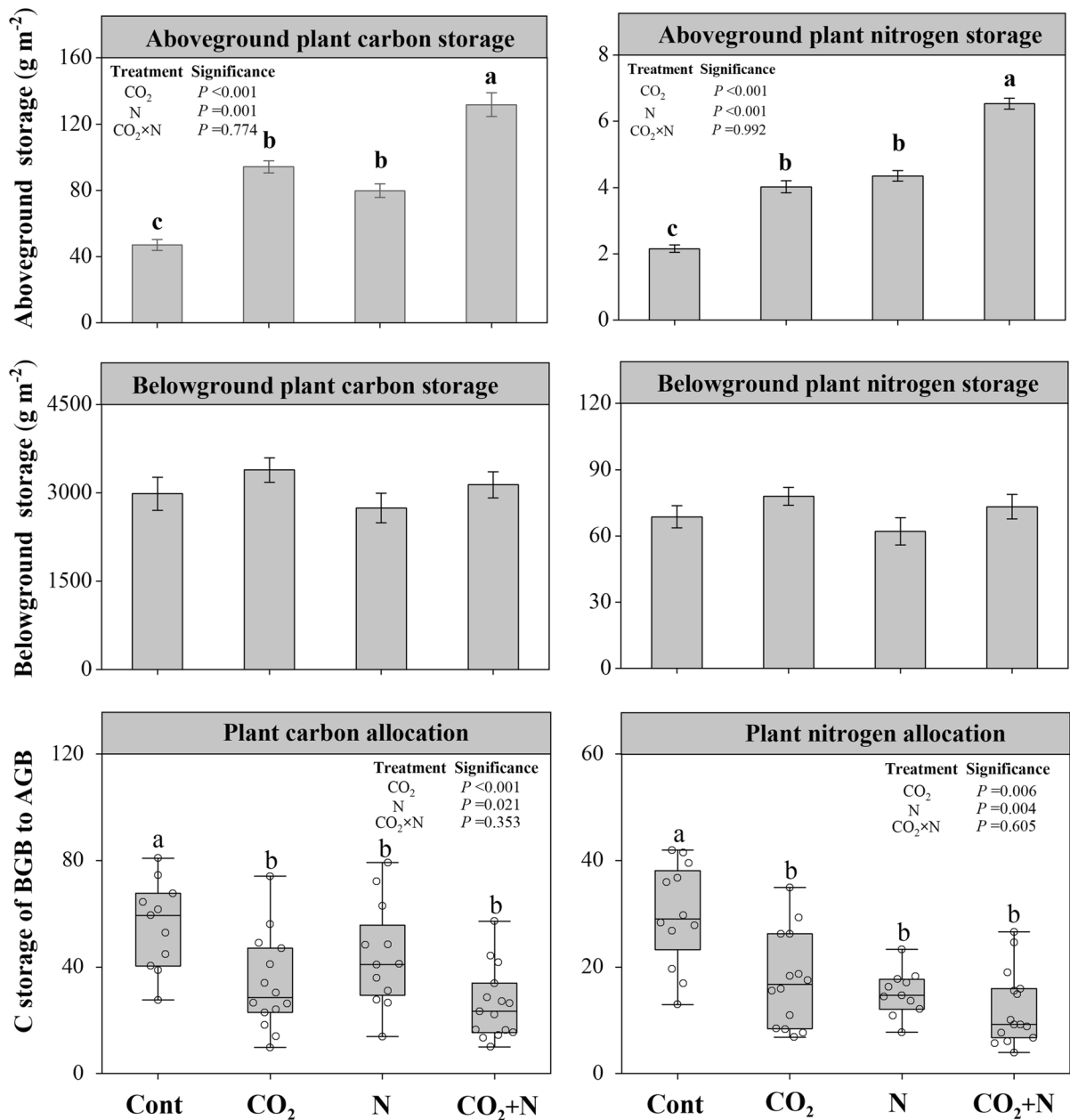


Fig. 5 Responses of plant carbon and nitrogen storage to elevated CO₂ and nitrogen addition. Error bars indicate standard error. Solid line in the box marks the median of each dataset, with the upper and lower ends of boxes representing the 0.25 and 0.75 percentiles, respectively. The upper and lower whisker caps denote the maximum and minimum values,

respectively. The inserted text is the results of the two-way ANOVA analysis. Different lowercase letters in the figure indicate a significant difference of C, N and C:N among treatments according to Turkey's *b* test at $P < 0.05$. Cont, control treatment; CO₂, elevated CO₂; N, nitrogen fertilization; AGB, aboveground biomass; BGB, belowground biomass

biomass allocation were further analyzed. In general, Ln (C/N) in belowground tissues showed no significant relationship with Ln (BGB/AGB) ($P > 0.05$;

Fig. 7). Under the control treatment, Ln (C/N) significantly correlated with Ln (BGB/AGB), generating an R^2 value of 0.335 ($P < 0.05$) for aboveground tissues.

Table 1 Effects of elevated CO₂ and N addition on soil C and N concentrations from 2015 to 2018. The values listed in the table were the four-year means with standard error. Bold numbers in the table indicate significance between control and treatments at level of *P* < 0.05 according to split-plot analysis

Stoichiometry	Treatment				<i>P</i> value		
	Cont	CO ₂	N	CO ₂ +N	CO ₂	N	CO ₂ ×N
C (mg g ⁻¹)	42.1 ± 2.5 ^a	51.0 ± 1.4 ^c	45.7 ± 2.2 ^{bc}	47.0 ± 1.5 ^{bc}	0.010	0.925	0.049
N (mg g ⁻¹)	3.82 ± 0.02	4.22 ± 0.01	4.05 ± 0.19	4.09 ± 0.01	0.108	0.692	0.188
C:N	11.2 ± 0.2 ^a	12.1 ± 0.2 ^b	11.3 ± 0.3 ^a	11.3 ± 0.1 ^a	0.018	0.121	0.028

of variance. Cont, control treatment; CO₂, elevated CO₂; N, nitrogen addition. Different lowercase letters indicate significant differences among treatments at level of *P* < 0.05 according to Tukey’s *b* test

The *R*² of these relationships decreased under elevated CO₂ and N fertilization from 0.335 to 0.216 and 0.260, respectively. The combined effects of elevated CO₂ and N fertilization further decoupled the correlation between the C:N ratio and BGB/AGB ratio, generating a nonsignificant *R*² value of 0.029 (*P* = 0.54).

Discussion

Elevated CO₂ increases C allocation to plant aboveground parts

The aboveground plant biomass in this alpine ecosystem showed a higher sensitivity to five years of CO₂ enrichment (increased by 98%; Fig. 8) in comparison with other experiments worldwide (increased by 20% on average; Sillen and Dieleman 2012). Meanwhile, elevated CO₂-induced high C concentrations in aboveground tissues suggest a reinforced capability of C sequestration for plants besides the increased

aboveground biomass. These support our first hypothesis that high-elevation plants can exhibit particular response to elevated CO₂. Although CO₂ concentrations in the atmosphere remain constant with rising elevation, CO₂ partial pressure and density drop dramatically in alpine regions (Körner and Diemer 1987). Given that atmospheric pressure decreases by roughly 11% per km of elevation in the atmosphere, the CO₂ partial pressure at this alpine meadow (approximately 50% of the pressure at sea level) is considerably less than that at lowland grassland. Since the concentration of CO₂ in the liquid phase is proportional to its partial pressure in the surrounding gas phase, the concentration of CO₂ in chloroplasts of alpine plants would be considerably lower than that in lowland plants (Terashima et al. 1995). Alpine plants are observed to enhance CO₂ utilization efficiencies (Körner 2003), to compensate for the negative influence of declined CO₂ partial pressures on photosynthesis. Therefore, alpine plants with increased photosynthetic CO₂ use efficiency may respond more

Table 2 Effects of elevated CO₂ and N addition on C and N concentrations of above- and belowground plant tissues from 2015 to 2018. The values listed in the table are the means with standard error. Cont, control treatment; CO₂, elevated CO₂;

N, nitrogen addition. Bold numbers indicate a significant difference between control and treatments at the level of *P* < 0.05 according to according to split-plot analysis of variance

Tissue	Stoichiometry	Treatment				<i>P</i> value		
		Cont	CO ₂	N	CO ₂ +N	CO ₂	N	CO ₂ ×N
Aboveground	C (mg g ⁻¹)	421.6 ± 3.6 ^b	424.3 ± 3.0 ^{ab}	414.4 ± 2.57 ^b	432.8 ± 2.7 ^a	0.013	0.888	0.042
	N (mg g ⁻¹)	21.2 ± 1.5 ^{bc}	19.4 ± 1.1 ^c	24.2 ± 1.6 ^a	22.3 ± 1.2 ^b	0.002	< 0.001	0.908
	C:N	20.9 ± 1.2 ^b	22.9 ± 1.2 ^a	18.1 ± 1.1 ^c	20.2 ± 1.1 ^d	0.001	< 0.001	0.888
Belowground	C (mg g ⁻¹)	353.3 ± 11.8	359.2 ± 37.7	371.6 ± 9.8	367.6 ± 1.1	0.674	0.094	0.997
	N (mg g ⁻¹)	8.11 ± 0.26	8.05 ± 0.22	8.42 ± 0.25	8.46 ± 0.79	0.766	0.108	0.900
	C:N	43.7 ± 1.1	43.3 ± 0.9	44.5 ± 1.6	43.9 ± 1.9	0.972	0.900	0.548

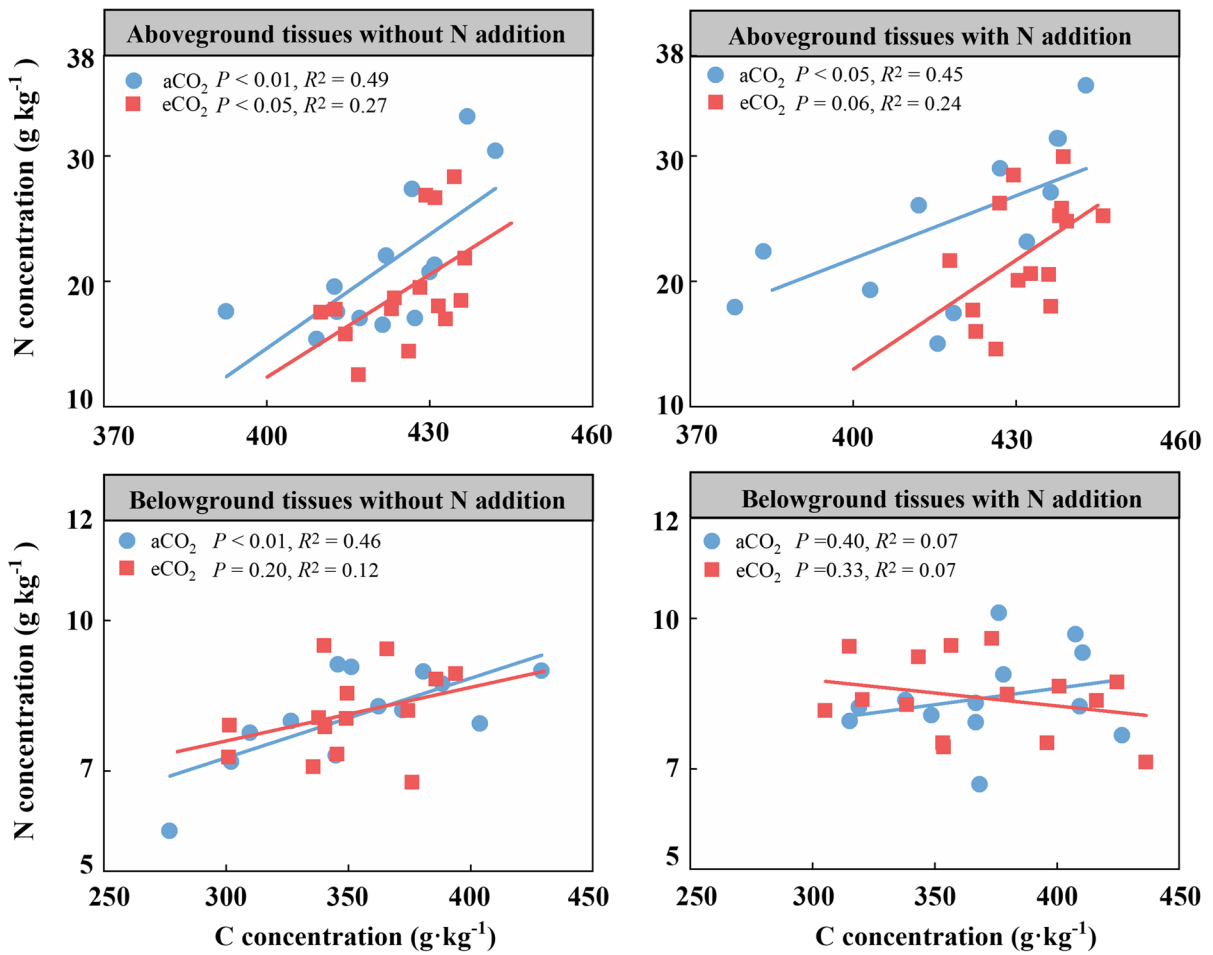
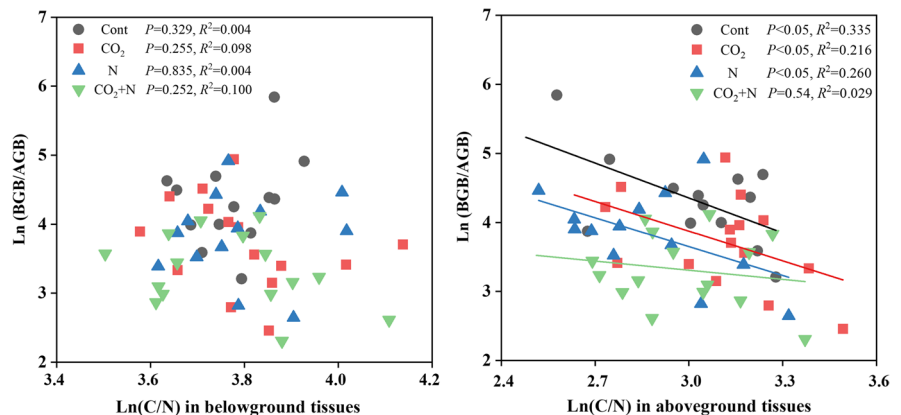


Fig. 6 The relationships between carbon and nitrogen contents of different plant tissues under elevated CO₂ and N treatments. Error bars indicate standard error. The inserted text is the results of the two-way ANOVA analysis. Different lowercase

letters in the figure indicate a significant difference of C, N and C:N among treatments according to Turkey’s *b* test at $P < 0.05$. aCO₂, ambient CO₂; eCO₂, elevated CO₂

Fig. 7 Relationships between C:N ratio and biomass allocation under elevated CO₂ and N addition for the entire plant from 2015 to 2018. Cont, control treatment; CO₂, elevated CO₂; N, nitrogen addition



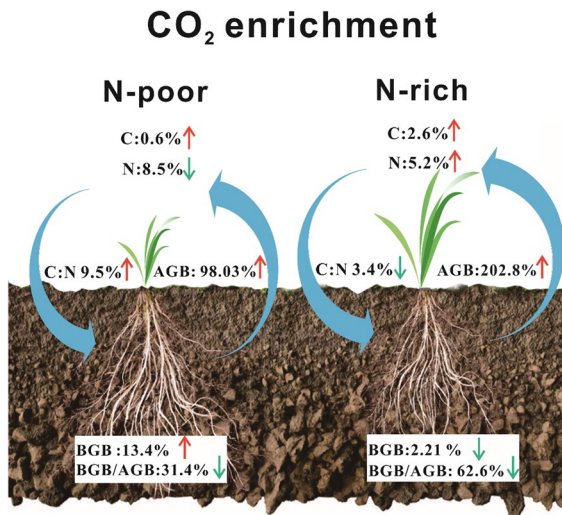


Fig. 8 The tradeoff of biomass, C and N stoichiometry between the above- and belowground plant parts under elevated CO_2 and N fertilization. Red and green arrows in the figure represent increasing and decreasing trend, respectively. CO_2 , elevated CO_2 ; N, nitrogen addition; AGB, aboveground biomass; BGB, belowground biomass

strongly to elevated CO_2 than comparable lowland plants.

According to optimal partitioning models, the plant would allocate more biomass to plant belowground components to optimize nitrogen acquisition, thereby alleviating CO_2 -induced N limitation (Leuzinger and Hättenschwiler 2013; Avila et al. 2020; Noyce et al. 2019; Frew et al. 2021). Inconsistent with the optimal partitioning predictions, elevated CO_2 causes more biomass distribution to aboveground components but not to belowground parts in the current experiment. Two reasons may account for this phenomenon. First, plants under elevated CO_2 may increase C allocation to soils as root exudates instead of increasing belowground biomass, and thus enhance N uptake through intensified soil organic matter decomposition rates (e.g., “priming effect”). Second, the unique changes in the C and biomass allocation patterns of the alpine ecosystem may be related to the special high-altitude and cold environments. Plants in alpine ecosystems have evolved better-developed root systems than shoots in adapting to the environmental conditions of the low fertility soils and cold temperatures (Yang et al. 2009). Under these harsh environments plants are prone to possessing vast biomass in long-lived organs, such as roots (Hermans et al. 2006), to strengthen nutrient uptake from the soil and

improve resistance to cold stress. Therefore, a high root/shoot ratio (from 31.2 to 73.6 in this study) for alpine plants may lead to the unresponsiveness of root systems to elevated CO_2 .

Regulation of N availability on plant C dynamics under elevated CO_2

Our results demonstrate that N availability largely regulates the plant C dynamics and allocation under elevated CO_2 . Elevated CO_2 alone increased plant C concentrations and C:N ratio but decreased plant N concentrations, consistent with previous reports (Knops et al. 2007; Leakey et al. 2009). This consistency indicates that CO_2 fertilization-induced N-limitations are reflected in the changes in tissue quality. It is worth noting that no significant effect of N addition on plant and soil C concentrations were observed, while the interacted N addition and CO_2 enrichment significantly increased the C concentrations in aboveground plant tissues and soils. That is, the C concentration of plants grown in N-enriched soils would be more responsive to elevated CO_2 than those grown in N-poor soils.

Enriched CO_2 and N influence not only the amount of plant tissue quality but also the plant biomass produced. A combination of N fertilization with CO_2 enrichment simulates the aboveground biomass, indicating an alleviated CO_2 -induced N limitation on plant growth and boosted C fixation capacity for the alpine ecosystem with future nitrogen deposition scenarios. We also found that the CO_2 fertilization effect had weakened with increasing treatment duration independent of N treatment, although the aboveground plant biomass under combined CO_2 and N fertilization could sustain at a high level in contrast to that under elevated CO_2 alone. This phenomenon suggests that besides the N availability (Reich et al. 2006a), the CO_2 fertilization effect on biomass may also be modulated by other factors, possibly water or phosphorus limitation (Reich et al. 2014; Farrior et al. 2015; Terrer et al. 2019).

Elevated CO_2 and N fertilization decouple plant C and N cycles

In natural conditions, C and N cycles are biologically coupled from molecular to global scales due to

the required balance in basic elements proportion and conserved elemental stoichiometry in organisms (Finzi et al. 2011; Delgado-Baquerizo et al. 2013). Here, we observed a significant positive correlation between plant aboveground C and N concentration and a positive relationship between above- and belowground biomass without CO₂ and N treatments. In addition, a significant correlation between the C:N ratio in aboveground tissues and the ratio of BGB/AGB exists, even under elevated CO₂ or N additions. That is, aboveground tissue C and N concentrations are primarily related to the relative changes in biomass allocation. As the C and N status has been suggested as an important indicator of plant source and sink balance (Wang et al. 2021), these CO₂-induced variations in chemometrics are therefore tightly correlated with the biomass allocation (Hilbert 1990; Sugiura and Tateno 2011). This phenomenon agrees with a previous hypothesis (McCarthy and Enquist 2007) that the biomass allocation is largely modulated by signals involving plant C and N balance and the C:N ratio would be an appropriate indicator to predict plant allocation strategies.

In this study, we found that elevated CO₂ decoupled C and N cycles in plant tissues and this uncoupled relationship further aggravated under an N-enriched environment. This may be the result of imbalanced N supply and plant demand under CO₂ and N fertilization. Similarly, the tight linkage between plant above- and belowground biomass become uncoupled under CO₂ and N fertilization, with C shifting away from belowground components and towards aboveground biomass production, indicating that perturbations from human activities can alter the natural linkages between C and N cycles associated with C reallocation. This imbalance observed in the C and N with elevated CO₂ and N fertilization may have important consequences on the alpine ecosystem, as the decoupling may cause asynchronous nutrient supply and demand, which can aggravate the nutrient loss and create new biogeochemical feedbacks (Delgado-Baquerizo et al. 2013).

Conclusions

This study provides novel insights into how alpine ecosystems respond to global changes (elevated CO₂ and N deposition) in terms of plant C and N relationships, and the biomass allocation in an alpine

meadow. Our results illustrate that aboveground plant tissues are more responsive to elevated CO₂ in contrast to belowground parts, with more biomass being allocated to the aboveground plant parts. We deduce that plants in high elevation alpine ecosystems may be more responsive to low CO₂ partial pressure there (approximately 50% of the pressure at sea level), and are inclined to shift the distribution of photosynthates to the aboveground compartment to strengthen the photosynthetic potentials. Nitrogen fertilization alleviates the N limitations on the plant growth induced by elevated CO₂ and further amplifies the capacity of plant C fixation simultaneously by increasing biomass and C concentrations in aboveground tissues. Overall, elevated CO₂ and N fertilization decouples plant C and N cycling and these effects may alter the impacts and feedbacks of plants on biogeochemical cycles, and either slow down or accelerate climate changes.

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Declarations

Competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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