



Responses of Herbaceous Species of Alpine Treeline to Elevated CO₂ 18

Sudeep Chandra, Vaishali Chandola, Ankit Singh, C. P. Singh, and M. C. Nautiyal

Abstract

Adaptation of plant species to cold environment and short growing season in the Himalayan high altitudes could render them highly sensitive to future climate change. In this study, we analyzed the effect of elevated CO₂ concentration on the growth, productivity, physiology, and various biochemical parameters of four alpine treeline herbaceous species, viz. *Acomastylis elata*, *Anaphalis nepalensis*, *Bistorta macrophylla*, and *Trillium govanianum*. We planted seedlings of the selected plant species in open top chambers and CO₂ concentration was raised from ambient (400 ppm) to elevated (650 ppm) levels. Elevated CO₂ stimulated net assimilation rate (34–38%), growth and productivity of *A. elata* and *A. nepalensis*, whereas *B. macrophylla* and *T. govanianum* showed decrease (18%) in photosynthesis. The sugar content in all the species increased (36–78%); however, foliar N decreased (17–37%), possibly due to dilution effect of high carbohydrate content. Reduced tissue N can probably affect the activity of key photosynthetic enzyme Rubisco and therefore, decreased carbon assimilation. From this study, it can be deduced that long-term effects of elevated CO₂ can be species specific and might be affected by availability of nutrients. More such studies in Himalayan regions involving different plant communities are needed to develop a better understanding of plant responses to climate change.

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439

KeywordsAlpine treeline · Biomass · Elevated CO₂ · Herbaceous species · Photosynthesis**18.1 Introduction**

The carbon dioxide (CO₂) concentration in the earth's atmosphere has varied over the geological time scale. CO₂ concentrations as low as 180–200 ppm had been known to exist during the last two ice ages around 13,000–30,000 and 140,000–160,000 years ago. Direct atmospheric sampling data have shown that CO₂ increased from 315 ppm in the late 1950s to 355 ppm in the early 1990s (Keeling et al. 1989) and has been rising continuously since then. The rise in atmospheric CO₂ has become more profound since the beginning of the industrial revolution and is expected to continue in the coming years with drastic effects on the earth's climate. The rise in atmospheric carbon dioxide will not only affect climatic events but is likely to have significant implications on the global carbon cycle and plants life too. Plants of the alpine regions are specifically more sensitive toward changes in the climatic factors chiefly temperature.

The upper limit of a closed forest is called as timberline, whereas a treeline is the upper limit of the “tree” life form showing an upright growth. The transitional zone between the timberline and treeline is called as the alpine treeline ecotone (Körner 2012). The trees that generally form treeline are *Abies spectabilis*, *Sorbus foliolosa*, *Betula utilis*, etc. Many herbaceous species are also found growing below canopies, along, and above treelines. Herbs are one of the major flowering plant life-forms in these regions. It has been estimated that approximately 2.6% land area besides Antarctica, covering the alpine belt, inhabits about 4% of all the flowering plant species on Earth. In other words, the alpine regions are quite rich in plant diversity than the lower elevation ecosystems (Körner 2018).

A number of herbaceous species can be restricted to the treeline or below it due to a more hospitable habitat than the open alpine meadows which are directly exposed to abiotic extremities, viz heavy snowfall, frost, and solar radiation. This higher distribution of herbaceous species is mainly based on their morphological adaptations, like small stature and dense structure, which help them restrict aerodynamic exchange with the atmosphere. This causes heat accumulation from solar radiation providing warm temperatures to operate unlike trees, which are upright and more ventilated. Alpine herbaceous plants produce short lived leaves and the sunlight heated soil provides a thermally buffered environment to their meristems which are positioned close to the ground in comparison to trees which form longer living and slowly maturing leaves along with meristems positioned above from the ground where they are fully exposed to cold temperatures (Körner 2018).

Increasing CO₂ concentration can cause positive effect on plant growth with an increase in leaf area (Allen Jr 1991). Plants that are exposed to higher levels of CO₂ usually have amplified growth, water use efficiency, and photosynthesis rate (Amthor 1995; Wittwer 1995). Being a very important raw material for the process

of photosynthesis, an increase in atmospheric CO₂ concentration can have direct effect on plant photosynthesis. However, plants growing in alpine region and glacier fore-fields in the Swiss Alps were found to be carbon saturated at ambient CO₂ concentrations of 390 ppm (Körner 2018). It was further reported that doubling the concentration of atmospheric CO₂ for over four successive seasons had no effect on net primary productivity of alpine plants with exception of some species. Hence, in long run, a possibility of slow but certain shifts in species composition is expected with some species getting suppressed and some gaining ground (Körner 2018).

At higher altitudes, the impact of elevated CO₂ on plants can vary due to low ambient partial pressure of CO₂, short growing season, and a number of abiotic stresses mainly extreme low-temperature regimes. CO₂ exposure duration can also cause varying responses in plants as initial exposure can enhance the net assimilation rate and biomass (Ainsworth et al. 2008; Chaturvedi et al. 2009; Chaturvedi et al. 2013). With increasing duration of CO₂ exposure plant can show photosynthetic acclimation, lowering of leaf chlorophyll and nitrogen followed by declined (Zelikova et al. 2014) or no significant variation in biomass (Schäppi and Körner 1997; Ward et al. 1999).

Initial studies on the effect of warming have shown that the Himalayas have a higher warming, more prominent at higher altitudes, as compared to the global average rate (Shrestha et al. 1999; Liu and Chen 2000) with higher increases in the winter and autumn temperatures than the summer. There have been only a few studies regarding the impact of CO₂ enrichment on the Himalayan alpine species (Joshi et al. 2007; Chaturvedi et al. 2009; Chaturvedi et al. 2013) and one long-term study on alpine grassland biomass and community structure (Zhu et al. 2020). However, a considerate amount of work and long-term studies on warming and CO₂ enrichment effect on the European Alps treeline, community structure, and species have been carried out (Schäppi and Körner 1996; Schäppi and Körner 1997; Inauen et al. 2012; Dawes et al. 2013). The treeline in the Himalayan highlands ranges from 3200 m to 4900 mamsl (Singh et al. 2019) bearing the highest peaks of the world, whereas the treeline in the Alps ranges from 1750 m to slightly above 2350 mamsl from north to south (Paulsen and Körner 2001). Therefore, the responses of treeline species and community structure to climate change particularly relating to species migration and distribution patterns at the Himalayas might differentiate from the other alpine regions.

Since the alpine regions are dominated by herbaceous species which are also a key component of biodiversity, this study aims to estimate the effect of elevated CO₂ on growth-productivity, physiology, and biochemistry of alpine-treeline herbs. Due to low temperature and partial pressure of gases in the alpine regions, the effect of CO₂ on plant species of these regions can vary from species of lower elevations, however, the photosynthesis rate, pigment content, and biomass of the herbs were expected to increase as a result of CO₂ fertilization effect as found in some earlier studies. Since in the earlier studies these responses have been found varying in different species; therefore, four species were subjected to CO₂ treatment in this study.

In order to properly understand the treeline dynamics with reference to anthropogenic warming of the planet, it is essential to carry out more reliable assessments of forest treelines across the globe to preserve the ecological state of alpine ecotone biodiversity and to forge forest conservation policies (Holtmeier and Broll 2010; Mishra and Mainali 2017).

18.2 Materials and Methods

18.2.1 Study Site

This study was conducted at alpine field station of High Altitude Plant Physiology Research Center at Tungnath, (3400 m amsl, 30°14'N and 79°13'E) in the Western-Central Himalaya, India. The study site is a part of Kedarnath Wild Life Sanctuary and exhibits a number of endemic species. The area usually remains snow covered from December to May and the growing period for herbaceous species is between May to October. The study area generally receives an annual rainfall ranging from of 300 to 500 cm with the month of July and August being the wettest. Daily mean temperatures from June to September fluctuate between 8 and 18 °C.

18.2.2 Growth Conditions

Two open top chambers (OTCs) were established at Tungnath. The OTCs were round in structure having 4 × 4 m (height × diameter) dimension. Due to high wind velocity at the study site, OTCs were preferred over free air CO₂ enrichment (FACE) experiment. The frame of the OTCs was made of galvanized iron pipes. The OTCs were covered with polycarbonate sheet which provided 90% transmittance of light. The OTCs were equipped with programmable logic controller coupled with supervisory control and data acquisition (SCADA) system which automatically recorded air temperature, relative humidity, and CO₂ concentration. The CO₂ concentration of one OTC was raised and maintained to 650 ppm during the growing period (June to mid-October). This performed automatically via a solenoid valve controlled by SCADA system.

18.2.3 Plant Materials

Morphologically similar seedlings of *Acomastylis elata* (Wall. ex G. Don) F. Bolle (Syn: *Geum elatum*, family: Rosaceae), *Anaphalis nepalensis* (Spreng.) Hand.-Mazz (Asteraceae), *Bistorta macrophylla* (D. Don) Soják (Polygonaceae), and *Trillium govianum* Wall. ex. D. Don., (Melanthiaceae) were collected from surrounding areas and planted in triplicates (each having 10 seedlings) in each OTC. These species are naturally distributed from treeline to upper alpine regions, while *T. govianum* grows in subalpine regions. Seedlings of all the 4 species were

transplanted to OTCs and left for a month to acclimatize, after which the treatment was given. The OTCs were established at native site of these species, the soil inside the OTCs was not modified, and no fertilizers were added. Transplantation was done in the month of July in the first year (2019) and therefore, the treatment in the first season was given only for 30 ± 2 days. In the second year (2020), the treatment lasted from June to September. The seedlings growing under elevated CO₂ chamber were compared with seedlings growing at ambient CO₂ concentration in an OTC.

18.2.4 Gas Exchange Measurement

Leaf gas exchange parameters were recorded on leaves of nine randomly selected individuals (3 from each replicate) of each species from each chamber using Licor, Li-6400, a portable infrared gas analyzer (IRGA). The instrument was equipped with red blue LED light source and CO₂ mixer (for maintaining desired CO₂ concentration). The photosynthetic rate (Pn, $\mu\text{mol CO}_2/\text{m}^2/\text{s}$), stomatal conductance (gs, $\text{mol H}_2\text{O}/\text{m}^2/\text{s}$), transpiration rate (E, $\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$), and intracellular CO₂ (Ci, $\mu\text{mol CO}_2/\text{mol air}$) were recorded between 9:00 and 11:00 h. The study was conducted under saturated light conditions (photosynthetic photon flux density—1250 $\mu\text{mol}/\text{m}^2\text{-s}$). The water use efficiency ($\text{WUE} = \text{Pn}/\text{E}$) and intrinsic water use efficiency ($\text{iWUE} = \text{Pn}/\text{gs}$) were calculated.

18.2.5 Pigment, Carbohydrate, and Nitrogen Estimation

Leaf samples of all the four species were collected (5 samples for each species) from both ambient (aCO₂) and elevated (eCO₂) chambers. Samples were further analyzed through UV-VIS spectrophotometer for chlorophyll a, b and total carotenoids following Holm et al. (1965) and total soluble sugars following (McCready et al., 1950). Total N was estimated from oven-dried leaves using Kjeldhal Nitrogen analyzer (Pelican Instruments).

18.2.6 Growth and Biomass

Plant height, leaf area, aboveground, and belowground biomass were recorded on five randomly selected individuals from each species grown under aCO₂ and eCO₂ chambers.

18.2.7 Data Analysis

All the growth parameters of plants from aCO₂ and eCO₂ were recorded after 120 days of CO₂ treatment. One way analysis of variance was performed to assess

the effect of elevated CO_2 and further significant difference between ambient and elevated CO_2 was tested using paired sample t-test for each parameter.

18.3 Results

The daytime air temperature under eCO_2 increased by 0.63°C and the temperature difference between aCO_2 and eCO_2 was greater in May and June. The total precipitation (Measured by tipping bucket rain gauge) during our study was 2130 mm and 60 to 80% of total precipitation took place between July and August (Fig. 18.1).

CO_2 enrichment experiment showed significant changes in physiology, growth, and biochemistry of herbaceous species; however, the effects are species specific.

18.3.1 Effect on Gaseous Exchange

CO_2 exposure strongly affected the physiological process of photosynthesis (P_n). P_n under eCO_2 significantly ($p = 0.05$) increased by 38 and 34% in *A. nepalensis* and *A. elata*, respectively; whereas it decreased (18%, $p < 0.05$) in *T. govianum* and remained unaffected in *B. macrophylla*. The stomatal conductance (g_s) under eCO_2 increased significantly in *A. elata* (+59%, $p < 0.01$); other species showed non-significant reduction in g_s . CO_2 is considered as an ideal antitranspirant and in our observation we found reduced transpiration rate (E) under eCO_2 for

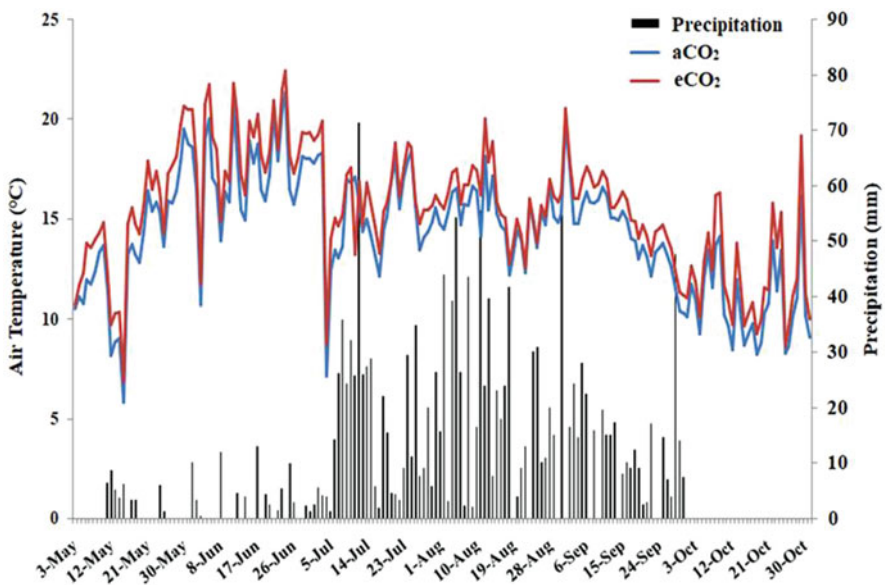


Fig. 18.1 Daily daytime air temperature in ambient and elevated open top chambers and the precipitation received by study area

Table 18.1 Gas exchange parameters of four herbaceous species grown under ambient and elevated CO₂

		<i>Acomastylis elata</i>	<i>Anaphalis nepalensis</i>	<i>Bistrotia macrophylla</i>	<i>Trillium govanianum</i>
Photosynthesis rate	aCO ₂	43.55 ± 7.45	15.10 ± 1.75	43.83 ± 10.99	18.4 ± 1.64
	eCO ₂	58.36 ± 5.73	20.90 ± 3.65	40.23 ± 24.40	15.6 ± 1.04
<i>p</i>		0.046*	0.045*	0.83	0.037*
Stomatal conductance	aCO ₂	1.42 ± 0.28	0.84 ± 0.17	1.64 ± 0.33	0.17 ± 0.02
	eCO ₂	2.27 ± 0.29	0.68 ± 0.11	1.19 ± 0.35	0.13 ± 0.01
<i>p</i>		0.008**	0.11	0.18	0.06
Transpiration rate	aCO ₂	5.47 ± 0.85	5.91 ± 1.31	9.18 ± 1.09	2.73 ± 0.21
	eCO ₂	7.45 ± 1.09	3.82 ± 1.00	7.68 ± 0.37	1.71 ± 0.14
<i>p</i>		0.002**	0.04*	0.087	0.001**
WUE	aCO ₂	9.95 ± 3.28	2.55 ± 0.65	4.86 ± 1.62	6.74 ± 0.62
	eCO ₂	7.75 ± 1.22	5.47 ± 1.25	5.24 ± 2.86	9.13 ± 0.83
<i>p</i>		0.338	0.008**	0.86	0.016*
iWUE	aCO ₂	29.54 ± 5.26	17.97 ± 3.79	28.45 ± 8.51	111.79 ± 19.37
	eCO ₂	25.86 ± 3.63	35.89 ± 7.54	32.15 ± 10.76	124.13 ± 12.34
<i>p</i>		0.382	0.02*	0.74	0.41

The significant difference is shown by asterisk, **p* < 0.05, ***p* < 0.01

WUE: Water use efficiency; iWUE: intrinsic Water use efficiency

A. nepalensis (−54%, *p* < 0.05), *B. macrophylla* (−54%, 0.08), and *T. govanianum* (−59%, <0.01); however, *A. elata* showed an increased *E* (+36%, <0.01). Elevated CO₂ improved the water use efficiency (WUE) in *A. nepalensis* (+2-folds) and *T. govanianum* (+35%), whereas WUE of *A. elata* and *B. macrophylla* was unaffected. The intrinsic WUE (iWUE) significantly improved by twofolds only in *A. nepalensis* (Table 18.1).

18.3.2 Effect on Pigments, Carbohydrates, and Nitrogen Content

CO₂ enrichment affected the pigment contents and the effect varied from species to species. The chl a and b (chlorophyll a and b) significantly (*p* < 0.05) increased in *A. elata* (+19%) and *A. nepalensis* (+21%) under eCO₂. Other species, i.e., *T. govanianum* and *B. macrophylla*, showed reduced chl a content under eCO₂; however, the chl b was unaffected. The total carotenoids showed most prominent response toward eCO₂. All species growing at eCO₂ chamber showed increased carotenoids (+23 to 46%). The TSS under eCO₂ increased significantly (*p* < 0.05) for *A. elata* (+46%), *A. nepalensis* (+36%), *T. govanianum* (+78%), and non-significantly for *B. macrophylla* (15%). The plant N in eCO₂ significantly

Table 18.2 Pigment contents, total soluble sugars and leaf nitrogen in four herbaceous species from ambient (aCO₂) and elevated (eCO₂) CO₂

		<i>Acomastylis elata</i>	<i>Anaphalis nepalensis</i>	<i>Bistrotta macrophylla</i>	<i>Trillium govanianum</i>
Chl a	aCO ₂	2.96 ± 0.17	1.33 ± 0.06	2.38 ± 0.37	4.37 ± 0.78
	eCO ₂	3.54 ± 0.15	1.62 ± 0.14	2.09 ± 0.20	3.01 ± 0.08
<i>p</i>		0.011*	0.031*	0.259	0.04*
Chl b	aCO ₂	2.17 ± 0.12	0.73 ± 0.08	1.47 ± 0.31	1.87 ± 0.12
	eCO ₂	2.54 ± 0.08	0.93 ± 0.06	1.42 ± 0.18	1.93 ± 0.16
<i>p</i>		0.01*	0.025*	0.827	0.628
Carotenoids	aCO ₂	1.11 ± 0.12	0.63 ± 0.05	0.98 ± 0.07	0.99 ± 0.2
	eCO ₂	1.45 ± 0.12	0.92 ± 0.03	1.29 ± 0.06	1.22 ± 0.18
<i>p</i>		0.026*	0.001**	0.004**	0.22
TSS	aCO ₂	26.1 ± 2.26	31.61 ± 3.48	22.57 ± 3.04	19.7 ± 4.43
	eCO ₂	38.17 ± 4	43.1 ± 2.82	26.02 ± 1.81	35.22 ± 5.46
<i>p</i>		0.01*	0.011*	0.167	0.019*
N content	aCO ₂	1.08 ± 0.05	1.11 ± 0.09	0.99 ± 0.12	1.10 ± 0.11
	eCO ₂	0.92 ± 0.06	0.9 ± 0.08	0.94 ± 0.06	0.80 ± 0.08
<i>p</i>		0.028*	0.038*	0.536	0.019*

Significant difference is shown by asterisk, **p* < 0.05, ***p* < 0.01

reduced for *A. elata* (−17%), *A. nepalensis* (−23%), and *T. govanianum* (−37%), although the effect of eCO₂ on N reduction of *B. macrophylla* was non-significant (Table 18.2).

18.3.3 Effect on Growth and Biomass

Under eCO₂, the plant height and leaf area of *A. nepalensis* and *A. elata* significantly increased; however, the species *B. macrophylla* and *T. govanianum* did not show any significant change in morphometrics. Height of *A. nepalensis* and *A. elata* increased by 55 and 24%, respectively under eCO₂ (Fig. 18.2). Similarly, leaf area in these two species also increased by 97 and 32%, respectively (Fig. 18.3). The accumulation of dry matter in shoot and root system was measured and in comparison to aCO₂, the aboveground biomass in eCO₂ increased significantly by 50 and 116% in *A. elata* and *A. nepalensis*, respectively. The below ground biomass in these two species also increased, although the increment was significant only in *A. nepalensis* (+55%), while the other two species did not show any significant change in above and belowground biomass (Fig. 18.4).

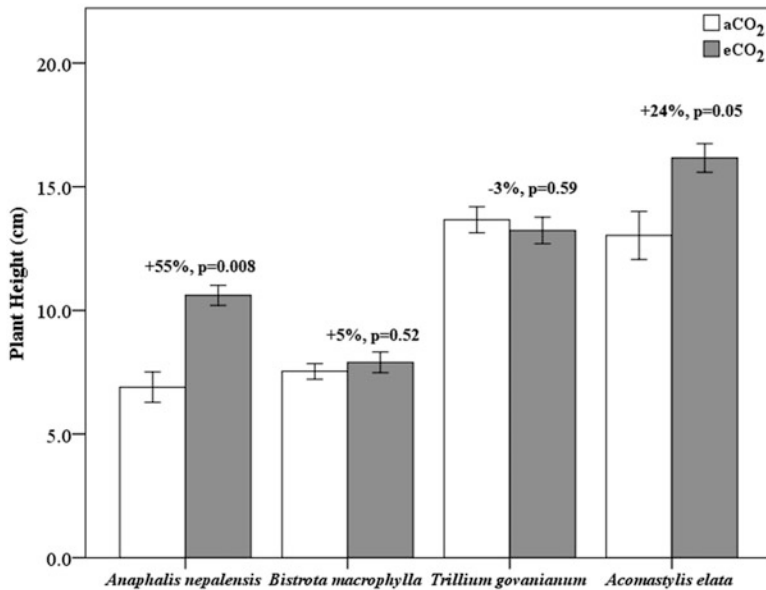


Fig. 18.2 Plant height of four herbaceous species under ambient (aCO₂) and elevated (eCO₂) CO₂. The significant difference was tested by paired sample *t*-test

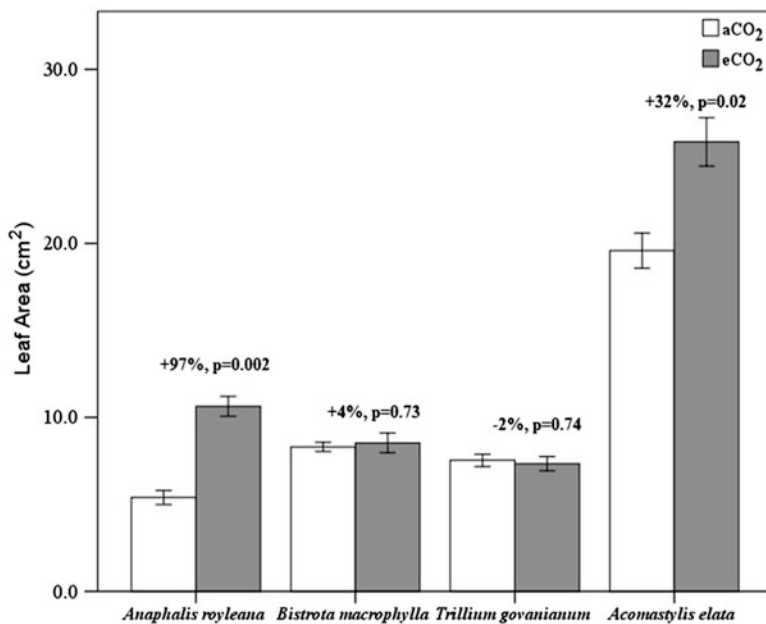


Fig. 18.3 Leaf area (cm²) of four herbaceous species under ambient (aCO₂) and elevated (eCO₂) CO₂. The significant difference was tested by paired sample *t*-test

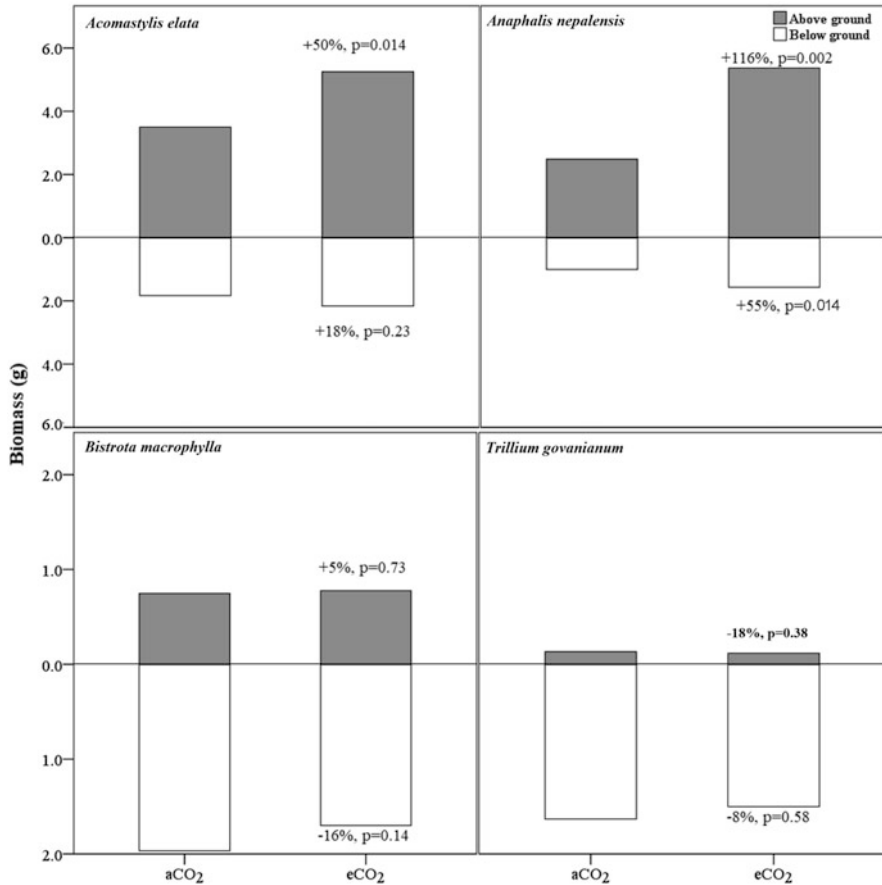


Fig. 18.4 Above and belowground biomass of four herbaceous species in ambient and elevated CO₂ chambers. The percentage increased or decreased was calculated and tested using paired sample *t*-test

18.4 Discussion

This study brings out the short-term exposure effects of CO₂ enrichment on four alpine treeline ecotone herbaceous species having different leaf structures and morphology.

Elevated CO₂ led to an increase in the daytime air temperature inside the chamber as compared to ambient CO₂ levels. This can be attributed to the heat absorbing and radiating nature of CO₂ and its activity as a greenhouse gas. Elevated CO₂ led to an increase in the photosynthesis rate in two species namely *A. elata* and *A. nepalensis*. Similar results were obtained in a number of other studies (Ainsworth and Long

2005; van der Kooi et al. 2016) where elevated CO₂ caused a rise in the photosynthetic rate with subsequent rise in growth and biomass. A higher CO₂ concentration has a fertilizing effect especially on C3 plants because Rubisco in C3 plants is not CO₂ saturated at its current atmospheric levels (Yu et al. 2012; Singh and Reddy 2016). Elevated CO₂ also reduces the instance of photorespiration, thereby having positive effects on the carboxylase activity of Rubisco (Zheng et al. 2019). However, the other two species, viz. *T. govianum* and *B. macrophylla* either had negative or no significant effect on their photosynthetic activity as a result of elevated CO₂. This could be possibly because of differences in their carbon saturation threshold and the ability to adapt to a carbon richer environment. The down-regulation photosynthetic activity could also be due to lower concentration and activity of Rubisco. Kanemoto et al. (2009) also reported a decline in the photosynthetic activity of soybean plants under elevated CO₂. In this study, a decline in the plant nitrogen (N) content was also seen. N is an important element of tissue protein and amino acid, and under elevated CO₂ a decline in tissue N may affect the concentration of Rubisco.

The stomatal conductance in three out of the four studied species was found to be declined. Increased levels of atmospheric CO₂ cause smaller stomatal apertures, thereby decreasing leaf conductance for water vapor (Morison 1987). A decrease in stomatal conductance of these species also led to a decline in the transpiration rate. Stomatal conductance of *A. elata* increased along with an increase in its transpiration rate. Changes in stomatal conductance and transpiration rate are also greatly influenced by the species type (Ward et al. 2013; Haworth et al. 2013). Stomatal conductance and transpiration rates are also influenced by changes in stomatal density, which have been found to be either decreased or increased in different species under elevated CO₂ (Xu et al. 2016). The WUE was significantly increased in *T. govianum* and *A. nepalensis* as a result of decline in the transpiration rate. In the other two species, viz. *A. elata* and *B. macrophylla* WUE remained unaffected. A significant increase in the pigment content, i.e., chlorophyll in *A. nepalensis* and *A. elata*, can be correlated with significantly higher Pn in both the species. Pigments trap light energy which is utilized to convert trapped carbon to carbohydrates by the process of photosynthesis. An increase in the carotenoid content of all the species has been found under elevated CO₂. Carotenoids moderate the effect of increased temperature by protecting the plants from photo-oxidative stress (Strzalka et al. 2003). Carotenoid content has shown variable responses under elevated CO₂ in a number of studies. Some studies have reported increase, whereas some had reported a decline in the carotenoid content with respect to elevated CO₂ (Loladze et al. 2019).

TSS increased under elevated CO₂ in all the studied species. Higher substrate availability can be the most probable reason for increase in TSS in comparison to ambient conditions. Higher Pn and TSS content leads to accumulation of carbohydrates stored as starch causing an increase in the biomass of the plants. The biomass accumulation was very significant in *A. nepalensis* and *A. elata* and so were the Pn rates and TSS content indicating the competence of these two species to utilize increased carbon more efficiently than the other two species. However, initial responses showed enhanced photosynthetic capacity and increase in biomass, but

this might tend to saturate after a certain time period; since, with the continuously increasing or higher CO₂ levels, some or most plants initially grow rigorously, and later soil microbes enhance the immobilization of limiting nutrients thus hampering further growth (Shaw et al. 2002). A decline in the tissue nitrogen was observed in all the species under elevated CO₂. Previous studies have found that long-term elevated CO₂ leads to down-regulation of photosynthetic activity, termed as photosynthetic acclimation. This acclimation causes less uptake of nitrogen and thus a reduction in tissue nitrogen (Temperton et al. 2003; Leakey et al. 2006; Zheng et al. 2019), which then limits photosynthetic capacity (Ewa Jach and Ceulemans 1999).

An increase in the leaf area under CO₂ enrichment has been reported in a number of studies (Centritto et al. 1999; Pritchard et al. 1999; Masle 2000; Usuda 2006). Leaves are the point of interaction for carbon transfer and capture. Elevated CO₂ can bring changes in the internal structure of leaves (Pritchard et al. 1999). Stomatal density has been found to either decrease or increase with elevated CO₂ depending on the species. An additional variation is alteration in epicuticular wax on elevated CO₂ grown leaves (Thomas and Harvey 1983; Prior et al. 1997). Plant height increased in *A. nepalensis* and *A. elata*, whereas there was no significant change in the height of *T. govanianum* and *B. macrophylla*. The stems of *A. nepalensis* and *A. elata* were slightly branched having internodes, whereas *T. govanianum* and *B. macrophylla* had erect unbranched stems. The leaves of *T. govanianum* and *B. macrophylla* were also thinner in comparison to the thick and hairy leaves of *A. nepalensis* and *A. elata*. Elevated CO₂ can cause the stimulation of cell division at the shoot apical meristem (Pritchard et al. 1999) by reducing the time period between consecutive cell divisions as demonstrated by Masle (2000). A number of studies have shown an increase in leaf area and stem length or branch elongation in plants exposed to CO₂ enrichment without any change in the number of nodes (Downton et al. 1990; Pritchard et al. 1999). But these results have also varied among species as can be seen in this study.

18.5 Conclusion

From the results of the present and previous studies, a general assumption that can be drawn is that the response of any plant species of any ecosystem or form depends on a number of factors, viz its morphology, habitat and community structure, microbial and other associations, nutrient and water availability, carbon saturation threshold, limit to withstand environmental extremes, etc. Under elevated CO₂ the photosynthetic yield increases due to availability of carbon substrate but later due to impaired phloem loading capacity, source—sink imbalance can be observed in the long term. Changes in microbial activity under elevated CO₂ might also affect soil nutrients consequently affecting their availability and uptake. Overall the effect of CO₂ enrichment in high altitude are majorly species specific and an increased growth and productivity might be beneficial for establishment of an individual species but can adversely affect community structure and distribution of other species. Therefore, studies especially in the Himalayan alpine regions on effect of CO₂ enrichment

and warming on community structure, species response, and distribution are required on a large scale to make precise predictions.

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