

Decoupling of Carbon and Nitrogen Under Elevated CO₂ 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, this hypothesis and the underlying mechanisms driving this response at an ecosystem scale are poorly understood. We aimed to exploring the biomass allocation and plant carbon-nitrogen relationships in response to elevated CO₂ in a Tibet meadow.

Methods: Here, 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 and N fertilization.

Results: Elevated CO₂ alone significantly enhanced aboveground plant biomass by 98.03 %, exhibiting a stronger CO₂ fertilization effect than the global average level (20 %) for grasslands. In contrast to the belowground parts, elevated CO₂ caused disproportionately aboveground tissues increment in association with C and N accumulation. These results suggest a potential C limitation for plant growth in alpine ecosystems. N fertilization alleviates 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 in the face of 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 for high-elevation alpine ecosystems, highlighting the need to incorporate altitude effects into Earth System Models in predicting C cycle feedback to climate changes.

1 Introduction

Human activities have raised CO₂ concentrations in the atmosphere more than 40% above their pre-industrial levels, and this rising is projected to continue for the coming decades (Terrer et al. 2018). Since elevated CO₂ can stimulate the rate of CO₂ assimilation by plants (Leakey et al. 2009; Franks et al. 2013), there is considerable interest in assessing whether and to what extent enhanced photosynthesis, and potentially reinforced carbon (C) sequestration ability of terrestrial ecosystems, could counterbalance the increasing CO₂ released by human activities (Schimel et al. 2015). A key part of the uncertainty in climate change projections concerns is how essential elements (e.g. C & N) and biomass allocation by plants respond to elevated CO₂ (Poorter et al. 2012; Sevanto and Dickman 2015), which is central to understanding ecosystem processes and contains with profound impacts on terrestrial ecosystem C storage and cycling.

Under global changes, a deeper understanding on plant allocation strategies is fundamental for predicting terrestrial C sequestration in future climate scenarios (Zhou et al. 2020). The plant C, N and biomass reallocation between the above- and belowground portions reflect evolution strategies for

resource acquisition and adaptation to environments (Dietze et al. 2014; Dickman et al. 2015). Theoretically, different plant organs are responsible for specific metabolic processes (Sevanto and Dickman 2015). For instance, leaves are the main organs providing metabolic energy through photosynthesis and root systems are closely associated with the acquisition of nutrients and water from the soil (BassiriRad et al. 2001). Their distinct functions result in their differential responses to global changes (Gifford et al. 2000). Meanwhile, the above- and belowground plant parts are exposed to dramatically different temperatures and moisture conditions, which largely determines their decomposition process and thus the permanence of sequestered C in the ecosystem (Wang and Taub 2010; Schmidt et al. 2011). As a consequence, 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 atmosphere (Friedlingstein et al. 1999). However, current investigations regarding plant allocations revolve mainly around forests, and grassland have been paid inadequate attention and limited conclusions also comprise much uncertainty (Kauwe et al. 2014).

Current empirical and theoretical methods have demonstrated distinct strategies of plant C, N and biomass allocation, which vary with environments and plant species (Peichl et al. 2012; Poorter et al. 2012). In CO₂ enriched environment, plant C concentrations and biomass production normally increases, and plant N concentrations decreases (Wang et al. 2021). Nevertheless, there is limited evidence on how elevated CO₂ would adjust the plant allocation between above- and belowground parts (Zhang et al. 2014). To optimize growth and survival, plants conventionally respond to elevated CO₂ by altering the ratio of C, N and biomass allocation to shoot and roots (Poorter et al. 2012; Bachofen et al. 2019). 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). In general, the plant C, N and biomass allocation patterns under elevated CO₂ still comprises high uncertainty and we need further field experiments to investigate the underlying mechanism, especially for the under-study ecosystems (Curtis and Wang 1998; Zhang et al. 2014; Bachofen et al. 2019).

C and N are crucial to many aspects of plant physiological function and microbial metabolism, with their tight coupled relationships in terrestrial ecosystem (Evans and Burke 2013). Due to close correlation between plant sink-source balance and C-N cycles, C:N stoichiometry is supposed to affect the above- and belowground biomass allocation in plants (Sugiura and Tateno 2011; Valentine and Mäkelä 2012). However, changes due to disturbance, such as elevated CO₂ and N deposition, can cause C and N to decouple and thus have critical impacts on ecosystem processes (Evans and Burke 2013). Most natural ecosystems are N-limited (Reich et al. 2006 a) and CO₂ enrichment can further aggravate this limitation. Therefore, the magnitude of CO₂ effects on terrestrial C sink is supposed to be largely dampened by N availability (Luo et al. 2004; Reich and Hobbie 2013; Langley and Megonigal 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), but also can decouple C -N cycles

and cause asynchrony in N supply and demand, with subsequent consequence on ecosystem C partitioning (Asner et al. 1997). Indeed, a growing body of evidence 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 (Sillen and Dieleman 2012; Zhao et al. 2020). Nonetheless, field experiments regarding N deposition effects on plant C-N coupling and the biomass allocation under elevated CO₂ are still lacking.

Because of the non-uniform environmental stresses worldwide, elevated CO₂ may have regional, but not global, effects on the plant allocation (Wang and Taub, 2010). We specially need to complement our knowledge by adding some experiments in those under-represented ecosystems. Alpine ecosystems distributed on high elevation are extremely vulnerable to global changes (Elser et al. 2020). In particular, alpine ecosystems are supposed to be more responsive to increasing CO₂ concentrations in the atmosphere due to therein low CO₂ partial pressure (Hättenschwiler et al. 2002). However, there are no experiments in alpine regions designed to test the co-regulations of elevated CO₂ and N addition on plant allocation. 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. In this study, we explored the responses of plant C, N dynamics and the biomass allocation to a 5-year elevated CO₂ and N deposition (simulated by N addition) treatments in a Tibetan meadow. We aimed at testing the two hypotheses: (i) elevated CO₂ stimulate disproportionately more biomass allocation to aboveground parts, and (ii) altered CO₂ and N availability may decouple the C:N relationships in plant tissues due to the imbalanced nutrient supply.

2 Materials And Methods

2.1 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, above sea level). This study constitutes the highest-elevation CO₂ enrichment experiment conducted on grassland worldwide (Zhu et al. 2020). The climate is characterized with a mean annual temperature of approximately - 1.16°C and the mean annual precipitation of circa 430 mm, mainly falling during summer season from June to September (Zhu et al. 2017). Moreover, the area has a gale-force wind 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 stoliczkai*. The growing season normally starts in mid-May and ends in mid-September.

2.2 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, a semi-FACE design composed of 8 octagonal open-top chambers (OTCs) was constructed with steel frames and glasses in 2013 (Fig. 1).

Each 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 was designed to be 100 ppm higher than the ambient levels. 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 eight octagons as eight blocks. Among them, four octagons were exposed to ambient CO₂ concentration, and the other four were exposed to rising CO₂ concentrations. Each octagon was divided into four plots with a size of 1.25m×1.25m, 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 dissolved in 200 ml water and sprayed in the plots during June of 2014–2018.

In each OTC with CO₂ enrichment, the polyvinyl chloride (PVC) pipes had 1 mm pinholes drilled every 0.1 m along the pipe and were framed 0.3m aboveground, to transport pressured CO₂ from tanks to chambers. The CO₂ flux from the tank was controlled by a flow 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 water in the chambers.

2.3 Plant and soil sampling

In the middle of each growing season (late August) from 2015 to 2018, a 10cm×10cm quadrat was randomly selected in each plot, and all aboveground plants within the selected quadrat was 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. All above- and belowground plant samples were oven-dried at 65 °C for 48 h to constant weight. Meanwhile, soil samples were thoroughly hand-picked to remove visible roots and organic debris and oven-dried. Lastly, 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.

2.4 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:

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

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.

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. A two-factor ANOVA was used to assess the effects of elevated CO₂ and N fertilization by Mixed Linear Model using statistical software 20.0 (SPSS Inc., Chicago, IL). All the differences and effects were considered statistically significant at $P < 0.05$.

3 Results

3.1 Biomass, carbon and nitrogen allocation in plant above- and belowground components

For this alpine meadow, the above- and belowground biomass range during 2015-2018 was 51.0-410.6 g·m², and 6649.9-13340.4 g·m², respectively (Fig. 2). In general, plant belowground biomass accounted for 93.8% to 99.2% of the whole community total. Elevated CO₂ significantly increased aboveground biomass ($P < 0.05$), but showed no significant effects on belowground parts (Fig. 2; $P > 0.05$). Combined elevated CO₂ and N fertilization significantly enhanced aboveground plant biomass by 84.4% to 339.9% ($P < 0.05$).

The ratios of belowground biomass (BGB) to aboveground biomass (AGB) varied between 31.2 and 73.6 (Fig. 3). Elevated CO₂ alone altered the biomass allocation between above- and belowground plant components ($P = 0.001$), causing a significant decrease (40.4%) in the ratio of BGB to AGB. N fertilization alone significantly lowered the ratio of BGB to AGB by 34.8% ($P = 0.006$). Combined elevated CO₂ and N fertilization significantly decreased the ratio of BGB to AGB by 57.7% ($P < 0.05$), 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. 3), the relative changes in the ratios of BGB to AGB were more induced 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 2818.7 to 3479.3 g m⁻², and from 66.3 and 79.78 g m⁻², respectively (Fig. 6). 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 increased aboveground plant C storage by 100.4% and 69.7%, respectively. Combined CO₂ and N fertilization significantly enhanced aboveground plant C storage by 180.1% ($P < 0.05$). Meanwhile, elevated CO₂ and N fertilization significantly increased aboveground plant N storage by 86.5% and 101.7% ($P < 0.05$), respectively. Combined CO₂ and N fertilization significantly enhanced aboveground plant C storage by 202.9% ($P < 0.05$). 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 ($P < 0.05$).

3.2 Dynamics of C:N stoichiometry in the alpine ecosystem

The total soil C concentrations varied from 3.11% to 5.86%, and the total soil N concentration varied from 0.29% to 0.48% (Table 1). Elevated CO₂ alone significantly increased soil C concentrations from 4.21% to 5.10% ($P < 0.05$) and increased soil C:N ratio from 11.16 to 12.07 ($P < 0.05$). However, N 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 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). N fertilization 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 42.16% to 43.28%, 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 C:N ratio of the belowground tissues ($P > 0.05$).

3.3 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. 4). 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. 4).

In general, C:N ratio in belowground tissues showed no significant relationship with the ratio of BGB to AGB ($P > 0.05$; Fig. 5). Under the control treatment, $\ln(C/N)$ significantly correlated with $\ln(BGB/AGB)$, generating R^2 value of 0.335 ($P < 0.05$) for aboveground tissues. 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 ($P = 0.54$).

4 Discussion

Alpine ecosystems on the high-altitude regions store a dominant proportion of biomass in belowground parts and they are also highly sensitive to global changes. How the two primary global change factors (e.g. rising CO₂ and N deposition) affect plant C-N dynamics and the biomass allocation between aboveground and belowground are critical to understand ecosystem C cycling in face of the accelerated

global changes on the plateau. The field experiment shows that the alpine grasslands exhibit distinct biomass allocation pattern from other highly investigated ecosystems.

4.1 Sensitivity of aboveground plant biomass to elevated CO₂ in the alpine meadow

This study revealed that aboveground plant biomass in this alpine ecosystem showed higher sensitivity to elevated CO₂ (increased by 98.03%) in comparison with other experiments worldwide (increased by 20% on average; Sillen and Dieleman 2012). Moreover, the elevated CO₂ causes a disproportionate biomass distribution to aboveground components, which refutes the long-held viewpoint that plants would invest more photosynthetic carbohydrates to root systems in CO₂-enriched environments (Leuzinger and Hättenschwiler 2013; Avila et al. 2020; Frew et al. 2021). Plants normally follow the rule of maximizing self-growth by adjusting biomass allocation strategies in response to changing environments (Friedlingstein et al. 1999), among which the most limiting resource plays the critical role (Leuzinger and Hättenschwiler 2013). Accordingly, under CO₂ enrichment environments, carbohydrates become increasingly abundant, while other nutrients, particularly the available N become progressively limiting (Luo et al. 2004). Thus, it can be inferred that plants would allocate a disproportionate proportion of biomass to roots to alleviate nutrient limitation under elevated CO₂ (McGuire et al. 1995), as root systems are responsible for absorbing nutrients from soil. The aboveground shoots are responsible for acquiring C from atmosphere, and plants are assumed to invest less biomass to aboveground compartments due to intensified photosynthetic capacity under elevated CO₂.

While, contrary to the above-mentioned mechanism and our hypothesis (i), the present study showed an opposite biomass allocation scheme. The unique change in biomass allocation pattern of the alpine ecosystem is related to the special high-altitude and cold environments. The lower stomatal conductance under elevated CO₂ can alleviate soil water limitation and decrease the investments to belowground compartments (Farrion et al. 2015). However, CO₂ enrichment-induced reductions in stomatal conductance may not systematically translate into a limitation of whole-plant transpiration, especially for grasslands that exhibit potentially greater increase in aboveground biomass production in response to elevated CO₂ (Ainsworth and Long, 2005; Lauriks et al. 2020). Nature normally can provide adequate C for the vegetation growth (Millard et al. 2007; Leuzinger and Hättenschwiler 2013), and CO₂ enrichment can further stimulate the root growth to absorb nutrients. In our study, elevated CO₂ increased biomass allocation to the aboveground parts, which are fast turnover tissues. So, plants grown in high-elevation regions may be relatively more C-limited than other ecosystems in low elevation regions. 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). As a result, CO₂ usable by plants decrease and plant photosynthesis are dampened (Inauen et al 2012). It can be inferred that CO₂ probably becomes a limiting resource for alpine ecosystems, and in return, more biomass is optimally allocated to aboveground compartments to strengthen the photosynthetic potentials of the community.

4.2 C and N dynamics in response to elevated CO₂ and N fertilization

C and N are the basic elements for metabolic activity of plant and soil microorganisms (Sardans et al. 2017). The elevated CO₂-induced changes in coupled cycling of C and N have significant effects on the plant growth and development (Reich et al. 2006 b). Our study demonstrates a clear pattern showing stoichiometric responses of plant C and N in aboveground tissues are more responsive to elevated CO₂ than the belowground ones (Fig. 7). We attributed this phenomenon to the specific adaptive strategies of a plant to external stressors from the Tibet Plateau. Plants in alpine ecosystems have evolved relatively 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 storing more biomass in long-lived organs, such as roots (Hermans et al. 2006), to strengthening nutrient uptake from the soil. Consequently, C and N stoichiometry in root systems may be more resilient to elevated CO₂. In addition, elevated CO₂-induced higher C concentrations in aboveground tissues suggest a reinforced capability of C sequestration for plants besides the increased biomass. In contrast to C content, elevated CO₂ lowered N concentrations in aboveground tissues. Collectively, C:N ratio is significantly increased and causes an increasing N constraint for the plant growth. Numerous studies attribute this phenomenon to the growth dilution hypothesis (Loladze 2002; Sardans et al. 2012), that is, elevated CO₂-induced increases in carbohydrate production surpass the N acquisitions of plants. This long hold explanation has been challenged by recent findings (Taub and Wang 2008; Feng et al. 2015). Plant N uptake can also be restrained by other mechanisms independent of CO₂-induced stimulation on biomass, such as a declined capacity of soil-root systems to provide N (Taub and Wang 2008). As a whole, our results show that elevated CO₂ facilitates more C and N accumulation in aboveground plant parts (Fig. 7).

Our results demonstrate that N availability largely regulates the biomass allocation under elevated CO₂. The aboveground and belowground plant biomass showed divergent responses to N fertilization, with increased biomass in shoots and reduced biomass in roots. According to the optimal allocation theory, plants would allocate more biomass towards organism acquiring the most limiting resources (McCarthy and Enquist 2007). For this study, enhanced N availability from N deposition reduced the plant dependence on root systems for absorbing nutrients and subsequently lowered belowground C investments to roots (Eastman et al. 2021), allowing plants to invest more C towards acquiring other limiting resources. This altered biomass allocation strategy suggests that plant biomass allocation patterns under global changes are closely linked with resource availability. Therefore, when N fertilization arrives simultaneously with CO₂ enrichment, biomass in the aboveground plants is stimulated. Further, enriched CO₂ and N influence not only the amount of plant material produced but also the plant tissue quality. The interacted CO₂ enrichment and N addition significantly increased the C concentrations in aboveground tissues, indicating an alleviated CO₂-induced N limitation on plant growth and boosted C

fixation capacity for plants in the future; that is, C accumulation of plants grown in N-enriched soils would be more responsive to elevated CO_2 than those grown in N-poor soils.

4.3 Decoupled relationships between biomass partitioning and plant C:N balance under elevated CO_2 and N fertilization

A significant correlation between the C:N ratio in aboveground tissues and the ratio of BGB/AGB exists, that is, aboveground tissue N concentrations under elevated CO_2 or N addition are primarily related to the relative changes in allocation to roots. As the C and N status has been suggested as an important indicator of plant source and sink balance (Wang et al. 2021), these CO_2 -induced variations in chemometrics are therefore tightly correlated with the biomass allocation (Hilbert 1990; Sugiura and Tateno 2011). This phenomenon conforms with the previous optimal partitioning theory (McCarthy and Enquist 2007), suggesting 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 strategy under global changes.

However, we found that elevated CO_2 decoupled C and N cycles in plant tissues, especially for belowground ones. This uncoupled relationship further aggravated under an N-enriched environment, which is supportive of the hypothesis (ii). 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), while perturbations from human activities can alter the natural linkages between C and N cycles (Peñuelas et al. 2012). The uncoupling phenomenon may weaken the plant regulation on biomass allocation through altering C:N stoichiometry. Furthermore, this imbalance observed in the C and N with elevated CO_2 and N fertilization could have important consequences on the alpine ecosystem, as the decoupling may cause asynchrony in the nutrient supply and demand, which can aggravate the nutrient loss and create new biogeochemical feedbacks (Delgado-Baquerizo et al. 2013). For example, although stimulating productivity and biomass accumulation, elevated CO_2 lowered the N content of plant tissue and the subsequent quality of litter. Litter with high C: N ratio is prone to intensifying N limitation and strengthening the positive “priming effect” in soils, which may preferentially release the old and recalcitrant C with century lifetimes in alpine regions to the atmosphere (Langley et al. 2009). In addition, as litter with lower N content may decompose slowly, elevated CO_2 presumably reduces the N cycle rates and soil N availability over time (Luo et al. 2004).

5 Conclusion

This study provides novel insights into how alpine ecosystems respond to global changes (elevated CO_2 and N deposition) in terms of plant C, N and the biomass allocation in an alpine meadow. Our results illustrate that aboveground plant tissues are more responsive to elevated CO_2 in contrast to belowground

parts, with more biomass proportionally being allocated to the aboveground plant parts. This finding refutes the hypothesis that plant would allocate more carbohydrate to root systems in CO₂-enriched atmosphere. We deduce that plants in high elevation alpine ecosystems may be C-limited due to low CO₂ partial pressure there and are inclined to shift the distribution of photosynthate to aboveground compartment to strengthen the photosynthetic potentials. N 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. Moreover, elevated CO₂ decouples plant C and N linkage to some extent, and N fertilization further aggravates this loosening. These effects may alter the impacts and feedbacks of a plants on biogeochemical cycles, and either slow down or accelerate climate changes.

Declarations

Declaration of 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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Tables

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 deviation. Control, control treatment; CO₂, elevated CO₂; N, nitrogen addition.

Stoichiometry	Treatment				P value of Two-way ANOVA		
	CK	CO ₂	N	CO ₂ +N	CO ₂	N	CO ₂ ×N
C (%)	4.21±0.83 ^a	5.10±0.56 ^c	4.57±0.80 ^{bc}	4.70±0.58 ^{bc}	0.010	0.925	0.049
N (%)	0.38±0.07	0.42±0.03	0.41±0.06	0.41±0.04	0.108	0.692	0.188
C:N	11.16±0.69 ^a	12.07±0.65 ^b	11.29±0.97 ^a	11.32±0.53 ^a	0.018	0.121	0.028

Table 2. Effects of elevated CO₂ and N addition on C and N concentrations of aboveground plant tissues from 2015 to 2018. The values listed in the table are the means with standard deviation. Control, 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 two-way ANOVA.

Tissue	Stoichiometry	Treatment				P value of Two-way ANOVA		
		CK	CO ₂	N	CO ₂ +N	CO ₂	N	CO ₂ ×N
Aboveground	C (%)	42.16±0.36 ^b	42.43±0.30 ^{ab}	41.44±0.71 ^b	43.28±36.76 ^a	0.013	0.888	0.042
	N (%)	2.12±0.16 ^{bc}	1.94±0.12 ^c	2.42±0.18 ^a	2.23±0.12 ^b	0.002	<0.001	0.908
	C:N	20.89±1.19 ^b	22.87±1.23 ^a	18.09±1.22 ^c	20.18±1.09 ^d	0.001	<0.001	0.888
Belowground	C (%)	35.33±1.19	34.89±0.70	37.16±0.98	36.76±1.05	0.674	0.094	0.997
	N (%)	0.81±0.03	0.81±0.02	0.84±0.02	0.85±0.02	0.766	0.108	0.900
	C:N	43.73±1.08	43.27±1.20	4.50±1.61	43.90±1.89	0.972	0.900	0.548

Figures

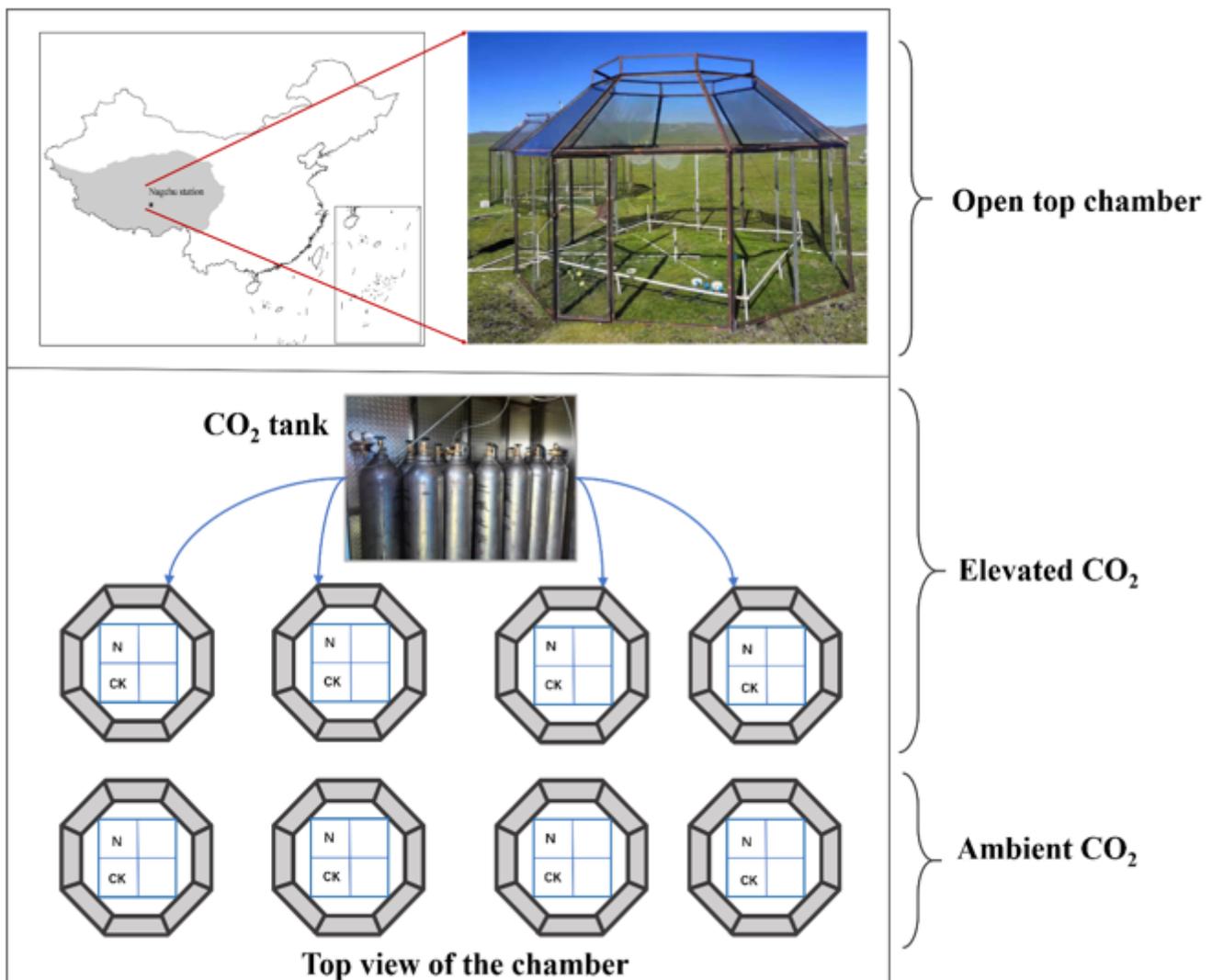


Figure 1

Open-top chamber (OTC) designed for enriching CO₂ in the Tibetan Plateau (4585 m above sea level). N, nitrogen addition; CK, control check.

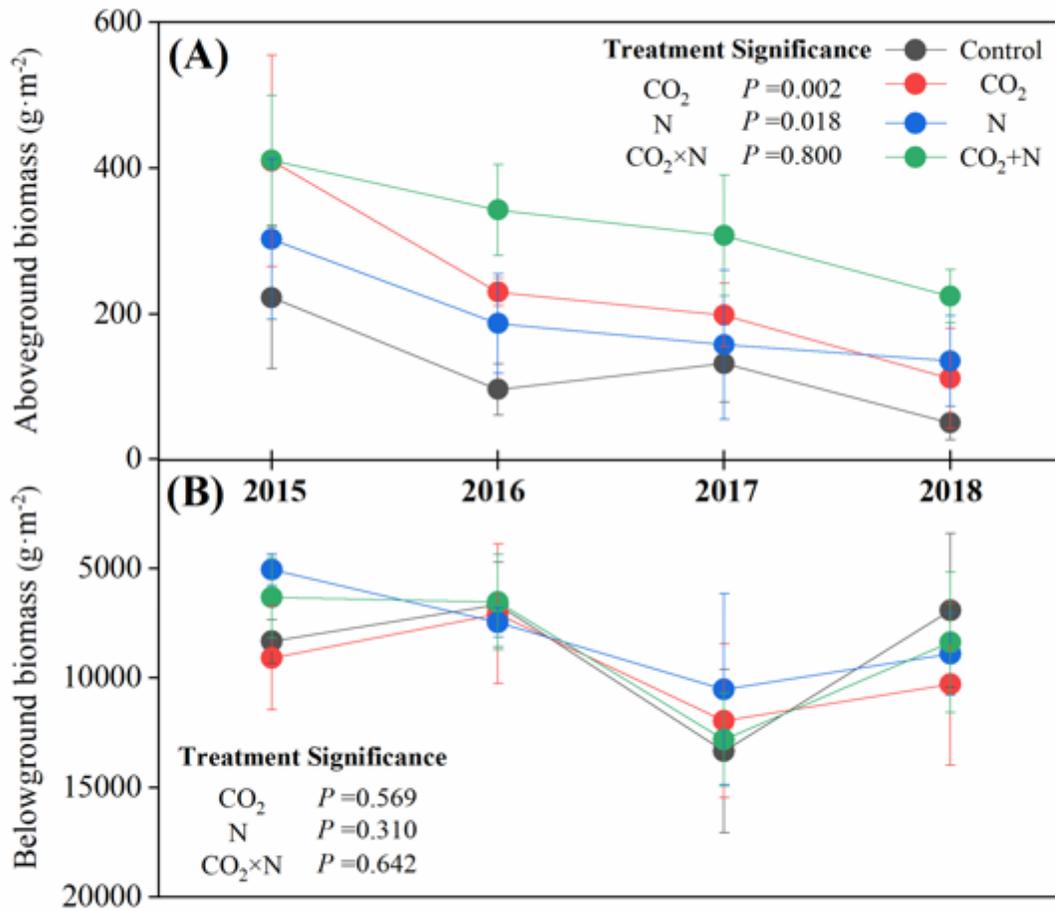


Figure 2

Effects of elevated CO₂ and N addition on above and belowground plant biomass for 4 years, from 2015 to 2018. Control, control treatment; CO₂, elevated CO₂; N, nitrogen addition; AGB, aboveground biomass; BGB, belowground biomass.

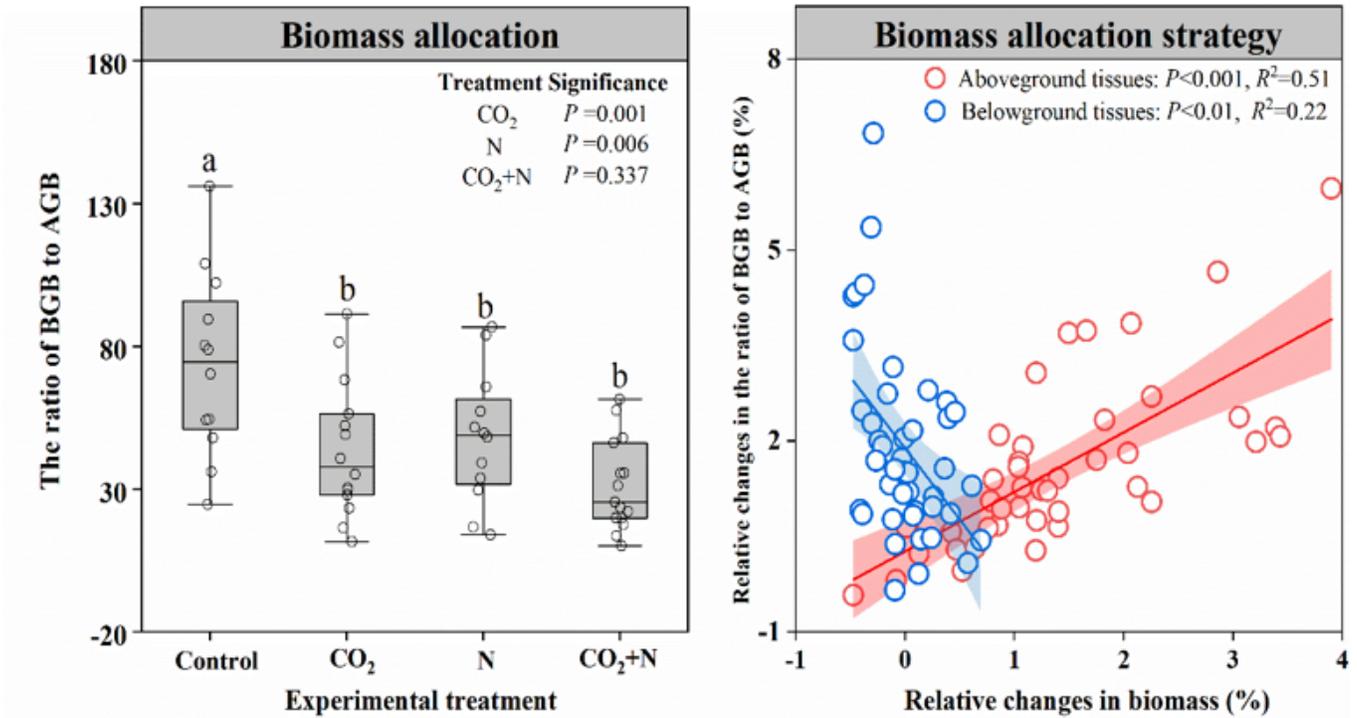


Figure 3

Effects of elevated CO₂ and N addition on biomass allocation (left) and allocation strategy (right). Control, control treatment; CO₂, elevated CO₂; N, nitrogen addition; AGB, aboveground biomass; BGB, belowground biomass.

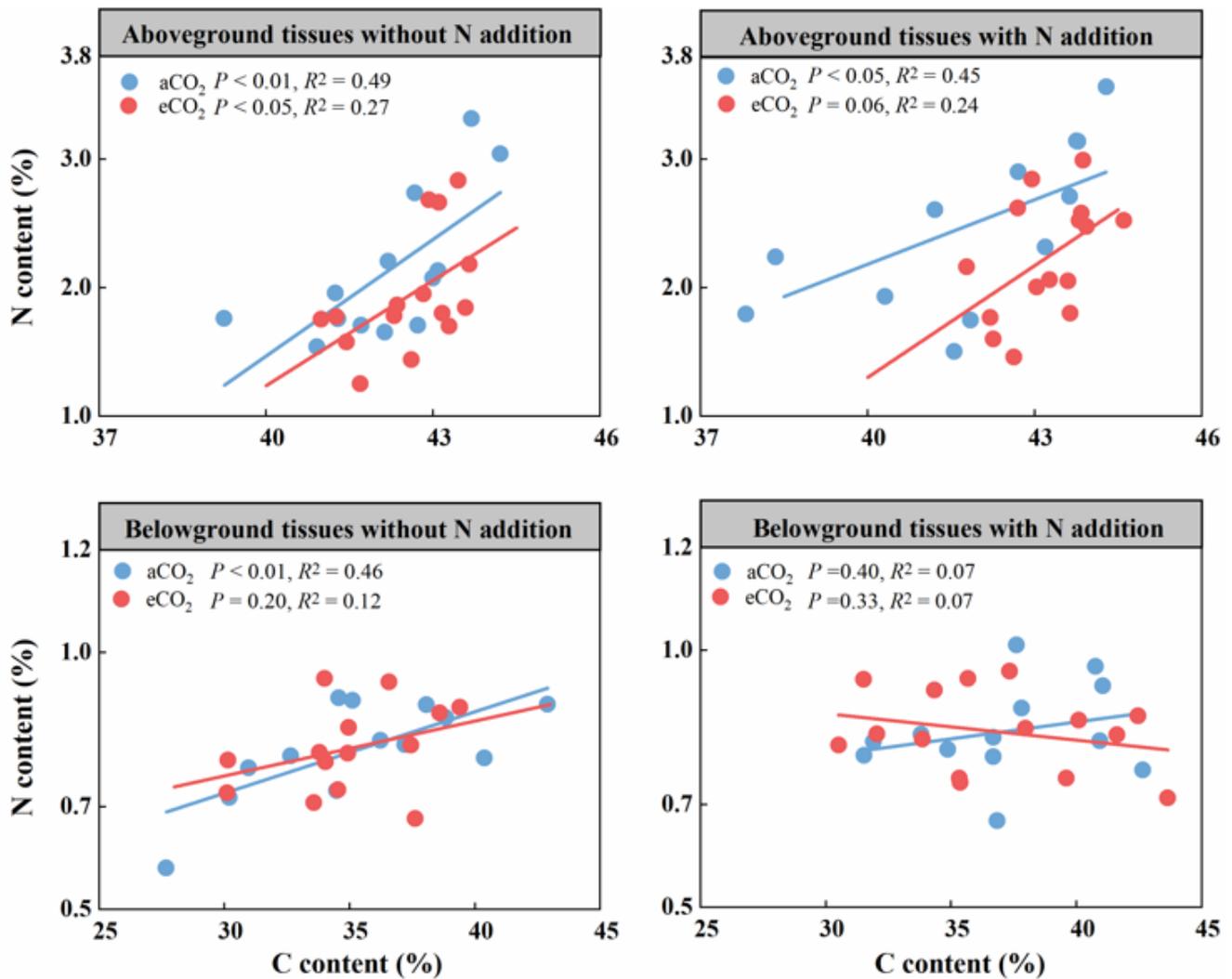


Figure 4

The relationships between C and N contents of different plant tissues under elevated CO₂ and N treatments from 2015 to 2018. aCO₂, ambient CO₂; eCO₂, elevated CO₂.

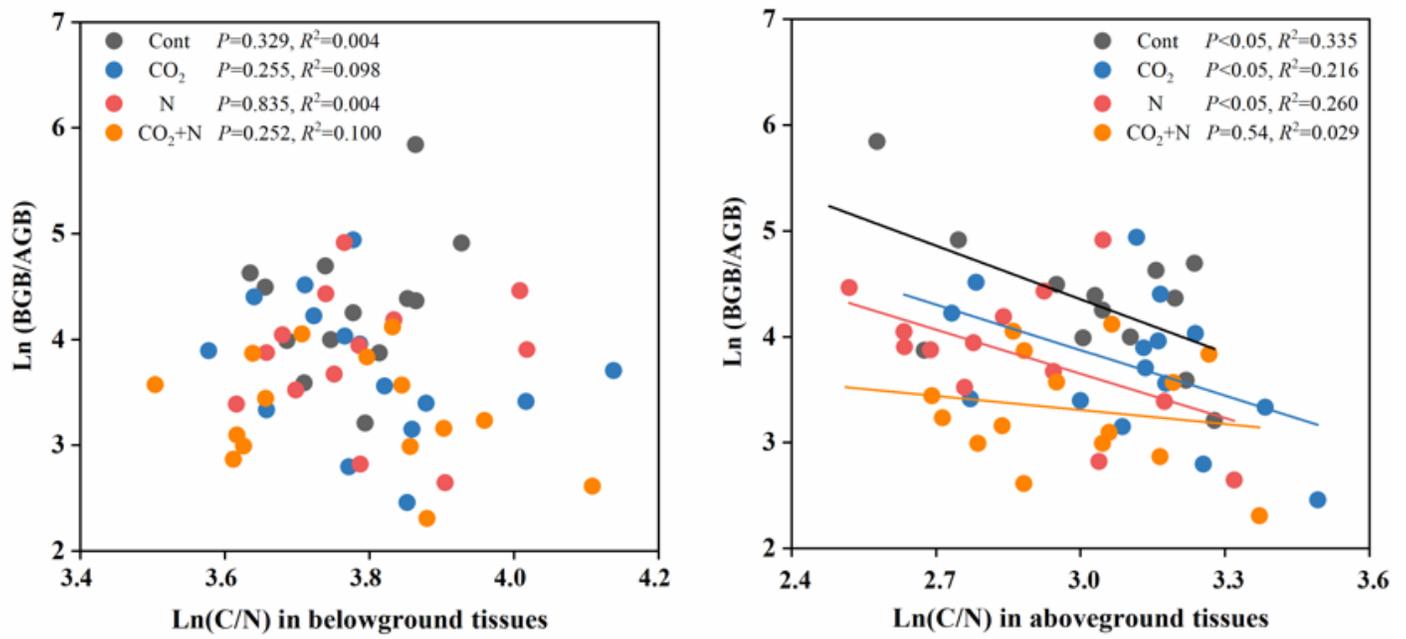


Figure 5

Relationships between C:N ratio and biomass allocation under elevated CO_2 and N addition for the entire plant from 2015 to 2018. Cont, control treatment; CO_2 , elevated CO_2 ; N, nitrogen addition.

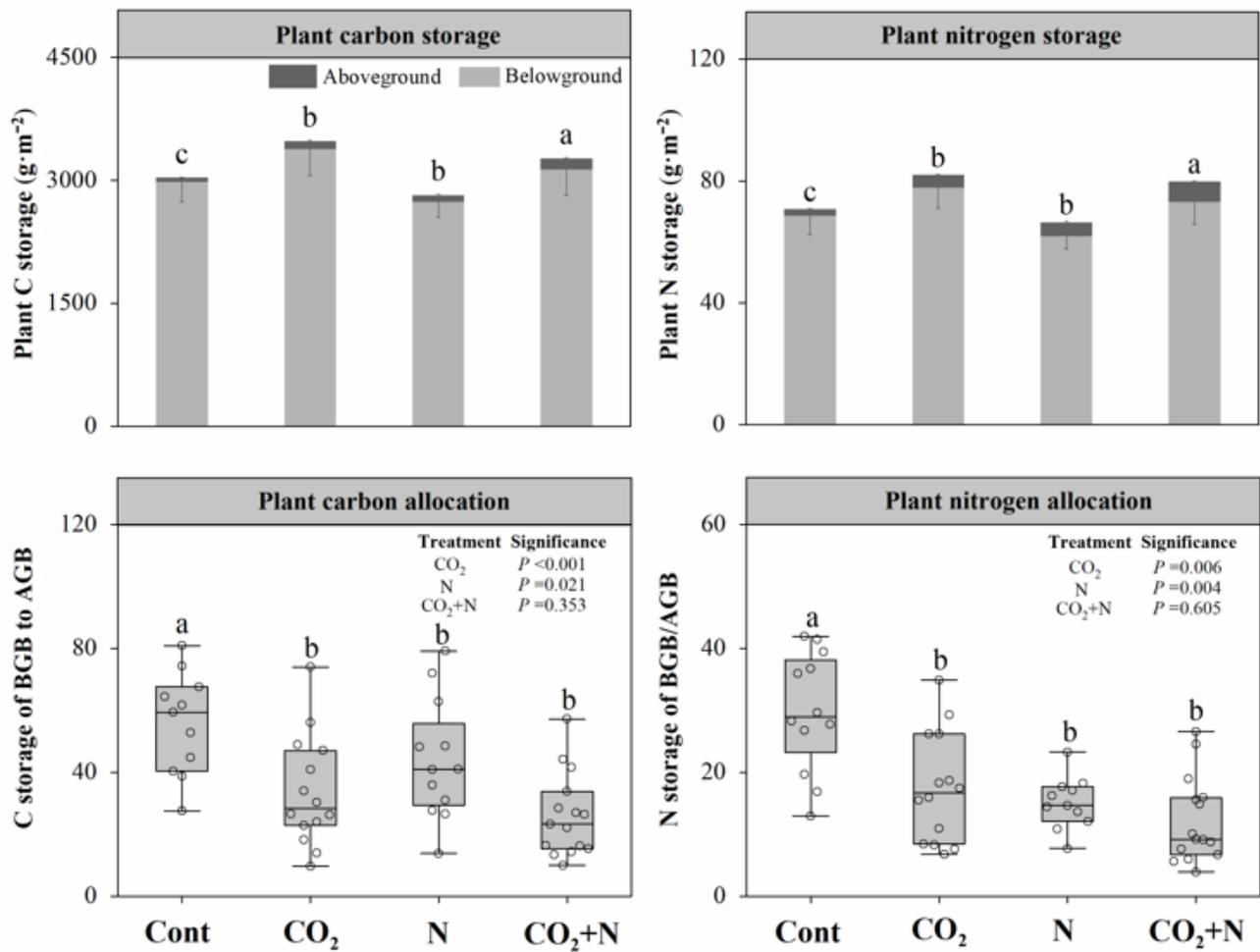


Figure 6

Relative changes of C, N stoichiometry and biomass allocation under elevated CO₂ and nitrogen addition from 2015 to 2018. CO₂, elevated CO₂; N, nitrogen fertilization; AGB, aboveground biomass; BGB, belowground biomass. 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.

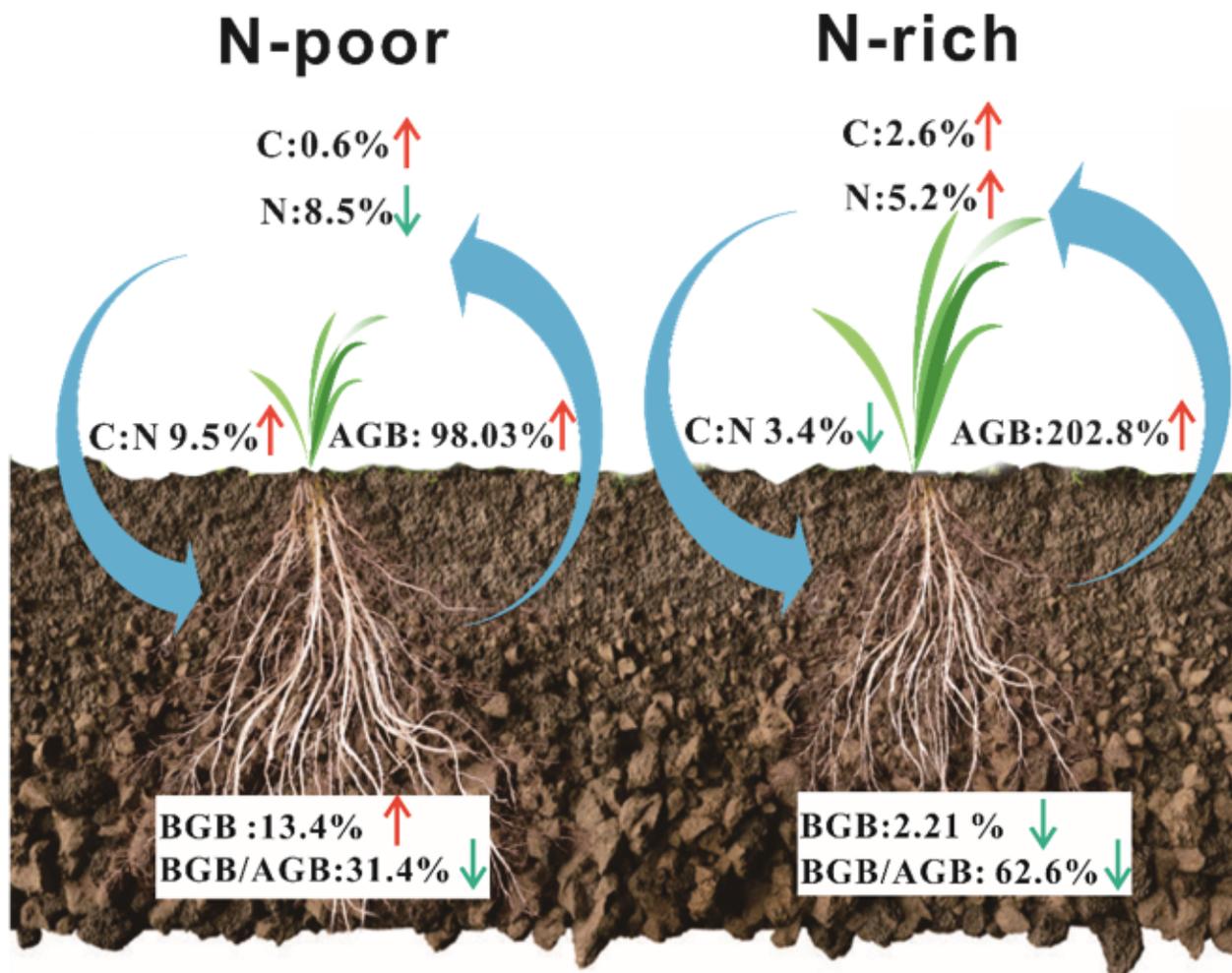


Figure 7

The tradeoff of biomass and stoichiometry between the above and belowground plant under elevated CO₂ and N fertilization. CO₂, elevated CO₂; N, nitrogen addition; AGB, aboveground biomass; BGB, belowground biomass.