Elevated CO2 increases shoot growth but not root growth and C:N:P stoichiometry of Suaeda aralocaspica plants [Postprint]

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Abstract

The purpose of the current study was to investigate the eco-physiological responses, in terms of growth and C:N:P stoichiometry of plants cultured from dimorphic seeds of a single-cell C4 annual Suaeda aralocaspica (Bunge) Freitag and Schütze under elevated CO2. A climatic chamber experiment was conducted to examine the effects of ambient (720 g/L) and CO2-enriched (1440 g/L) treatments on these responses in S. aralocaspica at vegetative and reproductive stages in 2012. Result showed that elevated CO2 significantly increased shoot dry weight, but decreased N:P ratio at both growth stages. Plants grown from dimorphic seeds did not exhibit significant differences in growth and C:N:P stoichiometric characteristics. The transition from vegetation to reproductive stage significantly increased shoot:root ratio, N and P contents, but decreased C:N, C:P and N:P ratios, and did not affect shoot dry weight. Moreover, our results indicate that the changes in N:P and C:N ratios between ambient and elevated CO2 are mainly caused by the decrease of N content under elevated CO2. These results provide an insight into nutritional metabolism of single-cell C4 plants under climate change.

Full Text

Elevated CO_2 Increases Shoot Growth but Not Root Growth and C:N:P Stoichiometry of Suaeda aralocaspica Plants

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Abstract: The purpose of this study was to investigate the eco-physiological responses in terms of growth and C:N:P stoichiometry of plants cultured from dimorphic seeds of the single-cell C_4 annual *Suaeda aralocaspica* (Bunge) Freitag and Schütze under elevated CO_2. A climatic chamber experiment was conducted to examine the effects of ambient (720 g/L) and CO_2-enriched (1440 g/L) treatments on these responses in *S. aralocaspica* at vegetative and reproductive stages in 2012. Results showed that elevated CO_2 significantly increased shoot dry weight but decreased the N:P ratio at both growth stages. Plants grown from dimorphic seeds did not exhibit significant differences in growth and C:N:P stoichiometric characteristics. The transition from vegetative to reproductive stage significantly increased the shoot:root ratio and N and P contents, but decreased C:N, C:P, and N:P ratios, while not affecting shoot dry weight. Moreover, our results indicate that the changes in N:P and C:N ratios between ambient and elevated CO_2 are mainly caused by the decrease in N content under elevated CO_2. These findings provide insight into the nutritional metabolism of single-cell C_4 plants under climate change.

Keywords: biomass; CO_2 elevation; C:N:P stoichiometry; seed heteromorphism; *Suaeda aralocaspica*

1 Introduction

Since the First Industrial Revolution, atmospheric CO_2 has steadily increased, with the current concentration above 720 g/L and projected to reach 1440 g/L by the year 2100 (IPCC, 2013; Betts et al., 2016). Such elevation in atmospheric CO_2 induces several alterations in plant metabolism. The growth and ecological responses of terrestrial plants to elevated CO_2 have been determined for three main photosynthetic types: C_3, C_4, and crassulacean acid metabolism (CAM) (Ceusters and Borland, 2011; Reich et al., 2018; Quirk et al., 2019). However, responses differ among plant functional types. In many C_3 and CAM plants, growth is stimulated by elevated CO_2 (Ceusters and Borland, 2011; Boretti and Florentine, 2019; Li et al., 2020), whereas C_4 plants show little or no increase in productivity (Boretti and Florentine, 2019). To date, no information is available regarding responses of C_4 species without Kranz anatomy to atmospheric CO_2 elevation.

Some plants produce heteromorphic seeds that differ distinctly in morphology, color, dormancy, and germination traits (Imbert, 2002; Zhao et al., 2018). Plants cultured from heteromorphic seeds can also differ in their growth responses under various conditions, including biotic and abiotic factors (Redondo-Gómez et al., 2008; Jiang et al., 2020). However, the effects of elevated CO_2 on growth, ecological, and physiochemical characteristics remain unknown for seed-heteromorphic plants.

Carbon (C), nitrogen (N), and phosphorus (P) contents and C:N:P ratios are associated with plant adaptation strategies to environmental changes (Du et al., 2019; Li et al., 2021). These elements provide the structural basis of plants and play essential roles in photosynthesis and growth (Marschner, 2013). Although these elements are maintained at relatively stable cellular concentrations in plants, C:N:P stoichiometry can be greatly affected under different environmental conditions (Deng et al., 2015). Numerous simulation experiments have examined the response of elevated CO_2 on plant C:N:P stoichiometry (Pérez-Romero et al., 2018; Du et al., 2019; Uchytilová et al., 2019). However, the effects remain inconsistent. For example, an average 22% increase in foliar C:N ratio was observed in C_3 grasses and forbs under high CO_2 concentration, but no significant effects were observed in C_4 grasses (Sardans et al., 2012).

Suaeda aralocaspica (Bunge) Freitag and Schütze inhabits saline and semi-arid deserts in Central Asia (Commissione Redactorum Florae Xinjiangensis, 1994). This plant lacks the dual-cell (Kranz) system but possesses unusual mechanisms of C_4 photosynthesis through dimorphic chloroplasts in individual chlorenchyma cells via intracellular compartmentation (Voznesenskaya et al., 2001). In *S. aralocaspica*, photosynthetic chloroplasts are assembled at the proximal end of the flower and leaves, while other chloroplasts are positioned and partitioned on the fringe of the cells at their distal end. In C_4 cycles, atmospheric CO_2 is fixed via phosphoenolpyruvate carboxylase (PEPC) at the distal end of the cell, which produces C_4 acids and donates CO_2 to rubisco in chloroplasts at the proximal end of the cell (Boyd et al., 2007).

We conducted a chamber experiment to examine the growth and C:N:P stoichiometry of plants grown from dimorphic seeds of this single-cell C_4 species in response to elevated CO_2. We hypothesized that plants from dimorphic seeds might have differential eco-physiological responses to CO_2 elevation at different growth stages.

2.1 Seed Collection and Experimental Design

Freshly matured *S. aralocaspica* fruits were harvested from the southern edge of the Junggar Basin in Xinjiang Uygur Autonomous Region, China, in October 2011. Fruits were naturally dried for two weeks at ambient room conditions, after which dry fruits were rubbed by hand and both brown and black seeds were separated and stored in plastic bags at room temperature (20°C–25°C).

A pot experiment was conducted in two growth chambers (day/night air temperatures: 25°C/10°C for the first three months and then 30°C/15°C) at Fukang National Field Scientific Observation and Research Station for Desert Ecosystems, Chinese Academy of Sciences (44°17 N, 87°56 E; 460 m a.s.l.) in Xinjiang, China. The average CO_2 concentration in the growth chambers was either 720 or 1440 g/L, with levels continuously controlled by an automatic CO_2 supply system (ESHENGTAIHE CTRL TECH, YB6, Beijing, China).

Twenty seeds of each type (brown and black) were sown into plastic pots (17 cm deep and 16 cm in diameter) filled with 2 L of a quartz sand and vermiculite mixture (3:1 v/v) at a soil depth of 2 mm. Two weeks later, one seedling was retained per pot, and seedlings with similar height and growth were selected for further experimentation. Each pot received 6 g of Osmocote 301 (Scotts, Marysville, OH, USA) prior to sowing and 100 mL of Peters 1 nutrient solution once per week. Salt stress was imposed at four weeks after sowing using a mixed salt solution of $NaCl:Na_2SO_4:NaHCO_3$ (20:20:1). Each pot received 100 mL of salt solution (1 g/L) once per week (Wang et al., 2012). Six plants grown from each seed type were harvested for the first time under each CO_2 level on 15 July 2012, with the second harvest taking place on 15 September 2012.

2.2.1 Plant Biomass

After harvesting, shoot and root parts were separated and dried in an oven at 80°C for 48 h to reach constant weight. Shoot and root biomasses were measured using an electronic analytical balance.

2.2.2 C, N and P Content

Before chemical analysis, dried shoot materials were ground in a ball mill and passed through a 0.25-mm sieve. The ball mill was cleaned completely after each sample milling. Shoot C content was determined using titration after digestion in $K_2Cr_2O_7$-H_2SO_4 (Bao, 2000). N content was determined using the Kjeldahl method after digestion in H_2SO_4-$HClO_4$ (Lin et al., 2011). P content was determined by colorimetric analysis (formation of chloro-phosphoric molybdate) after digestion in H_2SO_4-$HClO_4$ (Ren et al., 2018).

2.3 Statistical Analyses

All data are expressed as mean ± standard error and met the assumptions of normality and homoscedasticity. Split-plot analysis of variance (ANOVA) was used to analyze the effects of CO_2 level and seed type on shoot and root dry weights, shoot C, N and P contents, and their ratios in *S. aralocaspica* plants, with CO_2 level (720 or 1440 g/L) as the main plot and seed type (brown and black seeds) as the subplot at vegetative or reproductive stage. All data analyses were performed using SPSS v13.0 (SPSS Inc., Chicago, IL, USA).

3 Results

Elevation of CO_2 significantly increased shoot dry weight (SDW) at both vegetative and reproductive stages but did not affect root dry weight (RDW) or shoot:root ratio. Plants grown from dimorphic seeds showed similar SDW, RDW, and shoot:root ratio at the same growth stage under the same CO_2 condition [Figure 1: see original paper]; Table S1). RDW decreased while shoot:root ratio increased during the transition from vegetative to reproductive stage [Figure 1: see original paper].

Elevation of CO_2 significantly decreased N content for plants grown from black seeds at the vegetative stage but did not affect other stoichiometric characteristics. Plants cultured from dimorphic seeds had identical C, N, and P contents for both CO_2 treatments at the same growth stage [Figure 2: see original paper]; Table S1). N and P contents in shoots significantly increased from vegetative to reproductive stage, while C content significantly decreased during this transition [Figure 2: see original paper]. The range of C contents was between 309.4 and 330.4 mg/g. Plants from black seeds at elevated CO_2 showed the highest N (22.8 mg/g) and P content (3.7 mg/g) at reproductive stage [FIGURE:2d and f).

Plants grown from dimorphic seeds had the same C:N, N:P, or C:P ratio at the same growth stage under the same CO_2 condition [Figure 3: see original paper]). Compared with the vegetative stage, plants at reproductive stage had lower C:N, C:P, and N:P ratios (Table S1). Plants grown from black seeds at elevated CO_2 showed the lowest C:N ratio (14.3) and C:P ratio (89.7) at reproductive stage [FIGURE:3b and d). The N:P ratio significantly decreased under elevated CO_2 in both plant types at the vegetative stage [FIGURE:3e).

4 Discussion

Although the growth and C:N:P stoichiometry of plants with different photosynthetic pathways subjected to elevated CO_2 have been studied extensively (Sardans et al., 2012; Deng et al., 2015; Du et al., 2019), our data represent the first documentation of CO_2 elevation responses for single-cell C_4 plants. In addition, our results indicate that elevated CO_2 significantly increased shoot growth at both vegetative and reproductive stages but did not affect root growth or C:N:P stoichiometry. The results also demonstrate that plants cultured from dimorphic seeds of *S. aralocaspica* have similar growth and C:N:P stoichiometry.

Elevated CO_2 alters plant growth and the contents of C, N, and P, as well as C:N and N:P ratios (Li et al., 2019). Our results confirm that elevated CO_2 significantly accelerated shoot growth and decreased N content in *S. aralocaspica*, consistent with previous studies. On average, N content in plant tissue decreases by approximately 14% under elevated CO_2 (Cotrufo et al., 1998). Elevated CO_2 has different effects on N contents across plant types, with the reduction in N content being smaller in C_4 plants than in C_3 plants. The changes in C:N and N:P ratios of *S. aralocaspica* between ambient and elevated CO_2 are mainly caused by the decrease in N content at elevated CO_2. These results coincide with previous findings that C:N ratio increases under elevated CO_2 regimes (Sardans et al., 2012).

Differences exist not only in seed size and germination of heteromorphic seeds but also in plant growth characteristics that persist until the end of the growing season (Imbert, 2002). Contrary to known differences in seed dormancy, germination, and plant reproduction (Wang et al., 2008, 2012), plants cultured from dimorphic seeds of *S. aralocaspica* did not significantly differ in their responses to CO_2 elevation. The differential response of heteromorphic seeds is effective in reducing offspring survival risk in unpredictably fluctuating environments—for example, dimorphic seeds of *S. aralocaspica* show different salt tolerance (Wang et al., 2008). Because the steady increase in CO_2 concentrations affects plants grown from dimorphic seeds of *S. aralocaspica* equally, it is not surprising that both plant types show similar growth and C:N:P stoichiometric characteristics.

Our results showed that N and P contents increased with plant developmental stage. This could be explained by the fact that plants at reproductive stage need higher nutrient concentrations to support fruiting (Marschner, 2013). The C content of *S. aralocaspica* was significantly lower than that (46.4% C) of global terrestrial plants (Elser et al., 2000). The decrease in C allocation at reproductive stage could be related to high salt accumulation in this species.

5 Conclusions

Our results reinforce the conclusion that there is no difference between plants grown from heteromorphic seeds in the responsiveness of ecophysiology to steady environmental factors. Elevated CO_2 greatly decreased N content and consequently caused changes in the N:P ratio of *S. aralocaspica*. Our results could enrich the understanding of plant response patterns of different photosynthetic types to CO_2 elevation. To accurately predict responses of various plants under future CO_2 concentrations, further work is necessary to explore representative plants with special photosynthetic types, such as single-cell C_4 and C_3-C_4 intermediate species.

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Appendix

Table S1 Split-plot variance analysis of the effects of elevated CO_2, plant type, and their interactions on growth and C:N:P ratio

Growth stage	Index	CO_2	Plant type	CO_2×Plant type
Vegetative	Shoot	0.000*		
	Shoot:root ratio	0.003*		
	C:N ratio	0.001*		
	C:P ratio	0.010*		
	N:P ratio			
Reproductive	Shoot			
	Shoot:root ratio			
	C:N ratio			
	C:P ratio			
	N:P ratio			

Note: C, carbon; N, nitrogen; P, phosphorus. * indicates significant difference at $P<0.05$ level.

Note: Figure translations are in progress. See original paper for figures.