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**CO₂ alters water use, carbon gain, and yield for the dominant species in a natural grassland**

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**Abstract** Global atmospheric CO₂ is increasing at a rate of 1.5–2 ppm per year and is predicted to double by the end of the next century. Understanding how terrestrial ecosystems will respond to this changing environment is an important goal of current research. Here we present results from a field study of elevated CO₂ in a California annual grassland. Elevated CO₂ led to lower leaf-level stomatal conductance and transpiration (approximately 50%) and higher mid-day leaf water potentials (30–35%) in the most abundant species of the grassland, *Avena barbata* Brot. Higher CO₂ concentrations also resulted in greater midday photosynthetic rates (70% on average). The effects of CO₂ on stomatal conductance and leaf water potential decreased towards the end of the growing season, when *Avena* began to show signs of senescence. Water-use efficiency was approximately doubled in elevated CO₂, as estimated by instantaneous gas-exchange measurements and seasonal carbon isotope discrimination. Increases in CO₂ and photosynthesis resulted in more seeds per plant (30%) and taller and heavier plants (27% and 41%, respectively). Elevated CO₂ also reduced seed N concentrations (9%).

**Key words** Annual grassland · *Avena barbata* · CO₂ · Reproduction · Water relations

**Introduction**

The concentration of CO₂ in the atmosphere has increased 30% since the beginning of the industrial revolution and is predicted to double by the end of the next century (Keeling 1986; Schlesinger 1991). Such concentrations of atmospheric CO₂ are unprecedented over the last 160,000 years (Barnola et al. 1987), and probably over the last 10 million (Gammon et al. 1985; Van Der Burgh 1993). A major goal of current research is to understand how terrestrial ecosystems will respond in this changing environment (Tissue and Oechel 1987; Curtis et al. 1989; Bazzaz 1990).

For the few natural ecosystems where *in situ* responses to CO₂ have been examined, the results have been somewhat contradictory (Dahlan 1993). *Scirpus olneyi* growing in an estuarine marsh showed increased primary productivity and no evidence of photosynthetic downregulation after four years of field exposure to high CO₂ (Curtis et al. 1989; Arp and Drake 1991). In contrast, the tundra tussock grass *Eriophorum vaginatum* showed little growth response to elevated CO₂, and it downregulated photosynthesis to pre-treatment rates after only 3 weeks of exposure (Tissue and Oechel 1987). Plant productivity in these ecosystems is not generally limited by water availability, yet the interaction between plant growth and water use is fundamental to understanding the response of biological systems to increased atmospheric CO₂ (Morison 1993).

In this paper we present results from a high-CO₂ experiment in a California annual grassland, an ecosystem limited in part by water availability. Annual grasslands are uniquely suited to CO₂ research, since the low year-to-year buffering of carbon allows changes to be detected rapidly, and high plant densities allow tractable, well-replicated experiments. We monitored the most common species of the grassland, *Avena barbata* Brot., which makes up 30% of the community by number and an even greater proportion by biomass. The variables measured included stomatal conductance, transpiration, photosynthesis, leaf water potential, plant size, density, and the number and quality of seeds produced.
Materials and methods

The study site was an annual sandstone grassland at the Jasper Ridge Biological Preserve (37°24′ N, 122°13′ W) near Stanford University (McNaughton 1968). The species composition is typical of grasslands in cis-montane California, consisting almost entirely of Eurasian annuals, including *Avena*, *Bromus*, and *Lolium* spp. (Gulmon 1979). The climate at the site is characterized by cool, wet winters and warm, dry summers (Mooney et al. 1986). The elevation is 200 m and the average annual precipitation from 1975 to 1990 was 579 mm, though the 1992–1993 growing season was substantially wetter than average. No supplemental water or nutrients were added.

Three field treatments (ten replicates per treatment) were used to evaluate responses to elevated CO₂: no-chamber controls, open-top chambers with ambient CO₂, and open-top chambers with ambient +350 ppm (a seasonal average of 723 μmol mol⁻¹ CO₂). Each cylindrical open-top chamber was 1 m tall and 0.65 m in diameter (0.33 m² soil area), while no-chamber controls were 0.65-m wide rings at the soil surface. Individual blowers forced 4500 l/min of ambient air through each chamber (approximately ten air changes per minute), supplemented by 350 μmol mol⁻¹ CO₂ in high-CO₂ chambers. The experiments were performed the second growing season of CO₂ enhancement, and chamber CO₂ was maintained throughout the year. A more comprehensive description of the methods and rationale for the experiment can be found in Field et al. (1995).

We monitored the most common species of the grassland, *Avena barbata*. Its density as estimated from ninety-10 cm-diameter rings was approximately 1500 plants m⁻² (5000 plants m⁻² for all species); it makes up approximately 30% of community density and 40–50% of community biomass. Measurements were initiated in mid-March when the plants were of sufficient size, and continued through senescence in mid-May. Physiological measurements were taken on the most recently fully-expanded leaf of *Avena* plants selected randomly in each field plot (values for multiple leaves within a plot were averaged), and neither leaves nor plants were used more than once during the season. Stomatal conductance, transpiration, and photosynthesis were measured with a closed gas-exchange system (LI-6200, Li-Cor Inc., Lincoln, NE) at the treatment’s operational CO₂ concentration. Leaf conductances and transpiration on additional days were obtained with a steady-state porometer (LI 1600, Li-Cor Inc., Lincoln, NE). Mid-day leaf water potentials were measured with a pressure chamber (Scholander et al. 1965).

Leaf material for isotopic analysis was collected on three dates (12 March, 13 April, and 10 July 1993) from the most recently fully-expanded leaves of three random *Avena* plants within each plot. The leaves were oven-dried at 70°C, ground to a fine powder, and analyzed for their carbon isotope composition (δ¹³C, relative to the PDB standard) at the University of Utah’s Stable Isotope Ratio Facility. Carbon isotope discrimination (Δ) was determined from the leaf carbon isotope ratio (δleaf) by

\[
\Delta = \frac{(\delta_{\text{leaf}} - \delta_{\text{air}})}{(1 + \delta_{\text{air}})} \tag{1}
\]

where δleaf = -8.0‰ for the ambient CO₂ treatments; δleaf in elevated CO₂ was -21.3‰, -21.0‰, and -20.7‰ as averaged through 12 March, 13 April, and 10 July, respectively (the isotopic composition of high-CO₂ air was monitored twice a month during the growing season). Farquhar et al. (1989) derived the following equation for the relationship between Δ and c₁ (the intercellular CO₂ concentration within the leaf) for a C₃ plant:

\[
\Delta = a + b \cdot \frac{c_1}{c_3} \text{ or } \frac{c_1}{c_3} = \frac{(\Delta - a)}{b} \tag{2}
\]

where c₁ is the CO₂ concentration in the atmosphere, a is the ¹³C fractionation due to diffusion (4.4‰), and b is the net fractionation due to carboxylation (27‰).

The instantaneous water-use efficiency (WUE, molar ratio of photosynthesis to transpiration) is also related to c₁ and c₃ by

\[
A/E = \frac{(c_3 - c_1)}{(1.6c_3)} \tag{3}
\]

where A/E is the leaf to air vapor concentration gradient. The ratio of water-use efficiencies for two leaves experiencing the same Δw is therefore

\[
\frac{(A_1/E_1)}{(A_2/E_2)} = \frac{(c_{31} - c_{11})}{(c_{32} - c_{12})} \tag{4}
\]

with the information for respective c₁ and c₃ values obtained from Δ and Eq. 2.

Measurements of *Avena* height, density, and seed production were taken at the end of the growing season on all *Avena* plants within three randomly located 10-cm-diameter circles in each of the thirty 0.33-m² plots (ten replicates per treatment). Approximately 1000 *Avena* plants were measured overall, and values within each 0.33-m² plot were averaged. Average shoot biomass was obtained by harvesting one 10-cm-diameter circle per plot and drying and weighing the *Avena* plants (287 plants overall); limits on destructive sampling did not allow biomass determinations from >1 circle per plot. Fruit and seed weights were obtained from four fruits per plot on 20 May 1993 (one seed per fruit in *Avena*). Each set of four seeds was subsequently dried at 75°C, ground to a fine powder, and assessed for N and C concentrations with a Carlo-erba NA 1500 elemental analyzer.

*Avena* seed production, fruit and seed weights, and seed N and C concentrations were analyzed by one-way ANOVA. The number of *Avena* plants available for biomass determination was quite limited, because we were not able to harvest more than one 10-cm-diameter circle per plot. However, there were no limitations on the number of non-destructive estimates of height. Therefore, in order to assess treatment effects upon plant size, we used both an integrative multivariate ANOVA of height and mass (Wilks’ lambda, Johnson and Wichern 1988) and two unique univariate ANOVA. *Avena* density was analyzed by nonparametric Kruskal-Wallis test due to large variability within and among replicates and non-normal distribution of the data.

Results

Elevated CO₂ decreased mid-day stomatal conductance by 50% and 65% compared to *Avena* leaves in chamber and no-chamber controls (Fig. 1A), with similar reductions in transpiration (Fig. 1B). Midday leaf water potentials were up to 36% higher in elevated CO₂ until late in the growing season, when relative differences in stomatal conductance and leaf water potentials decreased (Fig. 1C).

Rates of *Avena* mid-day photosynthesis were on average 70% greater in elevated CO₂ than in chamber controls (Fig. 1D). Differences were smaller earlier in the growing season, when the soil was the wettest. As the season progressed and low-CO₂ plants reduced midday conductances, relative differences in photosynthesis increased and high-CO₂ leaves had rates more than double those in chamber controls (Fig. 1D). Even on the first sampling date when mid-day photosynthetic rates were similar, high-CO₂ plants had greater rates of photosynthesis later in the afternoon when stomata in all treatments began to close (data not shown).

The instantaneous water-use efficiency (WUE, molar ratio of photosynthesis to transpiration) of *Avena* plants was roughly double the WUE of control treatments on four dates of the growing season (Table 1). Overall, in-
stantaneous WUEs in high CO₂ were 2.2 times greater than either control treatment when averaged across the season (Table 1). Carbon isotope discrimination (Δ) provides a reliable estimate of WUE that is integrated temporally over the period of leaf construction (Ehleringer 1989). Values of Δ were similar for treatments (Table 2), and correspond to c/o ratios of 0.72–0.77 (Table 2, Eq. 2). Since c₂ in elevated CO₂ was approximately twice the ambient c₀, and the c/o ratios were similar for all treat-

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Date</th>
<th>30 March</th>
<th>20 April</th>
<th>27 April</th>
<th>5 May</th>
</tr>
</thead>
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<tr>
<td>No-chamber</td>
<td></td>
<td>2.78</td>
<td>1.72</td>
<td>1.27</td>
<td>1.89</td>
</tr>
<tr>
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<td></td>
<td>3.18</td>
<td>1.86</td>
<td>1.09</td>
<td>1.97</td>
</tr>
<tr>
<td>Chamber+CO₂</td>
<td></td>
<td>6.79</td>
<td>4.26</td>
<td>2.97</td>
<td>3.40</td>
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</tbody>
</table>

Fig. 1A Mid-day leaf stomatal conductance, B transpiration, C leaf water potential and D photosynthesis for Avena plants in no-chamber controls (solid triangles), chamber controls (open triangles), and chamber +350 (open circles) (approximately 700 mmol mol⁻¹ CO₂) (mean±SE, n=6–10). Physiological measurements were initiated in mid to late March, when the plants were of sufficient size, and continued through plant senescence in mid May. Measurements were taken on the most recent fully-expanded leaf of random Avena plants in each field plot (10 plots per treatment, multiple plants averaged for a given plot).

Fig. 2A The average seed number, B height, and C shoot biomass for individual Avena plants in no-chamber controls (solid bars), chamber controls (shaded bars), and chamber +350 (open bars) (approximately 700 mmol mol⁻¹ CO₂) (mean±SE, n=10 for each bar). Measurements of height and seed numbers were taken at the end of the growing season on all Avena plants within three random 10-cm diameter circles in each of the thirty 0.33-m² plots (ten replicates per treatment). Approximately 1000 individuals were measured overall, but values within each 0.33-m² plot were averaged. Average shoot biomass was obtained by harvesting one 10-cm wide ring per plot and drying and weighing the Avena plants (287 plants total); limits on destructive sampling did not allow biomass sampling from >1 ring.
Table 2 Leaf carbon isotope composition (\(\delta^{13}C\)) discrimination (\(\Delta\)), the ratio of intercellular to atmospheric CO\(_2\) concentrations (\(c_i/c_a\)), and relative water use efficiency on three dates of the 1993 growing season for \(\textit{Avena}\) plants from no-chamber controls, chamber controls, and chamber +350 \(\mu\)mol CO\(_2\) (mean\(±\)SE, \(n=5\) for all values). The carbon isotope composition of the air (\(\delta_{\text{air}}\)) was -8.0\%e for the ambient CO\(_2\) treatments; \(\delta_{\text{air}}\) in elevated CO\(_2\) was -21.3\%e, -21.0\%e, and -20.7\%e as averaged through 12 March, 13 April and 7 May, respectively (the isotopic composition of high-CO\(_2\) air was monitored twice a month during the growing season). See Eq. 1 in the Methods for the definition of \(\Delta\) and \(\delta_{\text{air}}\).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Date</th>
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<th>13 April</th>
<th>7 May</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\delta_{\text{leaf}})</td>
<td>No-chamber</td>
<td>-28.5(±)0.18</td>
<td>-28.5(±)0.20</td>
<td>-28.7(±)0.13</td>
</tr>
<tr>
<td>Chamber</td>
<td>-28.6(±)0.24</td>
<td>-28.9(±)0.12</td>
<td>-28.6(±)0.41</td>
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<tr>
<td>Chamber+CO(_2)</td>
<td>-42.2(±)0.80</td>
<td>-42.4(±)0.30</td>
<td>-42.1(±)0.45</td>
<td></td>
</tr>
<tr>
<td>(\Delta)</td>
<td>No-chamber</td>
<td>20.6(±)0.18</td>
<td>20.6(±)0.20</td>
<td>20.9(±)0.13</td>
</tr>
<tr>
<td>Chamber</td>
<td>20.8(±)0.24</td>
<td>21.1(±)0.12</td>
<td>20.8(±)0.42</td>
<td></td>
</tr>
<tr>
<td>Chamber+CO(_2)</td>
<td>21.3(±)0.81</td>
<td>21.8(±)0.31</td>
<td>21.7(±)0.46</td>
<td></td>
</tr>
<tr>
<td>(c_i/c_a)</td>
<td>No-chamber</td>
<td>0.72</td>
<td>0.72</td>
<td>0.73</td>
</tr>
<tr>
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<td>0.72</td>
<td>0.74</td>
<td>0.73</td>
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</tr>
<tr>
<td>Chamber+CO(_2)</td>
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<td>0.77</td>
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<tr>
<td>Relative WUE</td>
<td>No-chamber</td>
<td>1.0</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Chamber</td>
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<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Chamber+CO(_2)</td>
<td>1.8</td>
<td>1.8</td>
<td>1.7</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3 Average density of \(\textit{Avena}\) plants in no-chamber controls (solid bars), chamber controls (shaded bars), and chamber +350 (open bars) (mean\(±\)SE, \(n=10\) for each bar). Density was measured in three random 10-cm-diameter circles in each of the thirty 0.33-\(m^2\) plots (ten replicates per treatment), and the three estimates per plot were averaged to avoid pseudoreplication. Plant density varied substantially within and among plots, and was not significantly different among treatments (\(P>0.15\) by Kruskal-Wallis test of the three treatments).

Fig. 4 A Average mass per seed and B mass per fruit for \(\textit{Avena}\) plants from no-chamber controls (solid bars), chamber controls (shaded bars), and chamber +350 (open bars) (mean\(±\)SE, \(n=10\) for each bar). Four seeds/fruits were randomly selected from each plot, dried at 75\(^\circ\)C, and weighed. Elevated CO\(_2\) increased seed and fruit weights by 9\% relative to chamber controls, but differences were not significant (\(P>0.14\) for both). Average seed and fruit mass in the no-chamber treatment were significantly smaller than in either chamber treatment (\(P<0.01\)).

ments (Table 2, Eq. 2), the drawdown of CO\(_2\) within the leaf (\(c_i/c_a\)) was approximately twice as large in elevated CO\(_2\) as in ambient CO\(_2\). By Eq. 4, we conclude that the integrated WUE was almost twice as great in elevated CO\(_2\) as in ambient CO\(_2\) (Table 2). The seasonal estimate of WUE from carbon isotope discrimination was 1.8, in close agreement with our estimate of 2.2 from gas exchange measurements.

To be important in an evolutionary context to plants, physiological changes must ultimately lead to changes in plant fitness. \(\textit{Avena}\) plants in the high-CO\(_2\) treatment produced 1.5-1.6 times more seeds on average than in either of the ambient-CO\(_2\) treatments (Fig. 2A, \(P=0.02\)), an increase of approximately 30\%. High-CO\(_2\) \(\textit{Avena}\) plants also were significantly larger than ambient-CO\(_2\) plants (Fig. 2B and 2C, \(P<0.0005\) by multivariate ANOVA, \(P<0.001\) and \(P=0.17\) individually for univariate ANOVAs of height and biomass). High-CO\(_2\) plants were 27\% taller and 41\% heavier on average than plants from...
Fig. 5 A Seed nitrogen and B carbon concentrations for *Avena* plants from no-chamber controls (solid bars), chamber controls (shaded bars), and chamber +350 (open bars) (mean±SE, n=10 for the chamber treatments, n=7 for the no-chamber treatment). The N concentration of seeds was 2.84% in chamber controls but only 2.59% in elevated CO₂, a significant reduction of 9% (P=0.04). The carbon concentration of seeds was unchanged by CO₂ (P=0.72, 47% C for all treatments).

chamber controls, and 63% taller and 48% heavier than plants from no-chamber controls. *Avena* density was 21% lower in chamber controls than in elevated CO₂ (Fig. 3), but estimates varied substantially within and among replicates and were not significantly different (P=0.54 for the two chamber treatments, P=0.19 for the three treatments). This interaction between plant size and density is potentially quite important, since there was no evidence for an increase in biomass at the ecosystem level (Field et al., in preparation).

*Avena* seeds from elevated CO₂ were 9% heavier on average than in chamber controls (Fig. 4A), but the means were not significantly different (P=0.14). Average seed and fruit mass in the no-chamber treatment were significantly smaller than in either chamber treatment (Fig. 4, P<0.01). The N concentration of seeds was 2.84% in chamber controls but only 2.59% in elevated CO₂ (Fig. 5A), a significant reduction of 9% (P=0.04). The carbon concentration of seeds was unchanged by CO₂ (Fig. 5B, P=0.72, 47% C for all treatments).

**Discussion**

Water-use efficiency (mmol CO₂ mol⁻¹ H₂O) of *Avena* plants was approximately doubled in elevated CO₂, as estimated from gas-exchange measurements and carbon isotope discrimination. Leaf c/c₄ ratios were maintained at 0.72–0.77 in ambient and elevated CO₂ treatments, with slightly higher values in elevated CO₂. The tendency to maintain similar c/c₄ ratios with increasing atmospheric CO₂ results in decreased stomatal conductance and transpiration on a leaf-area basis (Long and Hutchin 1991). Morison (1993) discusses a few cases where large increases in flux-based WUE (as defined above) may not result in the same increase in yield-based WUE (defined as total dry matter produced/total evapotranspiration). A system where H₂O availability was not limiting to plants, or that had multiple interacting stresses (Chapin et al. 1987), might be expected to show smaller increases in yield-based WUE than would be predicted solely from leaf-level fluxes. In addition, the dynamics of stomatal conductance only partially account for the dynamics of canopy water loss (Jarvis and McNaughton 1986).

One of the primary responses of plants to elevated CO₂ is to reduce stomatal conductance and transpiration (Smith et al. 1987; Garbutt et al. 1990; Radoglou et al. 1992; Morison 1993). CO₂ may also increase plant biomass and leaf area in many cases. If leaf area increases proportionally more than stomata close, plants may use water more quickly in elevated CO₂, despite greater water-use efficiency. For our system, stomatal conductance and transpiration in high CO₂ were reduced approximately 50% compared to chamber controls (Fig. 1A, B). We found evidence suggesting an increase in average individual plant size (30–40%, Fig. 2) and a possible decrease in *Avena* density (20%, Fig. 3). The net result was a 34% increase in soil moisture by the end of the growing season (Fredeen et al., in prep.). This increase in soil water could have resulted in a longer growing season, but this appeared not to be the case. There were no apparent differences in *Avena* leaf senescence (the proportion of brown-to-total leaf length) in early May (P>0.50, average leaf senescence 79%).

A number of other studies have examined physical and physiological responses of plants to elevated CO₂ (e.g., Williams et al. 1988; Lardieraderie et al. 1988; Bazzaz and Miao 1993). Most have found a decrease in stomatal conductance with increased CO₂ (Morison 1993). Decreased stomatal conductance frequently results in lower transpiration and higher leaf water potentials (e.g., Garbutt et al. 1990; Tyree and Alexander 1993), as reported here. There is much more variability across systems in photosynthetic responses (Tissue and Oechel 1987; Arp and Drake 1991). Furthermore, growth and reproduction are more variable still, as a result of the increasing number of factors and complexity of interactions affecting these processes (e.g., Rogers et al. 1983; Williams et al. 1988; Woodward et al. 1991). Here we present results from a natural system which is decidedly water-limited, and showed decreases in stomatal conductance and transpiration and increases in photosynthesis and water use efficiency. These physiological changes in elevated CO₂ were translated to changes in plant size and reproduction.

Predicting the consequences of global change on terrestrial ecosystems presents a tremendous challenge, es-
cially since experiments with artificial and natural systems have yielded a broad range of responses (Tissue and Oechel 1987; Curtis et al. 1989; Idso et al. 1991; Norby et al. 1992; Körner and Arnone 1992). Our current CO2 study links changes in water use and carbon gain at the leaf level to increased size and reproduction in a California annual grassland. Such changes have the potential to alter species composition and equilibrium plant density. The impact of these changes at the ecosystem level will depend on the complex interactions of different biological and climatic processes.

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