

Elevated CO₂ increases productivity and invasive species success in an arid ecosystem

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Arid ecosystems, which occupy about 20% of the earth's terrestrial surface area, have been predicted to be one of the most responsive ecosystem types to elevated atmospheric CO₂ and associated global climate change¹⁻³. Here we show, using free-air CO₂ enrichment (FACE) technology in an intact Mojave Desert ecosystem⁴, that new shoot production of a dominant perennial shrub is doubled by a 50% increase in atmospheric CO₂ concentration in a high rainfall year. However, elevated CO₂ does not enhance production in a drought year. We also found that above-ground production and seed rain of an invasive annual grass increases more at elevated CO₂ than in several species of native annuals. Consequently, elevated CO₂ might enhance the long-term success and dominance of exotic annual grasses in the region. This shift in species composition in favour of exotic annual grasses, driven by global change, has the potential to accelerate the fire cycle, reduce biodiversity and alter ecosystem function in the deserts of western North America.

The well documented increase in atmospheric CO₂ concentration ([CO₂]) acts to increase photosynthesis, plant biomass and plant water-use efficiency in many plant species⁵. Primary production in deserts is strongly limited by water and nitrogen resources³. Conceptual models predict that desert ecosystems will be the most responsive to increased [CO₂] because the strong response of water-use efficiency in plants alleviates water limitations to primary production^{1,2}. In addition, differences among species in their response to elevated [CO₂] can modify competitive interactions, potentially changing community composition. For example, the dominance of the exotic grass species *Bromus tectorum* (cheatgrass), which has invaded many thousand hectares in western North America, may be enhanced by elevated [CO₂] and thus alter competitive balance and the fire cycle in semi-arid shrub-steppe environments⁶⁻⁸. If these predictions are realized, biodiversity in arid and semi-arid ecosystems could be significantly reduced, which in turn might alter ecosystem processes such as primary production, nutrient dynamics and landscape water balance⁹.

To examine the response of a desert ecosystem to elevated [CO₂], we established the Nevada Desert FACE (free-air CO₂ enrichment¹⁰) Facility (NDFF) in an undisturbed Mojave Desert ecosystem within the Nevada Test Site of southern Nevada, USA⁴. The climate is arid, with most precipitation occurring as rain during winter months. The NDFF is located within a desert scrub community (< 20% total perennial cover) dominated by the evergreen shrub *Larrea tridentata* (creosote bush) and several species of deciduous shrubs.

Perennial grasses are also common on the site, and dense stands of winter annuals occur in response to significant winter rains.

We have continuously maintained [CO₂] at a 550 μmol mol⁻¹ (p.p.m.) set point in three 23-m diameter experimental plots since April 1997, while maintaining three other plots at ambient [CO₂] and three control plots without the FACE apparatus. Elevated CO₂ treatment occurs 24 h d⁻¹, except during high winds (5-min average wind speed > 8 m s⁻¹) or at low air temperature (< 3 °C)⁴. CO₂ treatment occurred 93% of the time when air temperature exceeded 3 °C, and mean [CO₂] averaged 537 ± 41 and 375 ± 18 μmol mol⁻¹ in the elevated and ambient [CO₂] plots, respectively.

In the 1997–1998 hydrologic year (October to September) corresponding to the 1998 growing season (typically March to September for perennials; March to June for annuals), the south-western United States experienced a pronounced El Niño cycle during which the NDFF received 309 mm of rain, which was 2.4-fold higher than the long-term average for the area. In contrast, the 1998–1999 hydrologic year was a dry La Niña cycle in which 107 mm of rain fell, and only 17 mm occurred before the onset of the growing season.

For the dominant perennial shrubs of the NDFF, we measured above-ground production during the 1998 and 1999 growing seasons. The cumulative increase in new-shoot biomass for *L. tridentata* was significantly higher (roughly double) at elevated [CO₂] than at ambient [CO₂] in 1998 (*P* < 0.01), but no difference in new shoot production occurred between CO₂ treatments in the dry year of 1999 (*P* = 0.26) (Fig. 1). In addition, no differences in shrub production occurred between the two types of control plots, and thus the FACE apparatus did not influence these production measurements. We also monitored new root production with minirhizotrons during the same growth period (data not shown) and saw no evidence that plants shifted to new root production in the dry year. Results from the deciduous shrubs at the NDFF, primarily *Ambrosia dumosa* and *Lycium andersonii*, also showed substantially enhanced new shoot production at elevated [CO₂] in 1998, but not in 1999 (data not shown).

In response to the anomalous 1997–1998 wet cycle, a large number of annual plants germinated at the NDFF during the winter months. Four species of annuals, *Bromus madritensis* ssp. *rubens*, *Eriogonum trichopes*, *Lepidium lasiocarpum* and *Vulpia*

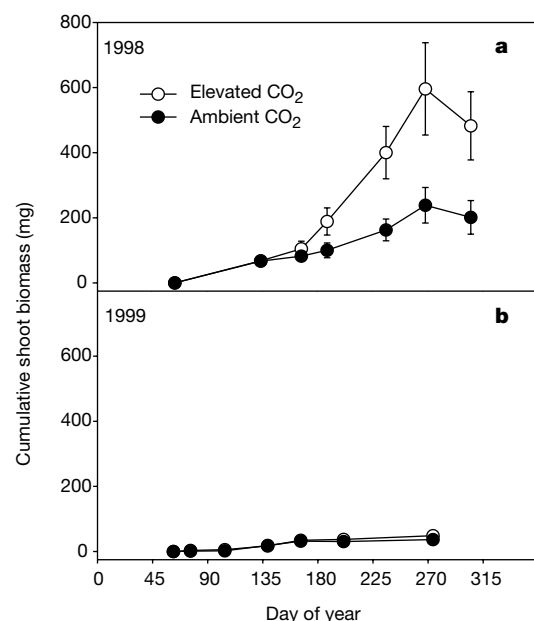


Figure 1 New shoot production of *Larrea tridentata* at ambient and elevated [CO₂] at the Nevada Desert FACE Facility in 1998 (a) and 1999 (b) (*n* = 27 for each CO₂ treatment).

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octoflora, constituted over 70% of the total density of annuals in the plots and so were selected for above-ground harvests throughout the growth cycle. *Bromus* (red brome) is an exotic annual grass that has invaded many areas of the Mojave and Sonoran Deserts¹¹; the other three taxa are native annuals. Because of the pronounced spatial variation in soil nutrients that occurs in desert ecosystems¹², two primary microsites were chosen for harvest locations: (1) open interspaces between perennial plants; and (2) beneath or within the canopy of perennial plants. Results from the NDFP confirmed this 'fertile island' effect—soils beneath deciduous and evergreen shrubs have similar concentrations of inorganic nitrogen in the surface 10-cm layer (15 p.p.m. NO₃-N; 3 p.p.m. NH₄-N), and these concentrations are three- to fivefold higher than in the open interspaces (3 p.p.m. NO₃-N; 1 p.p.m. NH₄-N).

Total density of the native annuals (*Eriogonum*, *Lepidium* and *Vulpia*) at peak biomass (May) in 1998 was 42% lower ($P < 0.01$) and total above-ground biomass was 40% higher at elevated [CO₂] ($P < 0.1$) than at ambient [CO₂] (Fig. 2a). The net result was that individual plants were 2.4-times larger at elevated [CO₂] (0.94 g per plant) than at ambient [CO₂] (0.39 g per plant) ($P < 0.01$). For *Bromus*, both plant density and biomass increased at elevated [CO₂] (Fig. 2a). The 2.3-fold increase in *Bromus* above-ground biomass at elevated [CO₂] ($P < 0.05$) was due to a 50% increase in density coupled with a 53% increase in individual plant biomass. Early in the growth season (March), plant density did not differ among elevated [CO₂] and both types of control plots (data not shown), and thus the differences at peak biomass were not caused by differences in seed germination or in seed banks. Because none of the measured parameters differed significantly between the two types of control plots throughout the growing season, the FACE apparatus did not influence these results. Therefore, we conclude that as annual populations exhibited natural self-thinning from germination to peak biomass, both native annuals and *Bromus* responded to elevated [CO₂] with larger plants, but *Bromus* became a higher proportion of total plant density at elevated [CO₂]. In the dry winter–spring of 1999, there was no germination of annual plants and therefore no production in either the ambient or elevated [CO₂] plots.

Table 1 Seed number and mean seed mass for three annual species and two microsites at ambient and elevated [CO₂] in May, 1998 at the Nevada Desert FACE Facility

Taxa	Microsite	[CO ₂]*	Seed number (plant)	Seed number (LSA)	Seed mass
<i>Bromus</i>	Interspaces	Ambient	97	32	2.13
		Elevated	196*	42	1.95*
	Fertile-islands	Ambient	235	29	2.15
		Elevated	535*	32	1.91*
<i>Vulpia</i>	Interspaces	Ambient	107	108	0.43
		Elevated	138	86	0.51*
	Fertile-islands	Ambient	133	86	0.46
		Elevated	230*	64*	0.53
<i>Lepidium</i>	Interspaces	Ambient	200	90	0.33
		Elevated	634*	76	0.33
	Fertile-islands	Ambient	578	56	0.33
		Elevated	897*	79	0.34

Taxa are *Bromus madritensis* ssp. *rubens*, *Vulpia octoflora* and *Lepidium lasiocarpum*. Microsites are open interspaces between perennial plants and beneath perennial plant microsites. Seed number is expressed per plant and per unit leaf surface area (LSA). Seed mass is the mean mass for individual seeds (in mg).

*Parameters are significantly different from the data point immediately above (that is, response at elevated [CO₂] versus ambient [CO₂] for that species and cover type).

Bromus exhibited a threefold higher total seed rain at the plot level at elevated [CO₂] (Fig. 2a). Although seed rain across the whole plot by native annuals was not significantly increased by elevated [CO₂] (Fig. 2a), [CO₂] effects occurred at an individual plant level. For example, individuals of *Bromus* and *Lepidium* produced greater numbers of seeds at elevated than at ambient [CO₂], regardless of microsite location (Table 1). In contrast, *Vulpia* showed an enhancement in seed production only in fertile island microsites. As *Bromus* and *Lepidium* showed no changes in seed production per unit leaf surface area (LSA) and *Vulpia* showed a slight decrease per LSA, the increases in seed number occurred as a result of larger plants at elevated [CO₂]. However, individual seed mass decreased at elevated [CO₂] in *Bromus*, a phenomenon that did not occur for the native species (Table 1). *Bromus* seeds at elevated [CO₂] also have lower N content¹³, and the reduction in seed quality for *Bromus* at elevated [CO₂] results in a 20% reduction in growth rate of subsequent seedlings¹⁴. However, *Bromus* produces so many more seeds at

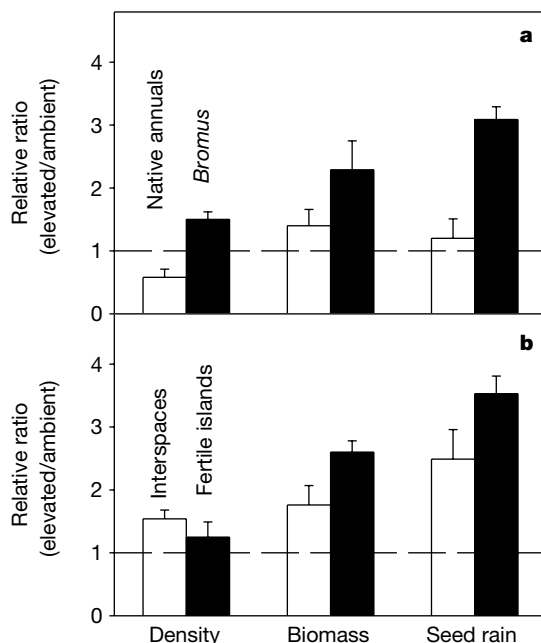


Figure 2 The relative ratio (elevated CO₂/ambient CO₂) of plant density (left), above-ground plant biomass (centre) and seed rain (total seed production; right) per unit area at the Nevada Desert FACE Facility in May, 1998. **a**, Native annuals (open bars) and *Bromus madritensis* ssp. *rubens* (solid bars). **b**, *Bromus madritensis* ssp. *rubens* in open

interspaces (open bars) and in 'fertile island' (solid bars) microsites. Note the dashed baseline at a ratio of 1; above the line plants at elevated CO₂ are greater than at ambient CO₂ for that parameter; below the line plants at ambient CO₂ are greater.

elevated [CO₂] that it potentially compensates for reduced seed quality. This phenomenon might have important implications for population structure and competitive relationships between *Bromus* and native annuals, as well as affect consumers in an ecological system in which granivores have a fundamental role¹⁵.

Using *Bromus*, we found an interactive effect of elevated [CO₂] and microsite on plant density, biomass and seed rain ($P < 0.01$) (Fig. 2b). Elevated [CO₂] increased *Bromus* biomass in open interspaces by 76% but by twice that in fertile island microsites (160%). The stimulation of *Bromus* seed rain by elevated [CO₂] in fertile islands (3.5-fold) was also greater than in interspaces (2.5-fold). In addition, we observed [CO₂]-by-microsite interactions for the native annuals (data not shown). This interactive effect is consistent with predictions that desert annuals will show their strongest response to elevated [CO₂] on sites with moderate to high soil fertility, whereas plant production on low-fertility desert soils is much less responsive to elevated [CO₂]¹³.

These results have several important implications for the structure and function of desert ecosystems. First, total ecosystem above-ground primary production (both annuals and perennials) during an anomalously wet year for this arid region was substantially higher at elevated [CO₂]. Desert ecosystems have been predicted to exhibit a 50% increase in primary production with a doubling of [CO₂], an increase that would be higher than for any other global biome type². Results from our FACE study exceeded this prediction in a wet year (a roughly 50% increase in native annual plant production, a doubling of new shoot production in the dominant shrub and a 2.3-fold increase in production of an exotic annual grass occurred with a 50% increase in [CO₂]). This also exceeds a roughly 25% increase in primary production observed in a young, rapidly growing pine forest in North Carolina using a similar FACE experimental design¹⁶. However, elevated [CO₂] did not enhance productivity at the NDFF in a drought year. Apparently, plant physiological processes are sufficiently constrained by water stress during dry years in the desert such that plant production is minimal at both [CO₂]. Indeed, our plant gas-exchange results indicate increased photosynthesis and reduced stomatal conductance (and therefore transpiration) at elevated [CO₂] in *Larrea* during wet seasons, but not during drought^{17,18}. By enhancing water-use efficiency in plants to the greatest extent when soil moisture is most abundant in this water-limited system, elevated [CO₂] stimulates an already high rate of production in a wet year, but not in a dry year. Desert ecosystems have extremely high temporal variability in rainfall and primary production¹⁹, and our observations of a wet-dry cycle in the Mojave Desert suggest that elevated [CO₂] will increase temporal variability of ecosystem production cycles.

Second, an invasive exotic annual grass showed significantly higher plant density, biomass and seed rain at elevated [CO₂] than did several species of native annuals. The growth of a closely related exotic *Bromus* (*B. tectorum*) is also particularly responsive to elevated [CO₂]⁶. The greater response of exotic *Bromus* species to elevated [CO₂] relative to native species could enhance the success of a group of highly invasive species in western North America. *B. tectorum* has invaded many thousands of hectares in this region²⁰, which has increased fire frequency²¹ and has converted large expanses of sagebrush-steppe vegetation to a fire-controlled annual grassland²². Post-fire competitive superiority of *Bromus* seedlings then precludes the reestablishment of perennial species²³. *Bromus madritensis* ssp. *rubens* has initiated a similar invasion cycle in the Mojave and Sonoran Deserts¹¹, although not yet to the extent as the invasion of *B. tectorum* farther north. The results from this study, showing both higher total biomass and seed rain in *Bromus* than in native annuals, confirm experimentally in an intact ecosystem that elevated [CO₂] may enhance the invasive success of *Bromus* spp. in arid ecosystems. Enhanced success of *Bromus* spp. would expose these deserts to an accelerated fire cycle to which they are not adapted, and thus convert long-lived shrublands to annual grass-

lands dominated by exotic grasses throughout the region. □

Methods

Above-ground shrub production

We determined above-ground shoot production in the dominant perennial shrubs (*Larrea tridentata*, *Ambrosia dumosa*, *Lycium andersonii*) by monitoring incremental changes in stem length over each growing season. Three shoot tips were marked, proximal to the newest emerged leaf pair, before each growing season on three shrubs per species per treatment plot. Total shoot length was measured at least monthly from the mark to the shoot tip (including any branching) with calipers. The number of leaves distal to the mark was also counted. Tissue harvests were used to create treatment-specific regression equations for each species that converted shoot length to total shoot biomass (dry weight). A repeated measures two-way analysis of variance (ANOVA) was used to evaluate the effect of time and [CO₂] for each year for perennial plant growth.

Annual plant density and production

We quantified annual plant density in each plot with two permanently placed 8 × 0.2 m transects positioned randomly from the centre of the plot to the periphery. Transects were divided into 0.5 × 0.2 m sub-plots in which all rooted annual plants were counted by species. We also categorized data by microsite type, allowing calculation of plant density in both open interspaces and within fertile island microsites (beneath evergreen shrubs, beneath deciduous shrubs and within perennial grass canopies). We determined biomass of the four dominant species (*Bromus madritensis* ssp. *rubens*, *Eriogonum trichopes*, *Lepidium lasiocarpum*, *Vulpia octoflora*) during five harvests at 2-week intervals from 7 April to 1 June 1998. At each harvest date, and for each microsite location within each plot, five plants of each species were harvested. We calculated plot-level annual biomass by multiplying plant density by mean individual plant biomass for each microsite, and then summing those values and correcting them for the proportion of microsite in each plot. For each response variable, a plot mean was used as the statistical unit. We determined the effect of elevated [CO₂] on total annual plant density and biomass production with a one-way ANOVA. The effect of elevated [CO₂] and microsite and their interaction for these parameters was determined individually for native and invasive annuals by a two-way ANOVA. Pairwise comparisons were determined by Student-Newman-Keuls tests ($\alpha = 0.05$).

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Evolutionary origins of vertebrate appendicular muscle

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The evolution of terrestrial tetrapod species heralded a transition in locomotor strategies. While most fish species use the undulating contractions of the axial musculature to generate propulsive force, tetrapods also rely on the appendicular muscles of the limbs to generate movement^{1,2}. Despite the fossil record generating an understanding of the way in which the appendicular skeleton has evolved to provide the scaffold for tetrapod limb musculature³, there is, by contrast, almost no information as to how this musculature arose. Here we examine fin muscle formation within two extant classes of fish. We find that in the teleost, zebrafish, fin muscles arise from migratory mesenchymal precursor cells that possess molecular and morphogenetic identity with the limb muscle precursors of tetrapod species. Chondrichthyan dogfish embryos, however, use the primitive mechanism of direct epithelial somitic extensions to derive the muscles of the fin. We conclude that the genetic mechanism controlling formation of tetrapod limb muscles evolved before the Sarcopterygian radiation.

Within the zebrafish fin bud, the first indication of a cell's commitment to the muscle lineage begins with the expression of the myogenic transcription factor *myoD*. Expression of *myoD* initiates within the fin mesenchyme at 29 hours post-fertilization (h.p.f.), about 5 h after myotomal muscle differentiation is complete (Fig. 1a)^{4–6}. Expression is confined to the fin, with no detectable *myoD*-positive cells extending from adjacent somites (Fig. 1a). By 36 h.p.f., two distinct regions of *myoD* expression are evident, corresponding to the future dorsal and ventral muscle masses (Fig. 1b). Differentiation of fin muscle begins 12 h later, where antibodies against myosin heavy chain (MyHC) reveal unfused myocytes within muscle masses surrounding a condensing chondrocyte core (Fig. 1c). Muscle differentiation is discrete within the extending fin bud (Fig. 1c), and by 72 h elongated myofibrils can be seen appearing to attach to individual actinotrichia of the extending fin blade (Fig. 1d). The timing and morphogenesis of muscle differentiation in the zebrafish fin argues against the derivation of these cells from direct epithelial somite extensions—a mechanism generally invoked to describe the formation of the fin musculature in all fish species^{1,2,7–11}. We there-

fore endeavoured to detect the presence or absence of fin-level somitic extensions by direct microscopic examination. Time-lapse recording of somitic cells adjacent to the fin failed to detect the presence of epithelial extensions (Fig. 1e–h). Cells were observed instead to exit the somite at somitic boundaries, a few proliferative mesenchymal-like cells at a time, moving towards the developing fin bud (Fig. 1g, h).

To determine at what somitic level, if any, fin muscle precursors arise, the lipophilic cell tracer DiI was injected into individual somites of 24 h.p.f. (26-somite stage) embryos of a transgenic strain of zebrafish that expresses green fluorescent protein (GFP) under the control of the muscle-specific α -actin promoter. This promoter drives GFP expression in all differentiating skeletal muscle cells, including that of the fin¹². Coincident DiI (red) and GFP (green) fluorescence in the fin at 48 h.p.f. thus reveals a contribution of precursors derived from the initial DiI injection site to the fin musculature (Fig. 2a, b). This analysis indicated that cells derived from somites 2–4 may migrate to form the fin musculature (Fig. 2a, b; $n = 10$). Contribution to the fin musculature was only detected when DiI was injected adjacent to the most ventral cells of the

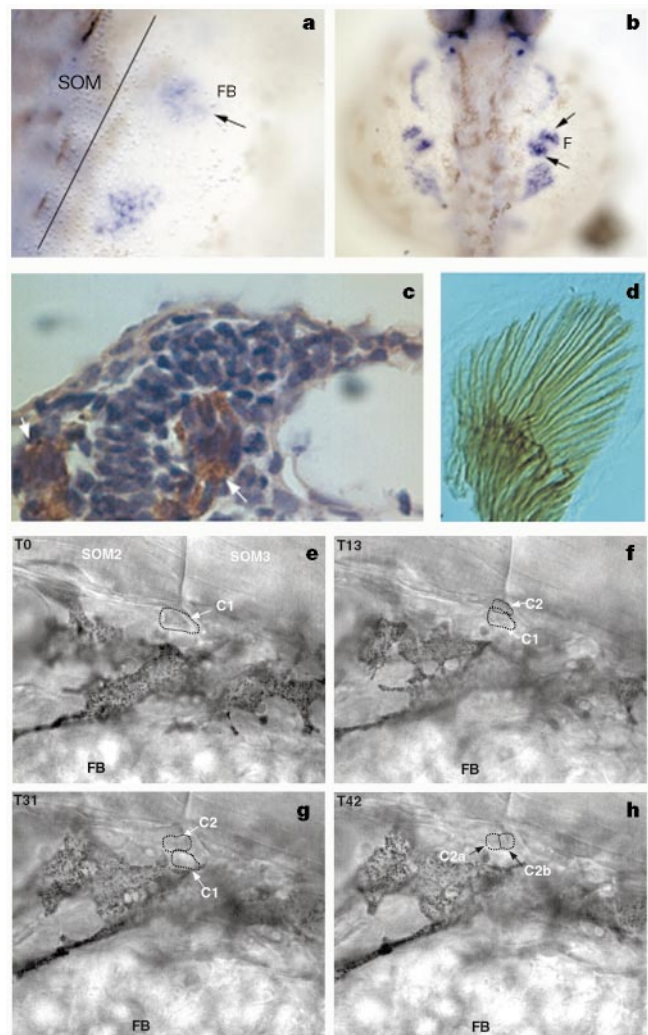


Figure 1 Zebrafish fin muscle formation. **a**, *myoD* expression initiates within the fin bud (arrow, FB) at 29 h.p.f. SOM, somites. **b**, *myoD* fin (F, arrows) expression at 36 h.p.f. **c**, Fin cross-section at 48 h.p.f. stained for MyHC (brown). Arrows indicate dorsal and ventral muscle masses. **d**, MyHC expression in the fin blade at 72 h.p.f. **e–h**, Cell movements from fin-level somites. Boundary between somites 2–3 of a 26-somite (24 h.p.f.) embryo in an oblique lateral view. T indicates time in minutes. C2a and C2b are daughters of cell C2.