

Intergenerational above- and belowground responses of spring wheat (*Triticum aestivum* L.) to elevated CO₂

Justin D. Derner^{1,*}, Charles R. Tischler², H. Wayne Polley², Hyrum B. Johnson²

¹ USDA-ARS, High Plains Grasslands Research Station, Cheyenne, WY USA

² USDA-ARS, Grassland, Soil and Water Research Laboratory, Temple, TX USA

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Abstract

We quantified intergenerational above- and belowground responses of two genotypes of semi-dwarf, hard red, spring wheats (*Triticum aestivum* L.) to elevated (700 $\mu\text{mol mol}^{-1}$) CO₂. These plants were progeny of seeds produced from previous generation plants grown at elevated CO₂ under well-watered and high nutrient conditions. Because neither genotype in the first generation exhibited enhanced performance with CO₂ enrichment, our objective in this investigation was to assess if exposure to CO₂ enrichment in subsequent generations resulted in temporal changes in the relative enhancement (elevated/ambient) of above- and belowground plant growth. Relative enhancement occurred in both the second and third generations for both above- and belowground variables. Above- and belowground variables were enhanced by similar relative amounts at elevated CO₂ within a generation at each harvest date. Relative enhancement of measured variables was generally greater in the third than second generation when plants were in the seedling or vegetative stage, but not when plants were reproductive. Additional research is needed to investigate physiological or other limitations of translating above- and belowground responses to CO₂ in vegetative growth stages to reproductive performance. Intergenerational above- and belowground responses of this C₃ annual plant to CO₂ enrichment are not driven by genetic change (selection) that occurred between generations, but rather CO₂-induced changes in seeds that affected seedling responses to CO₂ enrichment.

Wir quantifizierten die intergenerationelle ober- und unterirdische Reaktionen von zwei Genotypen mittellangen, hartrotten Winterweizen (*Triticum aestivum* L.) auf erhöhtes CO₂ (700 $\mu\text{mol mol}^{-1}$). Diese Pflanzen waren Abkömmlinge von Samen, die von Pflanzen der vorherigen Generation produziert wurden, welche ihrerseits bei erhöhtem CO₂ und bei ausreichender Wasserversorgung sowie guten Nährstoffbedingungen kultiviert wurden. Weil keiner der beiden Genotypen in der ersten Generation eine verbesserte Leistung bei CO₂-Anreicherung zeigte, war unser Ziel, in der Untersuchung abzuschätzen, ob die Exposition einer CO₂-Anreicherung in den nachfolgenden Generationen zu temporären Veränderungen in der relativen Förderung (erhöht/umgebend) des ober- und unterirdischen Wachstums führte. Eine relative Steigerung fand in der zweiten und in der dritten Generation sowohl bei den ober- als auch unterirdischen Variablen statt. Bei jedem Erntetermin waren die ober- und unterirdischen Variablen innerhalb einer Generation bei erhöhtem CO₂ mit ähnlichen relativen Anteilen positiv beeinflusst. Die relative Steigerung der gemessenen Variablen war im Allgemeinen bei Pflanzen im Keimlings- oder vegetativen Stadium in der dritten Generation größer als in der zweiten, jedoch nicht bei reproduktiven Pflanzen. Zusätzliche Forschung ist notwendig, um physiologische oder andere Limitierungen zu untersuchen, die ober- und unterirdische Reaktionen von vegetativen Wachstumsstadien auf CO₂ in die reproduktive Leistung übersetzen.

*Corresponding author: Justin D. Derner, USDA-ARS, High Plains Grasslands Research Station, 8408 Hildreth Road, Cheyenne, WY USA 82009, Phone: ++1-307-772-2433, Fax: ++1-307-637-6124, E-mail: jderner@npa.ars.usda.gov

Intergenerationelle, ober- und unterirdische Reaktionen dieser C₃-Pflanze auf CO₂-Anreicherung werden nicht durch genetische Veränderungen (Selektion) im Laufe der Generationen gesteuert, sondern eher durch CO₂-induzierte Veränderungen in den Samen, welche die Reaktion der Keimlinge auf eine CO₂-Anreicherung beeinflussen.

Key words: C₃ plant – carbon dioxide – physiological tradeoffs – relative enhancement – root responses – vegetative vs. reproductive growth

Introduction

The C₃ annual crop species spring wheat (*Triticum aestivum* L.) has been subjected to many elevated atmospheric carbon dioxide (CO₂) studies evaluating above-ground growth and grain yield (Samarakoon et al. 1995, Mulholland et al. 1998, Hudak et al. 1999, McMaster et al. 1999, Pleijel et al. 2000, Grossman-Clarke et al. 2001, Schütz & Fangmeier 2001). Generally, CO₂ enrichment increases leaf area, tillering, shoot growth and yield. However, in-depth investigations of belowground responses to CO₂ enrichment are few. Root biomass has been shown to be unresponsive to elevated CO₂ (Hudak et al. 1999), or to increase relatively more than does shoot biomass (McMaster et al. 1999, Wechsung et al. 1999).

Because there is genetic variation within species in responses to elevated CO₂ (Curtis et al. 1996, Schmid et al. 1996, Thomas & Jasienski 1996, Bezemer et al. 1998, Van der Kooij et al. 2000, Andalo et al. 2001, Klus et al. 2001, Goverde et al. 2002), impacts of CO₂ enrichment on spring wheat may be genotype-specific (Mulholland et al. 1998). Genotypic differences in responses of spring wheat to CO₂ enrichment have been recently assessed. Genotypes differ in response to CO₂ enrichment when they vary in gross morphology (e.g., height, Mayeux et al. 1997, De la Puente et al. 2000), but not in leaf physiology (Samarakoon et al. 1995).

Responses of plants to elevated CO₂ have been well-chronicled (Poorter 1993, Bunce 1997, 2001, Cotrufo et al. 1998, Pritchard et al. 1999, Makino & Mae 1999, Ward et al. 1999, Ward et al. 1999, Arnone et al. 2000, Campbell et al. 2000, Ghannoum et al. 2000, Norby & Jackson 2000, Pritchard & Rogers 2000, Zak et al. 2000, BassiriRad et al. 2001, Jablonski et al. 2002, Edwards et al. 2003), but most studies were conducted on plants grown for only a single generation, or more frequently, less. There is a paucity of information on the extent to which plants show intergenerational differences in their response to elevated CO₂. A few studies with C₃ annuals indicate that elevated CO₂ may differentially affect growth across generations (Curtis et al. 1996, Bezemer et al. 1998, Huxman et al. 1998, Ward et al. 2000), with potentially no further increases in plant biomass relative to first generational responses (Ward et al. 2000).

We quantified responses of three generations of two genotypes of spring wheat to CO₂ enrichment in glasshouse experiments. Second and third generations of plants were progeny of seeds produced by plants grown at elevated CO₂ (700 $\mu\text{mol mol}^{-1}$) under well-watered and high nutrient conditions the previous generation. Neither genotype in the first generation exhibited enhanced biomass or reproductive performance with CO₂ enrichment (see Materials and Methods, below). Because of this, our objective in this investigation was to assess if exposure to CO₂ enrichment in subsequent generations resulted in temporal changes in the relative enhancement (elevated/ambient) of above- and belowground plant growth.

Materials and methods

The CO₂ concentration of air in each of the four glasshouse bays was measured at 4-min intervals with a Li-Cor Model LI-6262 infrared gas analyzer (Li-Cor, Inc., Lincoln, NE). The CO₂ readings were corrected for atmospheric pressure measured with a Druck model DPI 260 pressure indicator (Druck, Inc., New Fairfield, CT). The infrared analyzer was calibrated daily against four CO₂ gas standards and monthly against a Li-Cor LI-610 dewpoint generator. Air temperature, manually set at 25 °C for both day and night, was measured in the center of each bay with fine-wire (25- μm diameter) thermocouples. Pure CO₂ gas was injected into appropriate bays as required to maintain the elevated CO₂ concentration. The CO₂ concentration of air in the ambient and elevated CO₂ treatments averaged 360 and 697 $\mu\text{mol mol}^{-1}$ in 1995, respectively, 363 and 699 $\mu\text{mol mol}^{-1}$ in 1999, respectively, and 365 and 701 $\mu\text{mol mol}^{-1}$ in 2000, respectively. Photosynthetic photon flux density (PPFD) was measured on the greenhouse roof with a quantum sensor (LI-190SB, Li-Cor) and within the glasshouse bays with 1-m long line quantum sensors (LI-191SA, Li-Cor) mounted about plant height. On average, the daily integral of PPFD inside the glasshouse bays was 70% of that measured above the greenhouse.

A rust resistant common wheat (AZ-MSFRS-82RR, Thompson 1983) and a highly productive, commercially available hybrid (DK49, Douglas King) were

studied. Both genotypes are semi-dwarf, hard red, spring wheats. Seeds collected from first generation plants (1995 experiment) grown at 700 $\mu\text{mol mol}^{-1}$ CO₂ were pooled and used for second generation plants (1999 experiment). The same approach was used for third generation plants (2000 experiment) as seeds produced from second generation plants grown at 700 $\mu\text{mol mol}^{-1}$ CO₂ were pooled. We justify the use of seed produced under CO₂ enrichment for use at both elevated and ambient CO₂ in subsequent generations as seed produced at elevated and ambient CO₂ differ in composition (e.g., Huxman et al. 1998, review by Jablonski et al. 2002), and thus also in properties of the resulting seedlings.

To prevent confounding influences of seed mass on initial seedling development (Villar et al. 1998), seeds of each genotype were weighed prior to planting of the first generation to ensure similar seed mass (0.050–0.060 g grain⁻¹). Three seeds of each genotype were planted in commercial potting mix into each of 40, 0.0025 m³ (0.15 m diameter $\times$ 0.14 m deep) pots in mid-February 1995. Although this pot size is small, both of these wheat varieties are spring wheats that mature quickly and have minimal tillering, thereby minimizing the effects of pot size on plant performance. Following planting, pots with each genotype were randomly assigned to one of four glasshouse bays, two maintained at ambient CO₂ and two at elevated CO₂, with 10 pots per genotype in each bay. Upon emergence, seedlings were randomly thinned to one per pot. Soil in pots was wetted three times per week with 200 ml of full-strength Hoagland's solution (Hoagland & Arnon 1950). Positions of pots within each bay were randomized twice weekly.

All plants in the first generation were harvested at physiological maturity, designated when the entire seed head was brown. Neither genotype exhibited differences between CO₂ treatments for aboveground mass (14.36 ± 0.86 vs. 14.87 ± 0.89 g plant⁻¹, ambient vs. elevated) or reproductive variables including number of seed heads (6.1 ± 0.4 vs. 6.5 ± 0.3) and seed mass (6.38 ± 0.40 vs. 6.49 ± 0.31 g plant⁻¹).

Seeds of each genotype grown under CO₂ enrichment the previous generation were weighed prior to planting to ensure similar seed mass for each generation (0.0450–0.055 g seed⁻¹, second generation; 0.025–0.035 g grain⁻¹, third generation). These values represent the mean ($\pm$ 1 SD) seed mass from plants produced the previous generation.

Three seeds of each genotype were planted in mid-February in a sandy loam soil into each of 120 same-sized pots for the second (1999) and third (2000) generations. Emerged seedlings were thinned randomly to result in one per pot. Properties of the soil include: pH = 7.1, organic carbon content = 0.57%, 76.2%

sand, 16.2% silt, 7.6% clay, field capacity = 18% on a volumetric basis. This soil was chosen for the second and third generations in contrast to the commercial potting mix because we wanted to examine root responses in these generations. This soil facilitates retrieval of roots through manual root washing, whereas the commercial potting mix presents methodological problems in the recovery of the entire root system. We used the same experimental protocol for the second and third generation plants as for the first generation plants, with the exception that there were 30 pots per genotype in each bay.

In contrast to the single harvest of first generation plants, we harvested one-third of the seedlings for each genotype ($n = 40$) at 10- and 21-days post-emergence and physiological maturity. At the 10- and 21-day harvests, aboveground biomass was separated into leaf and stem components. Area of each individual leaf blade was measured using a LI-3000A portable leaf area meter (Li-Cor, Inc. Lincoln, NE).

Soil was manually washed from roots, which were then digitally scanned using the WinRHIZO software (Regent Instruments, Inc. Quebec, Canada, version 3.9f) and hardware (Hewlett Packard ScanJet 6100C scanner). This software determines root length, surface area and root volume. Roots were scanned using a 10 cm $\times$ 15 cm tray for the 10- and 21-day harvests and a 15 cm $\times$ 25 cm tray for the final harvest at high resolution (600 dpi) in all cases. Roots were not stained prior to analyses, which result in underestimations (Bouma et al. 2000), but this was minimized by using high resolution and the WinRHIZO automatic threshold to increase sensitivity to pale roots. Aboveground tissues and roots were dried at 60 °C for 72 h prior to weighing.

Leaf area ratio (LAR) was calculated as total plant leaf blade area per total plant mass. Specific leaf area (SLA) was determined as total leaf blade area per total leaf mass. Leaf mass ratio (LMR) was calculated as total leaf blade mass per total plant mass.

Because the generations were not grown in the same growing season, no statistical tests were conducted between the generations. However, inferences are drawn between second and third generations at each harvest using the term relative enhancement, which was calculated by dividing the mean response of plants with CO₂ enrichment by the mean response of plants with ambient CO₂. Data were analyzed by harvest date each year using a split plot design with CO₂ as the main plot and the genotype as split plots in a balanced design (SAS Institute Inc. 1994). Means were separated by Duncan's multiple range test at the 0.05 level of probability. When needed to normalize residuals, data were logarithmically transformed before analysis; means and standard errors are reported after back-transforming.

Results

Second generation

Aboveground variables, with the exception of number of seed heads, were similar between the two genotypes at all harvests (data not shown). However, belowground variables were greater for DK49 than for AZ at day 10 (31–53%) and the final harvest (17–56%), but not day 21. Interactions between genotype and CO₂ were only observed for root variables at day 21 with greater responses (32–104%) to CO₂ enrichment displayed by DK49 than by AZ (2–27%) (data not shown).

Total plant mass increased by 21%, 38% and 63% with CO₂ enrichment at day 10, day 21 and the final harvest, respectively (Table 1). Total aboveground mass (31–60%), stem mass (39–77%) and leaf mass (18–27%) also increased with CO₂ enrichment across harvests. CO₂ enrichment decreased LAR, LMR and SLA by 7–22% at day 10 and day 21 (Table 2), but increased leaf area by 13% and 18% day 10 and day 21, respectively (Table 1). Also, the number of seed heads per plant, seeds per plant and seed mass per plant nearly doubled with CO₂ enrichment. CO₂ enrichment increased root mass by 19–88%, root volume by 35–110%, root surface area by 34–40% and root length by 36–44% across harvests.

Third generation

Both above- (15–60%) and belowground (18–36%) growth was greater for genotype DK49 than for AZ at the day 10 harvest, but these differences were not observed at day 21 nor at the final harvest, with the exception of the number of seed heads (data not shown). No interactions between genotype and CO₂ were observed for any variable at any of the harvests.

Total plant mass increased by 53%, 104% and 42% with CO₂ enrichment at day 10, day 21 and the final harvest, respectively (Table 1). Total aboveground mass (43–108%), stem mass (89–121%) and leaf mass (33–103%) exhibited a similar pattern in response to CO₂ enrichment at the day 10 and day 21 harvests. CO₂ enrichment decreased LAR and LMR, but not SLA, by 8–10% at day 10, with no differences displayed at day 21 (Table 2). Leaf area, however, increased with CO₂ enrichment by 26% and 93% at day 10 and day 21, respectively (Table 1). In contrast to vegetative responses to CO₂ enrichment, the number of seed heads per plant, seeds per plant and seed mass per plant were similar between CO₂ treatments. CO₂ enrichment increased root mass by 75–95%, root volume by 45–85%, root surface area by 39–66% and root length by 42–53% at the day 10 and day 21 harvests, but these differences did not persist in the final harvest.

Relative enhancement across generations

Third generation plants displayed increased relative enhancement, compared to second generation plants, of all aboveground variables at day 10 and day 21, but reductions in enhancement of all final harvest parameters (Table 3). Both second and third generation plants displayed similar relative enhancement of root variables at day 10, with the exception of root mass which displayed greater relative enhancement in the third than second generation. In contrast, relative enhancement markedly increased for belowground variables in the third generation at day 21. However, this trend reversed at the final harvest with the third generation exhibiting lower relative enhancement than the second generation. Plants exhibited a greater growth response at elevated than ambient CO₂ for all variables in both generations. Overall, relative enhancement was similar between above- and belowground variables within a generation at each harvest date.

Discussion

Intergenerational above- and belowground plant growth of spring wheat (*T. aestivum*) to elevated CO₂ exhibited intriguing trends. First, relative enhancement occurred in both the second and third generations for both above- and belowground variables. Second, above- and belowground variables were enhanced by similar relative amounts at elevated CO₂ within a generation at each harvest date. Third, relative enhancement of measured variables was generally greater in the third than second generation when plants were in the seedling or vegetative stage, but not when plants were reproductive. However, the occurrence of absolute reductions in plant size and production from the first to third generation, at both ambient and elevated CO₂ concentrations, advises that these results should be cautiously interpreted. The responsiveness of subsequent generations may be attributable to a compensatory response to limited growing conditions, such as the relatively nutrient-poor soil used. Even though supplemental nutrients were provided three times a week for the second and third generation plants, this soil was not comparable to the commercial potting mix used for the first generation plants. Thus, this discrepancy in soils likely contributed to the reduction in plant size and production in the second and third generation plants. Nutrient stress in C₃ plants tends to reduce responses to elevated CO₂ (Ward et al. 1999, Kimball et al. 2002). That we observed significant trends despite absolute reductions in plant and seed size and biomass production from the first to third generations does, however, suggest that elevated

Table 1. Mean ($\pm$ SE, $n = 40$) above- and belowground responses to ambient CO₂ (A) or CO₂ enrichment (E) (360 or 700 $\mu\text{mol mol}^{-1}$, respectively) of second and third generation spring wheat (*Triticum aestivum*) plants, averaged across two genotypes. Plants were grown from seeds derived from plants grown during the previous generation at elevated CO₂. Asterisks indicate a significant ($P < 0.05$) difference between CO₂ treatments within a harvest.

Variable	2 nd generation					3 rd generation				
	Day 10		Day 21		Final	Day 10		Day 21		Final
	A	E	A	E	A	A	E	A	E	A
Total Mass (g)	0.077 (0.009)	0.093* (0.009)	0.258 (0.016)	0.355* (0.018)	6.270 (0.404)	0.040 (0.001)	0.061* (0.005)	0.069 (0.005)	0.141* (0.009)	1.417 (0.231)
Aboveground										
Plant mass (g)	0.046 (0.002)	0.061* (0.005)	0.175 (0.010)	0.229* (0.013)	5.829 (0.365)	0.028 (0.001)	0.040* (0.004)	0.049 (0.004)	0.102* (0.008)	1.297 (0.212)
Stem mass (g)	0.013 (0.001)	0.023* (0.004)	0.059 (0.004)	0.082* (0.005)		0.009 (0.000)	0.017* (0.004)	0.014 (0.001)	0.031* (0.003)	
Leaf mass (g)	0.033 (0.001)	0.039* (0.002)	0.116 (0.007)	0.147* (0.008)		0.018 (0.001)	0.024* (0.001)	0.035 (0.003)	0.071* (0.006)	
Leaf area (cm ²)	12.83 (0.64)	14.52* (0.61)	42.61 (2.57)	50.63* (2.70)		7.83 (0.20)	9.88* (0.29)	11.94 (0.94)	22.99* (1.40)	
Seed heads (number)					2.3 (0.2)		4.3* (0.1)			1.3 (0.1)
Seed count (seeds/plant)					69 (4)		118* (4)			22 (3)
Seed mass (mass/plant)					1.74 (0.12)		3.23* (0.12)			0.560 (0.095)
Belowground										
Root mass (g)	0.031 (0.008)	0.037* (0.003)	0.083 (0.008)	0.126* (0.009)	0.441 (0.035)	0.012 (0.001)	0.021* (0.004)	0.020 (0.002)	0.039* (0.002)	0.120 (0.021)
Volume (m ³)	0.51 (0.05)	0.72* (0.05)	1.49 (0.14)	2.01* (0.12)	6.93 (0.57)	0.20 (0.01)	0.29* (0.02)	0.40 (0.04)	0.74* (0.04)	1.60 (0.26)
Surface area (cm ²)	42.7 (3.4)	59.7* (3.7)	124.5 (9.4)	166.8* (8.5)	346.8 (17.9)	22.6 (1.2)	31.4* (1.4)	37.3 (3.0)	61.8* (2.5)	134.4 (19.3)
Length (cm)	311 (22)	443* (28)	939 (60)	1280* (72)	2088 (125)	217 (10)	308* (11)	330 (21)	504* (19)	1228 (196)

CO₂ is beneficial to spring wheat relative to ambient concentrations.

Our finding that biomass, reproductive output and other measurements of above- and belowground plant growth remained greater at elevated than ambient CO₂ through three generations contrasts with results from the few previous studies because this investigation tested for maternal change (no genetic change) while others

analyzed for effects of genetic change. For example, second generation plants of the C₃ annual grass *Bromus rubens* that were selected at elevated CO₂ did not differ in shoot mass, leaf area, seedling mass, root mass, LMR, LAR, or SLA between elevated and ambient CO₂ concentrations (Huxman et al. 1998). In addition, plants of *Arabidopsis thaliana* selected at elevated CO₂ had similar plant mass as control plants, because of a faster life cycle (Ward et al. 2000). Yet, second generation plants of *Poa annua* produced more tillers in elevated than ambient CO₂ (Bezemer et al. 1998). Additionally, contrasting results between this investigation and previous studies may be attributed, in part, to differences among species studied. Spring wheat is a cultivated crop species that has undergone genetic selection to enhance plant growth, while the C₃ annual species used in previous investigations have not been selected by humans for yield.

Commercially available and common wheat genotypes responded similarly to CO₂ enrichment suggesting that resulting evolution of plant traits is not specific to CO₂, but rather is a response to characteristics common at both ambient and elevated CO₂ (Klus et al. 2001). Unfortunately, few studies have determined whether genotypes respond differently when grown under CO₂ enrichment or ambient levels (Bunce 2001, Klus et al. 2001). In contrast to our results, Klus et al. (2001) found that physiological parameters and tissue chemistry, but not mass allocation, of both families and populations of *Plantago lanceolata* responded differently to CO₂, suggesting that evolution of some plant traits is CO₂-specific.

Table 2. Mean (SE, n = 40) leaf area ratio (LAR, cm² g⁻¹), leaf mass ratio (LMR, g g⁻¹) and specific leaf area (SLA, cm² g⁻¹) responses to ambient CO₂ (A) or CO₂ enrichment (E) (360 or 700 μmol mol⁻¹, respectively) of second and third generation spring wheat (*Triticum aestivum*) plants, averaged across two genotypes. Plants were grown from seeds derived from plants grown during the previous generation at elevated CO₂. Asterisks indicate a significant (P < 0.05) difference between CO₂ treatments within a harvest.

Harvest	LAR		LMR		SLA	
	A	E	A	E	A	E
Second Generation						
Day 10	197.6 (8.0)	154.4* (5.7)	0.477 (0.016)	0.409* (0.013)	412.7 (6.0)	377.4* (4.6)
Day 21	170.1 (5.0)	144.9* (4.0)	0.458 (0.011)	0.417* (0.008)	370.4 (3.8)	346.1* (3.9)
Third Generation						
Day 10	196.5 (3.6)	176.9* (6.3)	0.462 (0.007)	0.426* (0.015)	425.0 (4.0)	417.8 (6.6)
Day 21	172.2 (3.4)	166.0 (3.7)	0.517 (0.008)	0.494 (0.011)	334.6 (6.3)	336.4 (6.9)

Table 3. Relative (elevated/ambient) enhancement of above- and belowground growth at three harvest dates for second and third generation spring wheat (*Triticum aestivum*) plants grown from seeds produced at elevated CO₂.

Variable	Relative Enhancement					
	Day 10		Day 21		Final	
	Second	Third	Second	Third	Second	Third
Total Mass (g)	1.21	1.53	1.38	2.04	1.63	1.42
Aboveground						
Plant mass (g)	1.33	1.43	1.31	2.08	1.60	1.43
Stem mass (g)	1.77	1.89	1.39	2.21		
Leaf mass (g)	1.18	1.33	1.27	2.03		
Leaf area (cm ²)	1.13	1.26	1.18	1.93		
Seed heads (number)					1.87	1.25
Seed count (seeds/plant)					1.71	1.45
Seed mass (mass/plant)					1.86	1.26
Mean enhancement	1.35	1.48	1.29	2.06	1.76	1.35
Belowground						
Root mass (g)	1.19	1.75	1.52	1.95	1.88	1.28
Volume (m ³)	1.41	1.45	1.35	1.85	2.10	1.16
Surface area (cm ²)	1.40	1.39	1.34	1.66	1.39	1.16
Length (cm)	1.42	1.42	1.36	1.53	1.44	1.23
Mean enhancement	1.35	1.50	1.39	1.75	1.70	1.21

Comparable carbon allocation to above- and belowground growth likely contributed to the display of similar relative enhancement of above- and belowground parameters within a generation at each harvest date. Relative enhancement of aboveground responses in the seedling and early vegetative harvests can be attributed to the direct effect of elevated CO₂ increasing growth. In contrast, relative enhancement of aboveground responses at the final harvest was likely driven by tillering differences of plants which is consistent with previous investigations of spring wheat and CO₂ enrichment (Gifford 1977, du Cloux et al. 1987, Samarakoon et al. 1995, Hudak et al. 1999, McMaster et al. 1999, Pleijel et al. 2000). Stimulation of root responses to elevated CO₂ at the seedling and early vegetative stage contrasts with previous work on C₄ grass seedlings (Derner et al. 2001), suggesting that differences in C₃/C₄ physiology and carbon allocation patterns may be manifest in differential belowground responses as for well-studied aboveground responses (e.g., Poorter 1993).

Biomass was enhanced by CO₂ enrichment relatively more in the third than second generation while plants were in seedling and vegetative stages, but the reverse was true for plants in the reproductive stage. However, absolute biomass decreased for both ambient and elevated CO₂ grown plants. Physiological or other limitations prevented biomass responses from being translated into greater reproductive growth and yield. Unfortunately, most previous studies have evaluated responses to CO₂ enrichment only at single, final harvest date (Bezemer et al. 1998, Huxman et al. 1998, Ward et al. 2000). Though biomass responded positively to CO₂ enrichment for successive generations, reproductive growth in the third generation may have been inhibited by environmental factors (e.g., light influence on tillering) that constrained the ability of plants to fully display their genetic potential. This view is supported by observations that growing points shifted from vegetative to reproductive structures within 3 weeks of emergence (Tischler, personal observations). Therefore, potential seed number was set before season-long effects of elevated CO₂ could be manifest on other characteristics, such as aboveground mass or seed mass, another component of grain yield. Data in Tables 1 and 3 supports this view.

Both genotypes of the C₃ annual spring wheat exhibited CO₂ responsiveness for multiple generations, suggesting that CO₂ enrichment effects on seed compositional changes (higher C:N ratio, Huxman et al. 1998, Jablonski et al. 2002) contributed to greater seedling growth in subsequent generations, especially under well-watered and high nutrient conditions. As a result of greater relative seedling growth in each generation, responsiveness to CO₂ enrichment was manifest

at subsequent harvests. Intergenerational above- and belowground responses of this C₃ annual plant to CO₂ enrichment are not driven by genetic change (selection) that occurred between generations, but rather CO₂-induced changes in seeds that affected seedling responses to CO₂ enrichment.

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