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Impact of Elevated CO_2 on Growth and Development of *Arabidopsis thaliana* L.

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With 5 Figures

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Summary

VAN DER KOOIJ T. A. W. & DE KOK L. J. 1996. Impact of elevated CO_2 on growth and development of *Arabidopsis thaliana* L.- Phyton (Horn, Austria) 36 (2): 173–184, with 5 figures.- English with German summary.

After germination, *Arabidopsis thaliana* L. (cv. Landsberg) was grown at 350 µl l^{-1} (control) or 700 µl l^{-1} (elevated) CO_2. Total shoot biomass at the end of the vegetative growth period was increased by 56 % due to a short transient stimulation of the relative growth rate by elevated CO_2 at the onset of the exposure. Thereafter the relative growth rate was comparable for both CO_2 levels during the remaining vegetative part of the life cycle (0.42 g g^{-1} day^{-1}). Flowering architecture was not affected by elevated CO_2, but seed production was 51 % higher. Starch content of the shoot was substantially increased upon exposure to elevated CO_2, while the soluble sugar content remained unaffected. Total nitrogen content, on a dry mass basis, was decreased at elevated CO_2 mainly as a result of the increased starch content. Photosynthesis was stimulated at elevated CO_2 and no acclimation of the photosynthesis at elevated CO_2 was observed. Even though the stimulation of relative growth rate was only temporary, elevated CO_2 resulted in an increased fitness of *Arabidopsis thaliana* by an increased reproductive output.

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Zusammenfassung

VAN DER KOOIJ T. A. W. & DE KOK L. J. 1996. Einfluß von erhöhtem CO_2 auf Wachstum und Entwicklung von *Arabidopsis thaliana* L. – Phyton (Horn, Austria) 36 (2): 173–184, 5 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Nach der Keimung wuchsen Pflanzen von *Arabidopsis thaliana* (cv. Landsberg) unter 350 µl . l^{-1} (Kontrolle) oder 700 µl . l^{-1} (erhöht) CO_2. Am Ende der vegetativen Wachstumsphase war die gesamte Sproßbiomasse entsprechend einer kurzen vorübergehenden Stimulation der relativen Wachstumsrate durch das erhöhte CO_2 zu Beginn der Exposition um 56 % angestiegen. Dann aber war die relative Wachstumsrate bei beiden CO_2-Konzentrationen gleichgeblieben (0.42 g . g^{-1} . Tag^{-1}). Die Blütenbildung wurde durch erhöhtes CO_2 nicht beeinflußt, die Samenproduktion jedoch stieg um 51 %. Der Stärkegehalt der Sproße stieg unter erhöhtem CO_2 wesentlich an, während der Gehalt an löslichen Zuckern unbeeinflußt blieb. Der Gesamtstickstoffgehalt, bezogen auf die Trockenmasse, war unter erhöhtem CO_2, hauptsächlich als Folge des höheren Stärkegehaltes, vermindert. Die Photosynthese war unter erhöhtem CO_2 stimuliert und es war keine Anpassung auf erhöhtes CO_2 zu beobachten. Obwohl die relative Wachstumsrate nur vorübergehend stimuliert wurde, wirkte sich erhöhtes CO_2 bei *Arabidopsis thaliana* wegen der gestiegenen reproduktiven Leistung positiv aus.

Introduction

Human activities are expected to result in a gradual increase in the atmospheric CO_2, which is assumed to be doubled by the second half of the next century. Elevated CO_2 may directly, or indirectly, by its effect on global climate, affect plant production and development. It has been observed that exposure of plants to elevated CO_2 resulted in a higher biomass production, an altered morphology, increased water use efficiency, increased photosynthetic activity and an enhanced carbohydrate content (KIMBALL 1983, CURE 1986, SMITH & al. 1987, LAWLOR & MITCHELL 1991, WOODWARD & al. 1991).

It has been suggested that interspecific variation in response to elevated CO_2 would be related to the morphology and strategy of different plant species (POORTER 1993, LOEHLE 1995). Differences in growth rate, life span and sink strength between different species have been shown to influence the effect of CO_2 (STULEN & al. 1994). In many species the CO_2-induced increase in the relative growth rate during the vegetative growth appeared to be transient; upon prolonged exposure the relative growth rate of plants growing at elevated CO_2 returned to or was lower than plants growing at ambient CO_2 (DEN HERTOG & al. 1993, POORTER 1993, STULEN & al. 1994). Plants are reported to acclimate to elevated CO_2 by a loss of photosynthetic activity and a decrease in Rubisco activity (SAGE & al. 1989, YELLE & al. 1989).

Arabidopsis thaliana L. is a plant species with a high relative growth rate and a short life cycle. In order to obtain more insight into the effect of

elevated CO_2 on plant growth and development, *A. thaliana* was exposed to elevated CO_2 after germination until seed set. The effects on the relative growth rate, biomass production, reproductive output, gas exchange, carbohydrate content and nitrogen content were studied.

Abbreviations: RGR, relative growth rate; SLA, specific leaf area; RLAIR, relative leaf area increase rate.

Material and Methods

Plant Material and Plant Culture

Arabidopsis thaliana L. (cv. Landsberg) was sown on commercial potting soil (Florafleur, Nevema, Zwolle, The Netherlands, containing 150-200 mg nitrogen l^{-1}, 100–200 mg phosphorus (P_2O_5) l^{-1}, 150–250 mg potassium (K_2O) l^{-1}) in 0.25 l plastic pots. The soil surface was covered with a thin layer of sand. Plants were pre-cultivated at ambient CO_2 to exclude effects of different CO_2 concentrations on seed germination. Seven days after sowing plants were thinned to four or five plants per pot. All pots were daily watered with tap water.

Plants were grown in two identical climate-controlled rooms (Klima 5005, Fridina, Groningen, The Netherlands). The day and night temperature of the chambers were 20 °C and the relative humidity 60–65 %. The photoperiod was 12 hours at a photon flux density of 300 ± 20 µmol m^{-2} s^{-1} (within the 400–700 nm range), provided by Philips HPI-T (400 W) and Osram incandescent lamps (40 W).

The CO_2 concentration inside the chambers was continuously controlled by a CO_2 measuring device (Siemens, Germany). Pressurized CO_2 (100 % CO_2, Hoekloos, Schiedam, The Netherlands) was injected by an electronically controlled valve system, linked to the CO_2-controllers, into the circulating air-stream of the chambers (see Den Hertog & al. 1993). In order to maintain the desired concentration, CO_2 was constantly removed from the circulating air stream by a soda lime filter (Sodasorb, Hoekloos, Amsterdam, The Netherlands). This filter was refreshed weekly. The CO_2 concentrations in the chambers were maintained at 350 ± 10 µl l^{-1} (control) and 700 ± 10 µl l^{-1} CO_2 (elevated). The CO_2 concentration in both chambers was continuously monitored by an infrared gas analyzer (ADC 225 MK 2 or ADC 7000 in combination with ADC WA-161 gas handling unit). From previous experiments with other plant species, it was evident that there were no chamber effects (Den Hertog & al. 1993).

Plants were transferred to elevated CO_2 at the appearance of the first real leaf pair, beside the cotyledons, 7 days after sowing. Half of the pots, randomly chosen, were transferred from ambient to elevated CO_2 at the end of the photoperiod. Ten shoots, randomly taken from the chamber, were harvested every 2 days. Harvesting was started after 4 days of exposure to CO_2 and ended at the onset of flowering. In another experiment plants were transferred to elevated CO_2 14 days after sowing. Ten shoots were harvested daily.

Shoots were harvested 8 hours after the lights were switched on. Until flowering, the occurrence of self shading was negligible. Shoot fresh weight was measured and the total leaf area was determined by using a Hewlett Packard SketchPro Digitizer (type 45911B). Shoot dry weight was determined after 24 h of drying at 85 °C. The mean relative growth rate (RGR) was calculated over the total exposure period.

Plants were kept at ambient or elevated CO_2 until the end of the life cycle to determine seed production. Flowering architecture was determined by counting the total amount of flowering stems. The total number of siliques per plant, total amount of seeds per silique and the dry weight of individual seeds were determined. The total number of seeds per plant was estimated by multiplying the total amount of siliques per plant with the number of seeds per silique.

Data were statistically analyzed using a Mann-Whitney's U-test (Table 3), a one-way ANOVA (Table 2) and all other data was analyzed using an unpaired student t-test.

CO_2 gas exchange

Photosynthesis and dark respiration were determined after 14 days of exposure to ambient or elevated CO_2. Plants were removed from the pot and the roots were carefully washed with tap water. Plants were placed in a petri dish (5 cm diameter) on tap water (5 ml) with their roots through a layer of parafilm. The parafilm prevented water evaporation and CO_2 exchange between the water in the petridish and the surrounding air. Each plant was placed in a cuvette temperated by a water bath (180 ml volume). The gas flow through the cuvette was 250 ml min^{-1}. Total shoot CO_2 exchange in the dark and light was measured using an infrared gas analyzer (ADC-225 MK 3, Hoddesdon, U.K.). The temperature inside the cuvette was maintained at 20 °C and the photon flux density was 300 µmol m^{-2} s^{-1} (within the 400–700 nm range), provided by a slide projector (Pradovit universal, Leitz Wetzlar, Germany). A water filter between the light source and cuvette prevented the cuvette from heating. The actual CO_2 concentration in the cuvette was obtained by injecting pressurized CO_2 into the incoming air stream, controlled by a mass flow controller (ASM type AFC-260, Bilthoven, The Netherlands). Plants were acclimated in the dark for 40 min before measuring of the dark respiration. Photosynthesis, calculated on a leaf area basis, was determined after CO_2 uptake had reached a constant rate.

Carbohydrate, nitrogen and nitrate

To determine carbohydrate, nitrogen and nitrate content dried plant material was used. Soluble sugars and starch of the total shoot were determined according to FALES 1951. Total nitrogen was determined with the Kjeldahl method (DONEEN 1933, BARNEIX & al. 1988). Nitrate was extracted in water and determined refractrometrically after HPLC separation according to STUIVER & al. 1992.

Results and Discussion

Exposure of *Arabidopsis thaliana* to elevated CO_2 resulted in a significantly higher shoot biomass production (Figure 1A). At the end of the vegetative growth period the fresh weight of the shoots was 38 % higher than that of plants at ambient CO_2. At elevated CO_2, shoots contained a higher dry matter content during the entire exposure (Figure 1C). A similar increase in yield upon exposure to elevated CO_2 has been reported for other *A. thaliana* ecotypes as well as for other plant species (KIMBALL 1983, ZHANG & LECHOWICZ 1995). The gain in biomass production at elevated CO_2 was obtained by a transient increase in RGR during the first days of

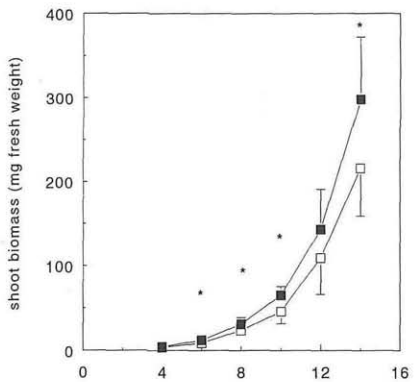

Fig. 1A

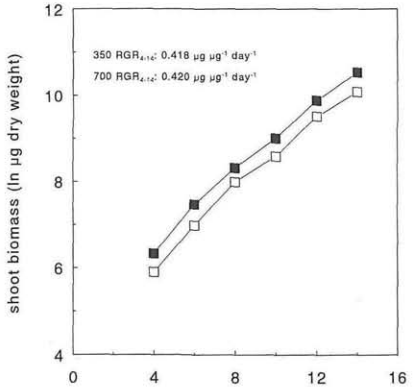

Fig. 1B

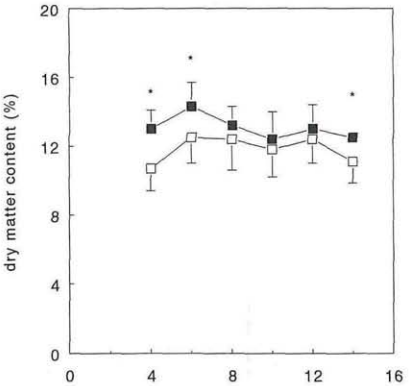

Fig. 1C

Fig. 1. The effect of elevated CO_2 on growth and dry matter content of *Arabidopsis thaliana* during the vegetative part of the life cycle. (A) Shoot fresh weight; (B) shoot dry weight production and relative growth rate; (C) shoot dry matter content. □, 350 µl l^{-1}; ■, 700 µl l^{-1}. Data represent the mean of 10 measurements (± SD). An asterisk (*) in Fig. 1A and 1C indicates a significant difference ($p < 0.05$) between the ambient and elevated CO_2 treatment.

exposure to elevated CO_2, since the RGR's at ambient and elevated CO_2 were comparable at the time interval between 4 and 14 days, whereover growth was determined (Figure 1B). Unfortunately, it was impossible to determine the RGR during the first four days of the exposure because of the low weights of the plants (less than 0.3 mg dry weight). In order to reconfirm that the observed effect of elevated CO_2 on growth was indeed obtained by a transient increase in RGR during the onset of exposure of

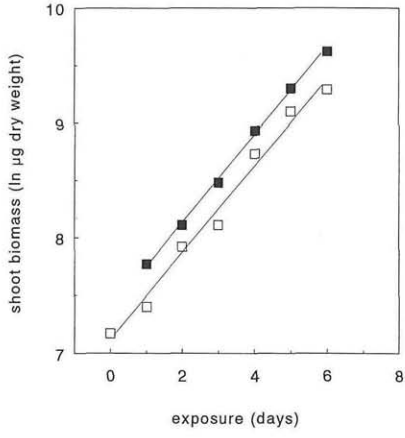

Fig. 2. The effect of elevated CO_2 on growth of *Arabidopsis thaliana* after the transfer of plants at the middle of the vegetative part of the life cycle. □, 350 µl l^{-1}; ■, 700 µl l^{-1}. Data represent the mean of 10 measurements (± SD). The r^2 of the linear fit at 350 and 700 µl l^{-1} are 0.979 and 0.998, respectively.

plants to elevated CO_2, plants were transferred from ambient to elevated CO_2 after 14 days. The observed increase of biomass production of shoots after one week of exposure to elevated CO_2 was obtained by an increase of RGR, which only occurred during the first day of exposure (Figure 2).

A transient stimulation of the RGR has also been reported for other plant species. For instance, elevated CO_2 induced a 30 days stimulation of RGR of *Abutilon theophrasti* (GARBUTT & al. 1990). The RGR of *Plantago major*, grown under similar growth conditions as in the present study, was only stimulated for 10 days (DEN HERTOG & al. 1993).

Plants exposed to elevated CO_2 had a significantly larger rosette leaf area (Figure 3). The enhancement in leaf area had already occurred during the first days of the exposure to elevated CO_2. Since, the relative leaf area increase rate (RLAIR) was comparable at ambient and elevated CO_2 at the time interval between day 4 and 14 of the exposure (Figure 3). On a dry weight basis, the specific leaf area (SLA) of plants at elevated CO_2 was smaller than that of plants at ambient CO_2 (Figure 4A). On a fresh weight basis a similar, but less obvious, effect of elevated CO_2 on the SLA was observed (Figure 4B).

Plants started to flower 21 days after sowing. Elevated CO_2 did not affect the flowering time of *A. thaliana* cv. Landsberg. Flowering architecture, expressed as total number of flowering stems, was also not affected by elevated CO_2 (Table 1). The number of seeds per silique and the number of siliques per plant were increased by 14 and 33 %, respectively, at

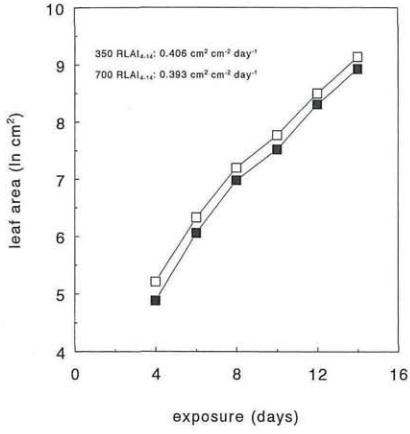

Fig. 3. The effect of elevated CO_2 on the leaf area development of *Arabidopsis thaliana*. □, 350 µl l^{-1}; ■, 700 µl l^{-1}. Data represent the mean of 10 measurements (± SD).

elevated CO_2 (Table 1). Reproductive output, defined as total seed production, at elevated CO_2 was 51 % higher than that of plants exposed to ambient CO_2. The dry weight of the individual seeds was hardly affected by elevated CO_2.

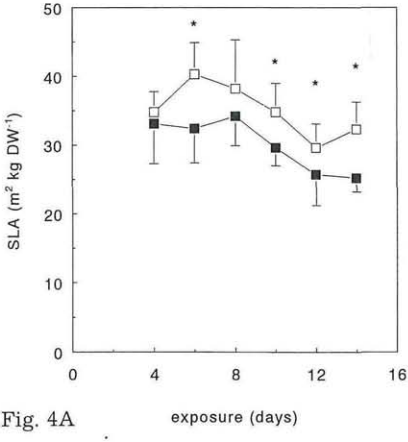

Fig. 4A

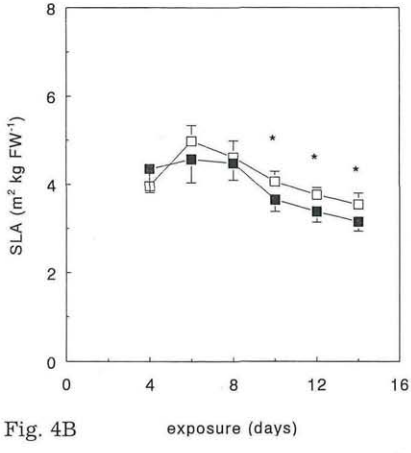

Fig. 4B

Fig. 4. The effect of elevated CO_2 on the specific leaf area (SLA). □, 350 µl l^{-1}; ■, 700 µl l^{-1}. The SLA is expressed on dry weight (A) and fresh weight (B) basis and the data represent the mean of 10 measurements (± SD). An asterisk (*) indicates a significant difference ($p < 0.05$) between the ambient and elevated CO_2 treatment.

Table 1

Effect of elevated CO_2 on flowering architecture and seed production of *Arabidopsis thaliana*. Data on flowering stems and siliques per plant represent the mean of measurements on 10 plants (±SD). Data on number of seeds per silique represent the mean of 7 measurements of 4 siliques in each (±SD) and seed dry weight on the mean of 10 measurements on 100 seeds in each (±SD). An asterisk (*) indicates a significant difference ($p<0.05$) between the ambient and elevated CO_2 treatment.

	CO_2 concentration	
	350 (µl l^{-1})	700 (µl l^{-1})
Flowering stems $plant^{-1}$	36.2±3.9	36.0±5.8
Siliques $plant^{-1}$	415±44	553±59*
Seeds $silique^{-1}$	50.9±4.6	57.8±6.3*
Seed dry weight (µg $seed^{-1}$)	14.9±0.53	15.6±0.54
Seeds $plant^{-1}$	21225	31963

The observed increase in dry matter content upon exposure to elevated CO_2 (Figure 1C) was solely accounted for an increase in the starch content (Figure 5A). At elevated CO_2 the starch content of shoots was consistently higher than at ambient CO_2 (Figure 5A). The soluble sugar content was unaffected by elevated CO_2 (Figure 5B). The observed lower SLA at elevated CO_2 (Figure 4) could largely be explained by the increase in starch content and to a less extent to changes in morphology, viz. thickening of the leaves.

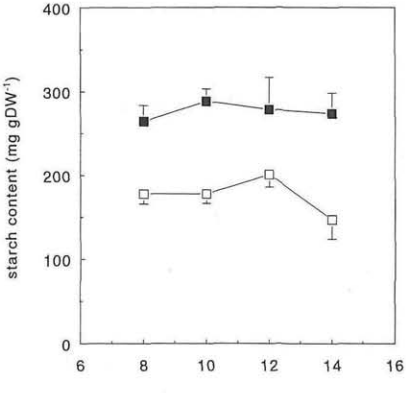

Fig. 5A

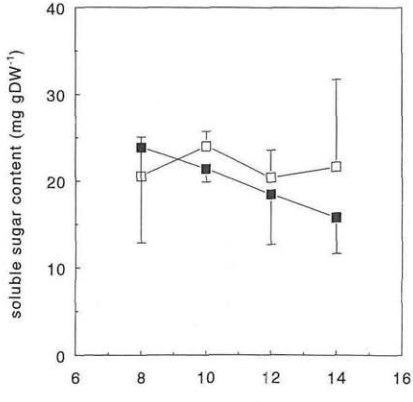

Fig. 5B

Fig. 5. The effect of elevated CO_2 on the carbohydrate content of shoots of *Arabidopsis thaliana*. □, 350 µl l^{-1}; ■, 700 µl l^{-1}. Starch (A) and soluble sugar (B) content of shoots are expressed as mg glucose g DW^{-1} and represent the mean of 4 measurements (± SD).

Table 2

Effect of elevated CO_2 on dark respiration and photosynthesis of shoots of *Arabidopsis thaliana*. CO_2 evolution and uptake was measured at 350 and 700 µl l^{-1} CO_2. Data represent the mean of 3 measurements (±SD). When tested by one-way ANOVA, photosynthesis was significantly increased ($p < 0.05$) at elevated CO_2 but the CO_2 concentration during growth had no significant effect on photosynthesis. Dark respiration was not significantly affected by elevated CO_2.

CO_2 concentration during growth (µl l^{-1})	CO_2 concentration during measurements (µl l^{-1})	Dark respiration (µmol $m^{-2}s^{-1}$)	Photosynthesis (µmol $m^{-2}s^{-1}$)
350	350	2.10±0.48	8.34±1.37
700	350	2.25±0.29	9.19±0.69
350	700	1.75±0.16	11.32±0.92
700	700	1.76±0.28	11.38±0.44

Photosynthesis of *A. thaliana*, measured at growth light intensity and growth CO_2 concentration, was 36 % higher in plants exposed to elevated CO_2 for 14 days compared to plants exposed to ambient CO_2 (Table 2). No significant effect of elevated CO_2 on shoot dark respiration was observed (Table 2). There were no significant differences in photosynthesis and dark respiration between plants exposed to various CO_2 levels but measured at the same CO_2 concentrations (Table 2). For other plant species it has been observed that photosynthetic acclimation may occur upon long-term exposure to elevated CO_2 (YELLE & al. 1989), e.g. by a loss of Rubisco activity (LAWLOR & MITCHELL 1991, SAGE & al. 1989) or a decrease of the amount of Rubisco (BOWES 1991). Apparently, acclimation of photosynthesis at elevated CO_2 did not occur in *A. thaliana*. This was supported by the observation that at a comparable RGR, shoots exposed to elevated CO_2 contained a higher starch content than those at ambient air. Apparently, there was an extra gain of photoassimilates at elevated CO_2, which could not be used for extra growth. The reported effects of elevated CO_2 on respiration are rather inconsistent and dependent on the plant species and measuring conditions (AMTHOR 1991).

Total nitrogen and organic nitrogen content expressed on a dry weight basis were significantly decreased at elevated CO_2, whereas nitrate content remained unaltered (Table 3). A decrease of leaf and total shoot nitrogen content, expressed on a dry weight basis, is observed in many plant species exposed to elevated CO_2 (GARBUTT & al. 1990, CHU & al. 1992, STULEN & al. 1994). After correction for starch content there were no consistent differences between total and organic nitrogen content in shoots of *A. thaliana* exposed to ambient or elevated CO_2 (Table 3). KUEHNY & al. 1991 reported that a decrease in nitrogen content in leaves exposed to elevated CO_2 could be explained by a dilution by carbohydrates. This

Table 3

The effect of elevated CO_2 on inorganic and organic nitrogen content of shoots of *Arabidopsis thaliana*. Plants were exposed for 16 days. Nitrate and total nitrogen were measured in the same shoots; the organic nitrogen content was calculated by subtracting the nitrate content from total nitrogen content. Data represent the mean of 5 measurements (±SD). An asterisk (*) indicates a significant difference ($p < 0.05$) between the ambient and elevated CO_2 treatment.

	CO_2 concentration ($\mu l\ l^{-1}$)			
	350 $\mu l\ l^{-1}$		700 $\mu l\ l^{-1}$	
	($\mu mol\ g\ DW^{-1}$)	($\mu mol\ g\ FW^{-1}$)	($\mu mol\ g\ DW^{-1}$)	($\mu mol\ g\ FW^{-1}$)
Nitrate	1.03±0.12	0.112±0.016	1.09±0.14	0.139±0.018*
Total N	5.36±0.18	0.578±0.075	4.53±0.15*	0.579±0.019
Organic N	4.33±0.14	0.466±0.063	3.44±0.15*	0.440±0.018

dilution of nitrogen by an increased carbohydrate content was likely also the main reason for the observed decrease in total and organic nitrogen content at elevated CO_2 in *A. thaliana*. The nitrate content in shoots of plants exposed to elevated CO_2 was significantly higher than that of plants exposed to ambient CO_2 after correction for the increased starch content in plants exposed to elevated CO_2. The rather high shoot nitrate content of plants exposed to both ambient and elevated CO_2 excluded the occurrence of nitrogen limitation in *A. thaliana* in the present experiments.

The enhanced carbon gain in *A. thaliana* upon exposure to elevated CO_2 resulted only in a transient increase in RGR and was mainly allocated in starch. Although the stimulation of the RGR was only transient, this resulted in a substantial increase of the fitness by an increased reproductive output.

From the present study it remained unclear why the stimulation of RGR was transient. The physiological processes which could underlay the transient stimulation of RGR, such as photosynthetic acclimation to elevated CO_2 (BOWES 1991) or morphological differences in plants growing at different CO_2 conditions (POORTER & al. 1988), were not observed in *A. thaliana*. Reported differences in weight ratios and nitrogen use efficiency between plants grown at different CO_2 concentrations could in fact be a shift in phenology and carbohydrate content and are not related to biochemical adaptations or acclimation to elevated CO_2 (LOEHLE 1995). It is proposed that the RGR of a plant is fixed at a certain level, and factors other than CO_2 and nitrogen availability are regulating factors limiting growth under the given growth conditions (LOEHLE 1995). This may be an environmental factor or an internal factor, such as a changed expression of genes at elevated CO_2 (VAN OOSTEN & al. 1994).

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