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ORIGINAL ARTICLE

T. Martijn Bezemer · T. Hefin Jones

The effects of CO_2 and nutrient enrichment on photosynthesis and growth of *Poa annua* in two consecutive generations

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Abstract We studied short- and long-term growth responses of *Poa annua* L. (Gramineae) at ambient and elevated (ambient +200 μmol mol^{-1}) atmospheric CO_2. In experiment 1 we compared plant growth during the early, vegetative and final, reproductive growth phases. Plant growth in elevated CO_2 was significantly enhanced during the early phase, but this was reversed in the reproductive phase. Seed mass and percentage germination were significantly reduced in elevated CO_2. Experiment 2 tested for the impact of transgenerational and nutrient effects on the response of *Poa annua* to elevated CO_2. Plants were grown at ambient and elevated CO_2 for one or two consecutive generations at three soil nutrient levels. Leaf photosynthesis was significantly higher at elevated CO_2, but was also affected by both soil nutrient status and plant generation. Plants grown at elevated CO_2 and under conditions of low nutrient availability showed photosynthetic acclimation after 12 weeks of growth but not after 6 weeks. First-generation growth remained unaffected by elevated CO_2, while second-generation plants produced significantly more tillers and flowers when grown in elevated CO_2 compared to ambient conditions. This effect was strongest at low nutrient availability. Average above- and belowground biomass after 12 weeks of growth was enhanced in elevated CO_2 during both generations, but more so during plant generation 2. This study demonstrates the importance of temporal/maternal effects in plant responses to elevated CO_2.

Keywords Annual meadow grass · Climate change · Epigenetics · Maternal effects · Photosynthesis · Plant phenology

T. M. Bezemer (✉)
Department of Terrestrial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), PO Box 50, 6700 AB Wageningen, The Netherlands
E-mail: m.bezemer@nioo.knaw.nl
Tel.: 317-473607

T. H. Jones · T. M. Bezemer
NERC Centre for Population Biology, Imperial College at Silwood Park, Ascot, Berkshire SL5 7PY, UK

T. H. Jones
Cardiff School of Biosciences, Cardiff University, PO Box 915, Cardiff CF10 3TL, UK

Introduction

Studies predicting plant responses to increasing atmospheric carbon dioxide (CO_2) concentrations have shown, in general, that in elevated CO_2 photosynthetic rates increase, enhancing plant growth and biomass (e.g. Bazzaz 1990). These studies, frequently carried out in greenhouses and controlled environments, have provided useful information on how plants can respond to increasing CO_2. However, in most experiments, the availability of nutrients has been relatively high compared to what plants generally encounter in nature (Poorter et al. 1996). Other studies have shown that soil nutrient availability may limit the magnitude of plant responses to elevated CO_2 (Stitt and Krapp 1999; Zangerl and Bazzaz 1984; Curtis et al. 1995; Bowler and Press 1996; Tang et al. 2006). Plants growing in conditions of low nutrient availability may show photosynthetic acclimation or downregulation with elevation of CO_2 (Bazzaz 1990). This can be due to photosynthate production exceeding sink demand to such a degree that nonstructural carbohydrates accumulate substantially in source leaves, leading to feedback inhibition of photosynthetic rates (Arp 1991). Alternatively, photosynthetic rates may be downregulated by the redistribution of nitrogen within the plant away from Rubisco and into the formation of additional leaves or other tissue (Sage and Pearcy 1987; Stitt 1991).

Many studies on the effects of elevated CO_2 on plant growth have been carried out for short periods of time, over only part of a plant generation, and have focussed on the effects of elevated CO_2 on vegetative biomass

enhancement ratios (reviewed by Kimball 1983; Poorter 1993). However, these ratios are of only secondary importance for predicting effects on annual plants, as the performance of a plant in a succeeding generation will be determined by the reproductive rather than the vegetative biomass response of the previous generation. In many plant species, environmental conditions such as the temperature, photoperiod and nutrient supply experienced by the parent plant during seed development and maturation can affect resource allocation to the seeds (e.g. Roach and Wulff 1987). CO_2 elevation can also lead to altered reproductive responses of a plant, such as allocation to reproductive biomass, reproduction time, total seed production, seed mass and seed germination (e.g. Curtis et al. 1994; Farnsworth and Bazzaz 1995; Andalo et al. 1996; Steinger et al. 2000; Kinugasa et al. 2003; Hikosaka et al. 2011). By affecting the reproductive output of a plant, the effects of CO_2 on plants can extend beyond a single growth period (Steinger et al. 2000; Lau et al. 2008).

A number of studies have shown that plant responses to elevated CO_2 can differ between plant generations, and that the response of a plant can be affected by the CO_2 environment that the maternal plant has experienced (e.g. Bezemer et al. 1998; Huxman et al. 1998, 2001; Steinger et al. 2000; Ward et al. 2000; Schulte et al. 2002; Derner et al. 2004; Lau et al. 2008). For example, seedlings of the annual grass *Bromus madritensis* respond to elevated CO_2 by increasing their growth rate, but this increase is less when the parent plant was also grown at elevated CO_2 than when the parent plant was grown at ambient CO_2 (Huxman et al. 1998). This can be explained by a negative effect of elevated CO_2 on seed size and the nutritional quality of seeds, which results in reduced photosynthetic rates of the next-generation seedlings (Huxman et al. 2001). Negative effects of CO_2 on seed quality were also reported for the related species *Bromus erectus* (Steinger et al. 2000). However, these effects did not lead to transgenerational effects of CO_2, possibly because reduced seed quality coincided with increased seed size under conditions of elevated CO_2 (Steinger et al. 2000). In contrast, a study of spring wheat (*Tritium aestivum* L.) showed that this plant species did not respond to elevated CO_2 during the first generation. However, plants that had been grown for two or three generations in elevated CO_2 produced more biomass as well as more and heavier seeds than plants grown for two or three generations in ambient CO_2 conditions (Derner et al. 2004). These authors also concluded that the intergenerational CO_2 responses of this plant species were driven by CO_2-induced changes in seeds that affect seedling responses to CO_2 (Derner et al. 2004).

Previously, we reported that plants of the annual grass species *Poa annua* did not respond to elevated CO_2 during the first growth period, but that second-generation plants produced more tillers in elevated CO_2 (Bezemer et al. 1998). In this paper, we present the results of two greenhouse-based experiments in which we examined the short- and longer-term effects of elevated atmospheric CO_2 and fertilisation on the growth, photosynthesis and seed production of *Poa annua*. In particular, we examined (1) the reproductive growth responses and seed quality of *P. annua* to elevated CO_2 within one growth period, and (2) how soil nutrient availability and CO_2 affect the photosynthesis, growth and seed production of *P. annua* during two successive plant generations.

Methods

The experiments were carried out in a CO_2-controlled greenhouse at Silwood Park, Ascot, UK. The greenhouse consisted of two separately controlled perspex-walled chambers, each of size 3.7 m × 1.3 m × 1.8 m, which could be maintained at different atmospheric CO_2 concentrations. Within each chamber, natural light was supplemented by two 400 W metal halide bulbs (16 h daylight). Light levels in the chambers were similar to outside conditions and typically varied between 400 and 1200 PAR. The temperature within the chambers was computer-controlled and regulated at 20 ± 1 °C during the day and 12 ± 1 °C during the night. Relative humidity was maintained at 60 ± 10 % throughout. Two sensors situated at equal distances along the chamber length continuously relayed information on temperature and humidity to a computer, enabling accurate monitoring and control of the environment. CO_2 levels were controlled using an infra-red gas analyser (PP Systems, Hitchin, UK) that measured the CO_2 levels in each chamber every 2 min. CO_2 levels were either ambient (outside air, 360–400 μmol mol^{-1}) or elevated (ambient + 200 μmol mol^{-1}), the latter being based on the predicted "moderate" scenario for the year 2060 in Houghton et al. (1996). The enhanced and ambient CO_2 treatments were exchanged between the two chambers once every seven days (i.e. 12 times during each experiment).

Experiment 1

Seeds obtained from John Chambers Seed Specialists (UK) were sown in 650 ml pots filled with a 40:60 sand–Surrey loam mix (41.61 ppm nitrogen, 17.63 ppm phosphorus, 12.45 ppm potassium) on a 2 cm base of gravel. Soil nutrient levels corresponded with levels found in weedy fields around the Silwood area. Fifty-two pots were sown (26 at each CO_2-treatment level). Seedlings emerged within a week and were thinned to one plant per pot ten days later. For 13 randomly chosen plants per treatment (referred to as "early growth"), the number of tillers and leaves were recorded upon the appearance of the first flower, as were the proportions of leaves that showed any senescence. Bud-initiation time was calculated as the number of days from seedling emergence until the day of first flower appearance. The

26 plants (13 for both treatments) were then harvested, separated into shoots and roots, oven-dried at 70 °C, and weighed. The remaining 13 plants per treatment (referred to as "final growth") were grown until all the seeds from one inflorescence had dispersed. The number of tillers and leaves, the proportion of senescent leaves, the reproduction time (days from seedling emergence until seed dispersal) and the total number of inflorescences per plant were then recorded, and the final dry weight (shoot and root) was determined. For both treatments, three mature inflorescences were collected from each of the 13 plants and used to estimate the seed production per plant. Seeds from all plants in each CO_2 treatment were pooled and ten groups of 25 seeds were taken from each treatment, placed on moist filter paper in a petri dish, and monitored for germination. One hundred randomly chosen seeds from each CO_2 treatment were weighed individually to estimate mean seed mass. Finally, approximately 0.5 g of seeds per treatment were analysed for carbon and nitrogen contents by total combustion. The original and remaining experimental seeds were used at a later date in experiment 2.

Experiment 2

To study the interaction between CO_2 and soil nutrient availability, first- and second-generation *P. annua* growth in ambient and elevated CO_2 at different soil nitrogen levels was compared. Plants were grown in pots filled with the same loam/silver-sand mixture as used in experiment 1. The experiment was a $3 \times 2 \times 2$ factorial design with three nutrient levels, two plant generations, and two CO_2 levels. Each CO_2 treatment consisted of 12 blocks, with each block containing one full set of replicates of all six combinations of the first two factors. Within each block, the position of the nutrient treatments for plants from both generations was randomised at the start of the experiment and after six weeks. Each week, at the time that the enhanced and ambient CO_2 treatment was exchanged between the chambers, the blocks were transferred between and randomly rearranged in the chambers. All plants from experiment 2 were grown simultaneously and under the same light conditions.

Three soil nutrient applications were used. Leaching the loam–sand mixture with water provided a very nutrient-poor soil ("nonfertilised"); the other two nutrient levels were obtained by fertilising the loam–sand mixture once every six weeks ("medium") or every two weeks ("high") with 0.78 g of a slow-release fertiliser per pot (Phostrogen, 8.0 % nitrogen, 4.8 % phosphorus, 19.1 % potassium). Plants in the medium fertilisation treatment received 0.12 g N, while plants in the high treatment received 0.37 g N during the course of the experiment. First-generation seeds were from the same seed source as those used in experiment 1. Second-generation seeds were obtained from experiment 1 plants grown at ambient or elevated CO_2. Seeds produced by plants grown at ambient CO_2 in experiment 1 were germinated and grown at ambient CO_2 for the second generation and vice versa. First- and second- (ambient and elevated) generation seeds were sown in trays with high, medium and nonfertilised soil in ambient and elevated CO_2. Seeds from all treatments germinated well (>80 %), and seven days after sowing, the seedlings were transplanted into 650 ml pots filled with corresponding high, medium or nonfertilised soil on a 2 cm base of gravel.

Individual-leaf photosynthesis was measured 6 weeks after germination when all plants were still in the vegetative phase, and after 12 weeks when all plants were flowering. Photosynthesis was measured using a portable infrared gas analyser (CIRAS-1, PP Systems, Hitchin, UK) equipped with an artificial light source (600 PAR). A 2.5 cm^2 cuvette was clamped over a mature leaf and photosynthetic rates were measured within the greenhouse chambers at the CO_2 concentration that the plant was growing in (ambient at 360–380 μmol mol^{-1} and elevated at 560 μmol mol^{-1}). To study photosynthetic acclimation, photosynthetic rates of plants grown at ambient CO_2 were also measured at 560 μmol mol^{-1} CO_2 by changing the CO_2 concentration within the cuvette. To avoid any potential effects of the chamber on measurements, after 6 of the 12 blocks in each CO_2 treatment had been measured, the CO_2 treatment was changed between the chambers and the blocks were transferred appropriately. Two photosynthesis analyses were carried out. First, we compared the photosynthetic activity of plants grown at either ambient or elevated CO_2. For this analysis, only measurements made at the actual CO_2 level at which the plant was grown were included. To investigate whether photosynthetic acclimation occurred, and whether this differed between treatments, the photosynthetic rates at 560 μmol mol^{-1} CO_2 (A[560]) were compared for all plants, irrespective of whether they had been growing at ambient or elevated CO_2.

Vegetative and reproductive growth was measured every seven days by counting the number of tillers and flowering stems, respectively. For each plant, the date of appearance of the first flower (bud initiation time) was also recorded. Thirteen weeks after germination, plant height was measured. All plants were then individually harvested, separated into shoot and root material, oven-dried at 70 °C, and weighed.

Statistical analysis

Data from both experiments were analysed using Statistica 64 (Statsoft 1984–2011), and were first tested for normality using probability plots and transformed when appropriate. Data from experiment 1 were analysed using analysis of variance (ANOVA), with CO_2 and harvest (vegetative vs. flowering plants) as the main effects. Count data (leaves, tillers, inflorescences and seeds) were square-root transformed. Proportional data (senescence and germination) were arcsine-transformed;

the other data (developmental time, biomass and seed weight) were log-transformed.

Data from experiment 2 were analysed using a nested design with blocks nested in CO_2. Data were separated into measurements taken at one specific time during the course of the experiment (bud initiation time and biomass) and repeated measurements (photosynthesis, tillers and inflorescences). A nested ANOVA on log-transformed data was carried out on specific time measurements with blocks nested in CO_2. Nested repeated measures analysis of variance (RMANOVA) was performed on both the square-root transformed count data (tillers and inflorescences) and on log-transformed photosynthesis data. The repeated measures analysis takes into account overall effects between the main treatments and their interactions (hereafter referred to as "between") as well as temporal differences (hereafter referred to as "within") to test whether treatments differed in the patterns of their responses (Gurevitch and Chester 1986).

Results

Experiment 1

At the end of the vegetative growth period, plants grown in elevated CO_2 had significantly more tillers ($F_{1,23} = 9.19$, $P < 0.01$) and leaves ($F_{1,23} = 6.77$, $P < 0.05$) and more aboveground biomass ($F_{1,23} = 4.54$, $P < 0.05$), than plants grown in ambient CO_2 (Table 1). Both root biomass and the percentage of leaves that showed senescence increased by 40 % in elevated CO_2, but this was not statistically significant. Bud initiation time did not differ between treatments. At the end of the reproductive growth phase, the number of tillers decreased by 13 % in elevated CO_2, just outside of the significance level ($F_{1,24} = 3.83$, $P = 0.06$). The number of leaves and leaf senescence, shoot and root biomass, number of inflorescences, and number of seeds per inflorescence were not affected by the CO_2 treatment. Seeds produced by plants grown in elevated CO_2 had lower germinability ($F_{1,18} = 11.92$, $P < 0.01$) and reduced seed mass (Table 1; $F_{1,198} = 4.60$, $P < 0.05$) than seeds from plants grown in ambient CO_2. The C:N ratio of the seeds remained unaffected by CO_2 concentration. Thus, while early vegetative growth responses tended to be enhanced in elevated CO_2, final, reproductive growth responses tended to be reversed (Table 1). This resulted in a significant CO_2 and time interaction for most of the variables measured (Table 2).

Experiment 2

Photosynthetic activity (A) was significantly higher at elevated CO_2 ($F_{1,22} = 10.16$; $P < 0.001$; Table 3), but not significantly so for all nutrient and generation combinations (Fig. 1). The mean photosynthetic rate of first-generation plants was lower than that of second-generation plants after 6 weeks of growth, but higher after 12 weeks, resulting in a significant within effect for

Table 1 Experiment 1: growth measurements of *P. annua* during the early, vegetative phase and the final, reproductive phase, as well as reproductive output of *P. annua* when grown in ambient and elevated CO_2 ($n = 13$)

	Ambient CO_2	Elevated CO_2	% Change	P
Growth during vegetative phase				
Bud initiation time (days)	52.3 ± 1.9	53.3 ± 2.3	+2.0	NS
Number of tillers	15.8 ± 0.7	20.9 ± 1.5	+32.4	**
Number of leaves	67.0 ± 4.6	94.4 ± 9.8	+40.3	*
Senescent leaves (%)	12.7 ± 2.7	17.8 ± 2.0	+40.2	NS
Shoot biomass (g)	0.4 ± 0.1	0.6 ± 0.1	+71.4	*
Root biomass (g)	0.2 ± 0.03	0.3 ± 0.04	+39.7	NS
Growth during reproductive phase				
Reproduction time (days)	81.4 ± 1.5	79.4 ± 1.3	−2.5	NS
Number of tillers	33.4 ± 1.5	29.0 ± 1.8	−13.1	NS
Number of leaves	176.1 ± 7.1	161.3 ± 11.0	−8.4	NS
Senescent leaves (%)	63.3 ± 2.6	59.3 ± 2.7	−6.1	NS
Shoot biomass (g)	1.3 ± 0.1	1.1 ± 0.1	−14.5	NS
Root biomass (g)	0.6 ± 0.05	0.5 ± 0.07	−9.1	NS
Reproductive output				
Number of inflorescences	14.3 ± 1.0	12.9 ± 0.9	−10.2	NS
Seeds per inflorescence	120.0 ± 4.0	111.6 ± 5.9	−7.0	NS
Seed mass (μg)	336.4 ± 4.7	323.0 ± 4.1	−4.0	*
Germinability (%)	94.0 ± 1.5	82.8 ± 2.9	−13.5	**
Seed C/N ratio ($n = 1$)	31.3	33.5	+7.0	

Vegetative measurements were taken at the appearance of the first flower. Reproductive measurements were taken when all of the seeds of one seed-head had dispersed. See "Methods" for more details regarding the measurements. Shown are the mean ± SE, the percentage change in elevated CO_2 conditions, and the P value calculated from one-way ANOVA

* $P < 0.05$

** $P < 0.01$

NS $P > 0.05$

Table 2 Comparison of measurements taken during the early, vegetative phase and the final, reproductive phase for experiment 1

	Development time	Tillers	Leaves	Leaf senescence	Shoot biomass	Root biomass
CO_2	NS	NS	NS	*	NS	NS
Time	***	***	***	***	***	***
$CO_2 \times$ Time	NS	**	**	NS	**	*

Shown are the significance of P values calculated from two-way ANOVA on the effects of CO_2, time (vegetative vs. reproductive responses) and $CO_2 \times$ time
* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$
NS $P > 0.05$

Table 3 Summary table of the statistics for experiment 2

Factor	Photosynthesis (actual)		Photosynthesis [560]		Vegetative growth		Reproductive growth		Bud initiation time	Aboveground biomass	Belowground biomass
	Between	Within	Between	Within	Between	Within	Between	Within			
CO_2	***	NS	**	***	NS	*	NS	**	NS	**	NS
Generation (Gen)	NS	***	NS	*	NS	NS	*	NS	*	NS	NS
Nutrient (Nutr)	***	**	***	*	***	***	***	***	***	***	NS
$CO_2 \times$ Gen	NS	NS	NS	NS	**	***	NS	**	NS	NS	NS
$CO_2 \times$ Nutr	NS	NS	NS	*	NS	NS	*	NS	NS	NS	*
Gen $\times$ Nutr	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	*
$CO_2 \times$ Gen $\times$ Nutr	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Time series data were analysed with repeated measures ANOVA; other data were analysed using ANOVA. For RMANOVA, "between" effects consider whether treatments and interactions differ in their overall responses, while "within" effects consider whether treatments or interactions differ in their responses over time. More details about the statistical analysis are given in "Methods"
* $P < 0.05$
** $P < 0.01$
**NS P* $P < 0.001$
> 0.05

generation ($F_{1,110} = 10.95$; $P < 0.001$; Table 3). After six weeks of growth, the mean photosynthetic rate of plants grown in high fertilized soil was significantly higher than that for plants grown in medium fertilized or nonfertilised soil, while there was no significant difference at the end of the experiment between the rates at high and medium soil nutrient levels, but both were significantly higher than rates at nonfertilised soil. This resulted in significant between and within nutrient effects (between: $F_{2,110} = 8.55$; $P < 0.001$; within: $F_{2,110} = 4.89$; $P < 0.01$; Table 3).

When measured at 560 μmol mol^{-1} CO_2, photosynthetic rates (A[560]) were affected by CO_2 and nutrients, but also differed between first- and second-generation plants (Table 3; Fig. 2). Overall, A[560] was higher for plants grown at ambient CO_2 (between: $F_{1,22} = 12.76$; $P < 0.01$). Moreover, for plants grown at elevated CO_2, A[560] at 12 weeks was lower than A[560] at 6 weeks of growth (within CO_2 effect: $F_{2,21} = 15.68$; $P < 0.001$). Similarly, A[560] of first-generation plants was higher than that of second-generation plants after 6 weeks but lower after 12 weeks of growth, leading to a significant within effect for generation ($F_{1,110} = 6.87$; $P < 0.05$; Table 3). At high nutrient levels, A[560] declined from week 6 to week 12 for both generations and at both CO_2 levels. In contrast, in nonfertilised soil, A[560] declined from week 6 to week 12, but only for plants grown at elevated CO_2 (Fig. 2), indicating that plants grown at elevated CO_2 and under conditions of low nutrient availability showed photosynthetic acclimation after 12 weeks of growth but not after 6 weeks.

Nutrient fertilization had a significant positive effect on the number of tillers (between: $F_{2,110} = 61.68$; $P < 0.001$; within: $F_{18,990} = 93.96$; $P < 0.001$; Fig. 3) and inflorescences (between: $F_{2,110} = 19.27$; $P < 0.001$; within: $F_{8,440} = 11.19$; $P < 0.001$; Fig. 4). The number of tillers and inflorescences of first-generation plants remained unaffected or even decreased in conditions of elevated CO_2, but second-generation plants produced significantly more tillers (Fig. 3) and flowers (Fig. 4) when grown in elevated CO_2. The effects of elevated CO_2 in second-generation plants were strongest in nonfertilised soil. Bud initiation times were reduced by nutrient fertilization ($F_{2,110} = 8.01$; $P < 0.001$), and at high nutrient soil they were significantly delayed in conditions of elevated CO_2 for generation 1 plants only (Table 4). Bud initiation occurred significantly earlier in generation 2 than in generation 1 plants ($F_{1,110} = 6.17$; $P < 0.05$; Table 4). Aboveground biomass was higher in conditions of elevated CO_2 for all nutrient treatments

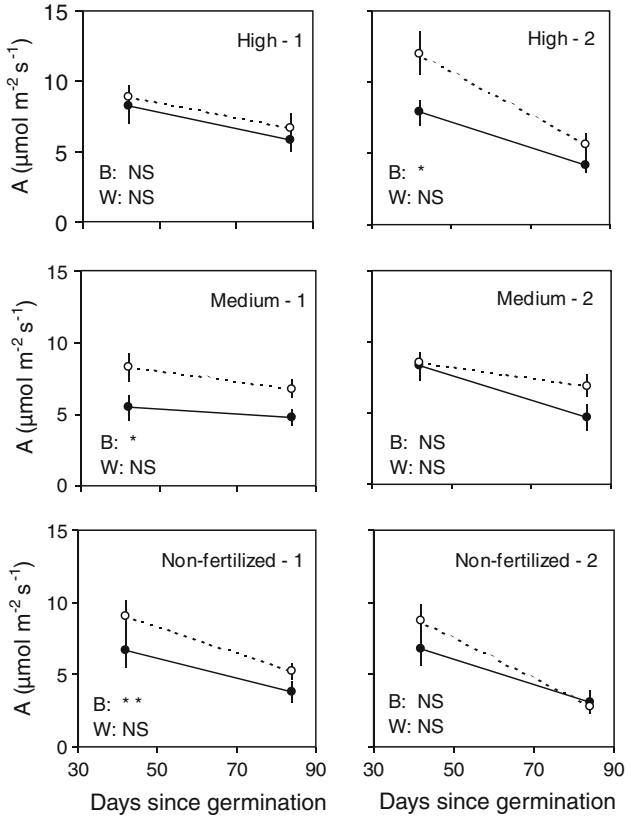

Fig. 1 Leaf photosynthetic rates (*A*) of *P. annua* plants grown in high or medium fertilized soil or in nonfertilised soil, and in ambient (*solid lines*) or elevated (*dotted lines*) CO_2 for the first and second generations. Means (n = 12) ± SE and P values based on RMANOVA are shown. A significant between effect (*B*) indicates that the CO_2 treatment results differ in their overall responses, while a significant within effect (*W*) indicates that the CO_2 treatment results differ in their responses over time. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS $P > 0.05$

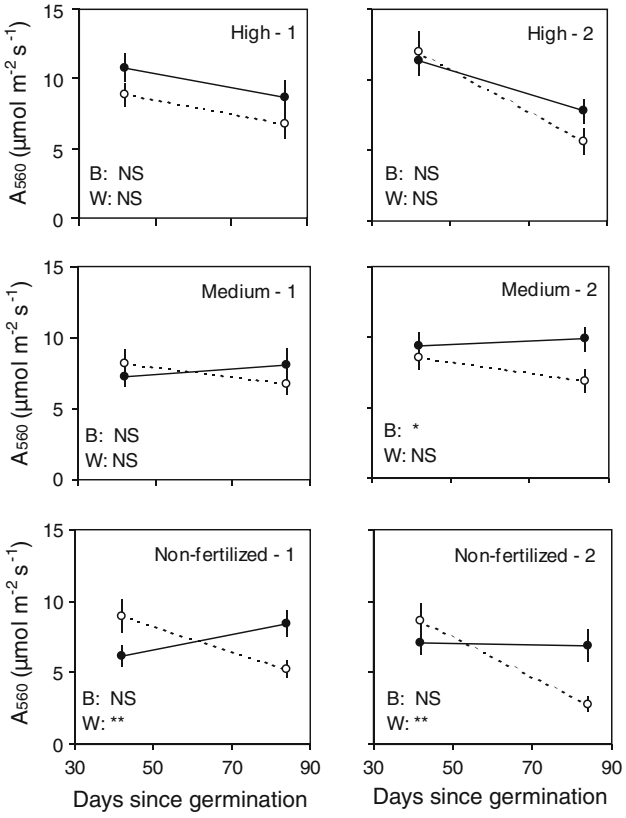

Fig. 2 Photosynthetic responsiveness of *P. annua* plants to CO_2 elevation when grown in high or medium fertilised soil or in nonfertilised soil, and in ambient (*solid lines*) or elevated (*dotted lines*) CO_2 for either one or two generations. Means are shown (n = 12) ± SE and P values are based on RMANOVA. See the legend for Fig. 1

and during both generations, resulting in a significant CO_2 effect ($F_{1,22}$ = 11.32; $P < 0.01$; Table 3). The effect was, however, only significant for generation 1 plants grown in nonfertilised soil and for generation 2 plants grown in either nonfertilised or high fertilized soil (Table 4). Significantly more root dry weight was produced at conditions of elevated CO_2, but only for generation 2 plants grown at high nutrient levels and in nonfertilised soil, resulting in significant interactions between nutrient level and CO_2 ($F_{2,110}$ = 3.55; $P < 0.05$), and nutrient level and generation ($F_{2,110}$ = 4.57; $P < 0.05$).

Discussion

Short-term responses

Early, vegetative-phase responses of *P. annua* to changes in atmospheric CO_2 concentrations differ distinctly from later, reproductive-phase responses. This makes extrapolating from short-term measurements to longer-term predictions for *P. annua* growth and development under conditions of elevated atmospheric CO_2 difficult. Measurements taken early in experiment 1 suggest enhanced growth for *P. annua* in conditions of elevated atmospheric CO_2, while later observations, taken during the reproductive phase, suggest a negligible or even negative response. This weak or even lack of response during the reproductive phase may have arisen from root growth being limited in the confines of the experimental pot (Arp 1991); shoot biomass during the early vegetative phase was more enhanced (71 %) in elevated CO_2 than was root biomass (40 %) (Table 1). Difficulties in predicting reproductive growth responses from early, vegetative responses is not unheard of (e.g. Farnsworth and Bazzaz 1995; Garbutt et al. 1990; Hunt et al. 1991), but the results of the present study emphasise the need for considerable caution when attempting to extrapolate plant growth responses in general and to elevated CO_2 in particular.

Multigeneration responses

While it is difficult to use early, vegetative responses as a basis for making predictions about reproductive growth

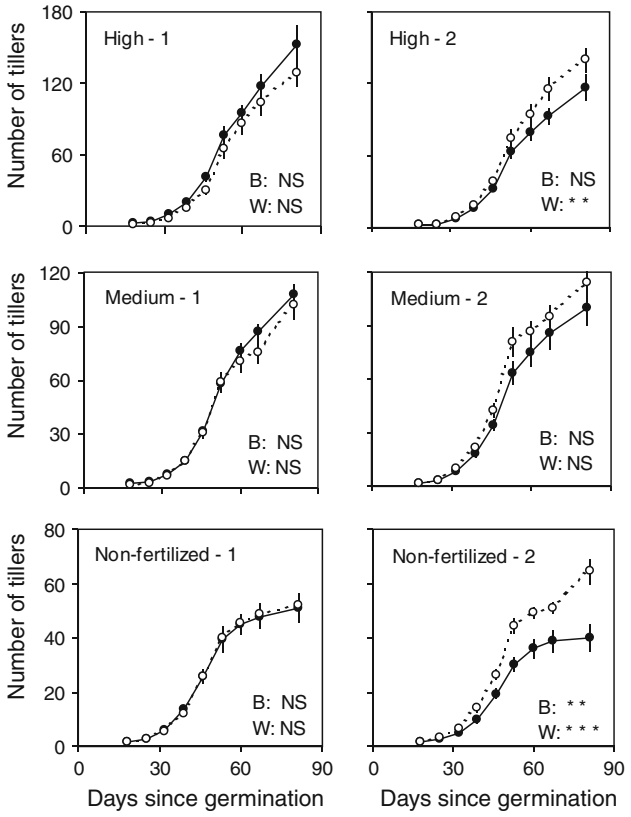

Fig. 3 The effect of ambient (*solid lines*) and elevated (*dotted lines*) atmospheric CO_2 on the vegetative growth responses of first- and second-generation *P. annua plants* grown in high or medium fertilised soil or in nonfertilised soil. Means are shown (n = 12) ± SE and P values are based on RMANOVA. See the legend for Fig. 1

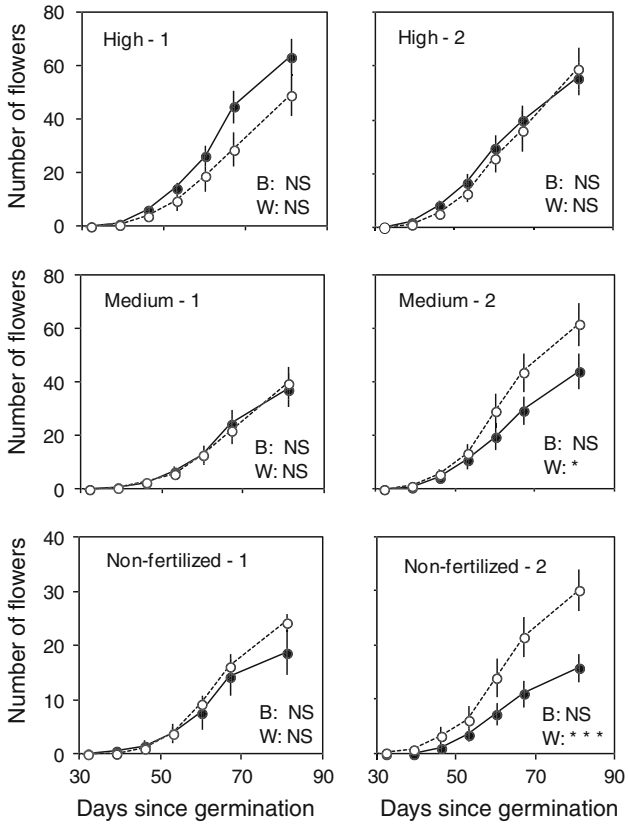

Fig. 4 The effect of ambient (*solid lines*) and elevated (*dotted lines*) atmospheric CO_2 on the reproductive growth responses of first- and second-generation *P. annua* plants grown in high or medium fertilised soil or in nonfertilised soil. Means are shown (n = 12) ± SE and P values are based on RMANOVA. See the legend for Fig. 1

responses, it is even more difficult to extrapolate from one generation to the next. Plants grown from seeds produced by parental plants grown for a whole generation in elevated CO_2 performed significantly better in terms of tiller and inflorescence production than plants grown in ambient conditions for two generations. This response could not be predicted from the performance of the first-generation plants. A plant's response to elevated CO_2 seems to be not only species-specific (e.g. Bazzaz et al. 1992; Farnsworth and Bazzaz 1995; Jones et al. 1998) and dependent on the plant's growth stage and nutrient availability (this study; Körner 1995; Thomas and Jasienski 1996), but can also differ between succeeding plant generations.

Biomass responses showed a similar intergenerational trend to elevated CO_2, with second-generation plants being more responsive than first-generation plants. This pattern was not apparent from leaf photosynthetic activity, as there were no significant CO_2 × generation interactions for these measurements, although there was a highly significant generation effect (Table 3). It is important to note that all of the leaves selected for photosynthetic measurements were of a similar age, so the data represent leaf photosynthetic capacity rather than plant net carbon uptake (a variable more closely related to plant growth). From experiment 1, we also know that under conditions of elevated CO_2, leaf aging of *P. annua* is enhanced. This, in turn, strongly influences leaf photosynthetic activity (Evans 1989; Schulze et al. 1994). It is therefore not surprising that measurements of leaf photosynthesis and plant growth did not correspond. This confirms the findings of Stirling et al. (1997), which show that increased photosynthesis of *P. annua* in elevated CO_2 need not necessarily be translated into increases in aboveground dry matter or enhanced growth.

The long-term effects of elevated CO_2 on the performance of a plant species will depend on changes in its fecundity and competitive ability (Bazzaz et al. 1992; Schmid et al. 1996). A number of studies have now been published which show that a variety of plant species show intergenerational differences in their responses to conditions of elevated CO_2 (Andalo et al. 1998; Bezemer et al. 1998; Huxman et al. 1998, 2001; Derner et al. 2004; Lau et al. 2008). In our study, seed weight and germinability of *P. annua* were both significantly lower for plants grown in conditions of elevated CO_2, and this might—at least partly—explain the differences observed in CO_2 responsiveness during the succeeding

Table 4 Experiment 2

	Generation 1			Generation 2		
	Ambient	Elevated	*P*	Ambient	Elevated	*P*
Bud initiation time (days)						
High	50.2 ± 1.3	57.5 ± 3.2	*	47.2 ± 1.7	50.6 ± 2.4	NS
Medium	57.7 ± 2.8	57.3 ± 2.7	NS	56.7 ± 2.6	53.1 ± 2.1	NS
Nonfertilised	62.3 ± 3.6	58.2 ± 2.0	NS	59.1 ± 1.9	55.1 ± 2.9	NS
Aboveground biomass (g)						
High	7.2 ± 0.5	7.8 ± 0.9	NS	5.7 ± 0.7	7.9 ± 0.9	*
Medium	4.6 ± 0.3	5.1 ± 0.6	NS	5.3 ± 0.5	6.1 ± 0.6	NS
Nonfertilised	1.7 ± 0.1	2.3 ± 0.1	**	1.5 ± 0.2	2.3 ± 0.1	*
Belowground biomass (g)						
High	0.9 ± 0.1	1.1 ± 0.2	NS	0.6 ± 0.1	1.2 ± 0.3	*
Medium	0.9 ± 0.1	0.8 ± 0.1	NS	1.5 ± 0.3	1.2 ± 0.2	NS
Nonfertilised	0.7 ± 0.1	1.1 ± 0.2	NS	0.6 ± 0.1	1.0 ± 0.1	*

Effect of elevated CO_2 on bud initiation time and biomass measurements of *P. annua* grown for one and two generations in ambient or elevated CO_2 in high and medium fertilised and nonfertilised soil. For pair-wise comparisons, the *P* value indicates the significance level based on ANOVA
* $P < 0.05$
** $P < 0.01$
NS $P > 0.05$

generation. Decreased germination (Farnsworth and Bazzaz 1995; Andalo et al. 1996) and seed weight (Wulff and Alexander 1985) have been reported for other plant species growing in elevated CO_2, although enhanced seed weight has also been reported (Steinger et al. 2000; Miyagi et al. 2007). Although most studies point to CO_2-induced effects on seed characteristics as the mechanism through which intergenerational differences occur, it may also be possible that these effects are driven by genetic changes in a plant's response to elevated CO_2 (Ward and Kelly 2004; Lau et al. 2008; Leakey et al. 2009). Genetic changes often occur over multiple generations, but recent epigenetic studies have shown that heritable changes in gene expression and function can also occur from one generation to the next. These effects are not driven by changes in DNA sequence but by molecular processes that can activate, reduce or disable the activity of particular genes (Bossdorf et al. 2008). Molecular ecological studies are needed that address the question of whether intergenerational responses of plants to elevated CO_2 are indeed driven by epigenetic changes.

Nutrient responses

The CO_2 responsiveness of a plant is often determined by the availability of nutrients, as these may limit the magnitude of a plant's response (Sionit et al. 1981; Zangerl and Bazzaz 1984; Bowler and Press 1996). As *P. annua* is a species with a ruderal (R) primary strategy (Grime 1974), little or no response would be expected to elevated CO_2, even in environments when other growth conditions are nonlimiting (Hunt et al. 1991). It is therefore interesting that in experiment 2, the CO_2 responsiveness of *P. annua* was most obvious at low nutrient levels (see Figs. 3, 4). There was also evidence for photosynthetic acclimation at elevated CO_2 at the end of the experiment at this nutrient level. The results allow us to suggest that photosynthetic rates were downregulated by the redistribution of nitrogen within the plant away from Rubisco and into the formation of additional reproductive biomass and leaf tissue (Sage and Pearcy 1987; Stitt 1991; Ellsworth et al. 2004). Plants grown at elevated CO_2 in nonfertilised soil were able to convert the limiting amount of nutrients available into biomass more efficiently than plants grown at ambient levels of CO_2. Photosynthetic acclimation, however, appeared to occur independent of the maternal environment, emphasising that differences between generations in how plants respond to elevated CO_2 are at least in part caused by other phenomena such as seed quality or epigenetics.

In conclusion, this study demonstrates temporal differences in the growth response of a plant to elevated CO_2 highlighting the importance of long-term and multigeneration experiments in determining the effects of this and probably other climate change factors on plant growth and development. Further studies are urgently required to fully understand the mechanisms by which these changes occur and how general such phenomena are.

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