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No cumulative effect of 10 years of elevated [CO_2] on perennial plant biomass components in the Mojave Desert

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Abstract

Elevated atmospheric CO_2 concentrations ([CO_2]) generally increase primary production of terrestrial ecosystems. Production responses to elevated [CO_2] may be particularly large in deserts, but information on their long-term response is unknown. We evaluated the cumulative effects of elevated [CO_2] on primary production at the Nevada Desert FACE (free-air carbon dioxide enrichment) Facility. Aboveground and belowground perennial plant biomass was harvested in an intact Mojave Desert ecosystem at the end of a 10-year elevated [CO_2] experiment. We measured community standing biomass, biomass allocation, canopy cover, leaf area index (LAI), carbon and nitrogen content, and isotopic composition of plant tissues for five to eight dominant species. We provide the first long-term results of elevated [CO_2] on biomass components of a desert ecosystem and offer information on understudied Mojave Desert species. In contrast to initial expectations, 10 years of elevated [CO_2] had no significant effect on standing biomass, biomass allocation, canopy cover, and C : N ratios of above- and belowground components. However, elevated [CO_2] increased short-term responses, including leaf water-use efficiency (WUE) as measured by carbon isotope discrimination and increased plot-level LAI. Standing biomass, biomass allocation, canopy cover, and C : N ratios of above- and belowground pools significantly differed among dominant species, but responses to elevated [CO_2] did not vary among species, photosynthetic pathway (C_3 vs. C_4), or growth form (drought-deciduous shrub vs. evergreen shrub vs. grass). Thus, even though previous and current results occasionally show increased leaf-level photosynthetic rates, WUE, LAI, and plant growth under elevated [CO_2] during the 10-year experiment, most responses were in wet years and did not lead to sustained increases in community biomass. We presume that the lack of sustained biomass responses to elevated [CO_2] is explained by inter-annual differences in water availability. Therefore, the high frequency of low precipitation years may constrain cumulative biomass responses to elevated [CO_2] in desert environments.

Keywords: carbon, deciduous, evergreen, FACE, functional group, isotopes, nitrogen, standing crop

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Introduction

Increases in atmospheric CO_2 concentration ([CO_2]) due to anthropogenic factors have prompted extensive research on the effects of elevated [CO_2] on ecosystem structure and function. Initial predictions suggested that elevated [CO_2] would increase photosynthetic rates and decrease stomatal conductance, leading to increased water-use efficiency (WUE) and thus increased productivity (Nowak *et al.*, 2004a). Elevated [CO_2] was also predicted to decrease tissue nitrogen content, resulting in higher carbon to nitrogen ratios (C : N) (Stiling & Cornelissen, 2007), as well as increased leaf area index (LAI) (Norby *et al.*, 2003). Resulting increases in LAI under elevated [CO_2] were anticipated to further enhance productivity.

Early experiments focused on the effects of elevated [CO_2] on cropland, forest, and grassland species, often in controlled growth environments. The advent of FACE (free-air carbon dioxide enrichment) experiments allowed for exploration of the effects of elevated [CO_2] in intact ecosystems under more natural conditions. In 1997, the Nevada Desert FACE Facility (NDFF) was established in the Mojave Desert to address responses

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of the driest desert ecosystem in North America to elevated [CO_2]. Although deserts were initially predicted to be highly responsive to elevated [CO_2] due to potential increases in WUE (Melillo *et al.*, 1993), studies have shown variable responses of arid and semiarid ecosystems to elevated [CO_2] (Nowak *et al.*, 2004a). Because short-term interactions between elevated [CO_2] and precipitation vary among arid and semiarid ecosystems (Morgan *et al.*, 2004b), long-term observations of elevated [CO_2] effects on arid ecosystems are needed.

Although controlled elevated [CO_2] experiments often revealed increased productivity, field experiments have shown variable responses on an annual basis (Shaw *et al.*, 2002; Belote *et al.*, 2003; but see Morgan *et al.*, 2004a). Forested ecosystems and trees appear to be consistently responsive to elevated [CO_2] and dominate the elevated [CO_2] literature (Nowak *et al.*, 2004a; Ainsworth & Long, 2005; Norby *et al.*, 2005). Other ecosystems, such as deserts, are less represented in the elevated [CO_2] literature even though these ecosystems represent substantial land cover (Lioubimtseva & Adams, 2004; Smith *et al.*, 2009b). At the NDFF, which is the only FACE experiment in an intact desert ecosystem, photosynthesis was enhanced by elevated [CO_2] (Naumburg *et al.*, 2003), but production of new shoots during the first 4 years of the experiment only increased in a wet year (Housman *et al.*, 2006). However, the long-term effects of elevated [CO_2] at the NDFF, and for deserts in general, are unknown.

Previous research has indicated variable species responses to elevated [CO_2], making it difficult to predict the response of entire ecosystems (Bradley & Pregitzer, 2007; Smith *et al.*, 2009a). Desert plant communities are comprised of a diversity of species that vary in their morphological and physiological adaptations to hot and dry environments, and differences in such traits among species and plant functional types may result in diverse ecosystem responses to elevated [CO_2]. For example, C_3 species were originally predicted to respond more strongly to elevated [CO_2] than C_4 species because the CO_2 concentrating mechanism of C_4 plants makes them relatively insensitive to the magnitude of changes in [CO_2] expected to occur within this century (Bowes, 1993); however, evidence exists both for and against this initial prediction (Owensby *et al.*, 1993; Wand *et al.*, 1999; Nowak *et al.*, 2004a; Ainsworth & Long, 2005). In addition, compared with drought-deciduous shrubs and grasses, evergreen species may have more opportunities to respond to elevated [CO_2] throughout the growing season in response to transitory increases in water availability following small precipitation events.

To understand how elevated [CO_2] may alter plant biomass in a desert ecosystem, we examined the cumulative effects of 10 years of elevated [CO_2] on aboveground and belowground measures of perennial plant biomass in an intact Mojave Desert ecosystem at the NDFF. Our study objectives included the following: (i) providing comparative biomass measures for various species in a poorly studied desert; (ii) assessing the relative response of aboveground and belowground standing biomass of dominant species to elevated [CO_2], and (iii) evaluating the long-term effects of elevated [CO_2] on total plant community biomass. To address these objectives, we destructively harvested aboveground and belowground standing biomass of all perennial species at the end of the 10-year NDFF experiment. For five to eight dominant species, we also measured biomass allocation, canopy cover, and LAI, as well as carbon and nitrogen content and isotopes of plant tissues. We predicted that elevated [CO_2] would increase aboveground and belowground standing biomass, the proportion of biomass contained in roots, canopy cover, LAI, leaf WUE (as inferred from carbon stable isotopes), and C : N ratios. We also predicted that C_3 species would respond more strongly to elevated [CO_2] than C_4 species and that the evergreen shrub, *Larrea tridentata*, would respond more strongly to elevated [CO_2] than drought-deciduous shrubs and grasses.

Materials and methods

Experimental site

The NDFF is located in southern Nevada on the US Department of Energy Nevada National Security Site (formerly Nevada Test Site; 36°46′12″ N, 115°57′54″ W, 970 m), which has been closed to the public and protected from livestock for over 50 years. The Mojave Desert receives approximately 60% of its annual precipitation during the winter (November–February) (Blainey *et al.*, 2007), and summer rain can be highly variable across years. Total annual precipitation, mean annual temperature, mean minimum temperature (December), and mean maximum (July) temperature during the study period are shown in Table 1.

The study was conducted in an intact *Larrea tridentata–Ambrosia dumosa* desert scrub community with undisturbed soils and crust/pavement surfaces. Dominant perennial species included a C_3 evergreen shrub (*L. tridentata*), C_3 drought-deciduous shrubs (*A. dumosa*, *Lycium andersonii*, and *L. pallidum*), and a C_4 bunchgrass (*Pleuraphis rigida*). Other perennial shrubs and grasses, as well as annual grasses and forbs, comprised the remainder of the plant community.

The NDFF consisted of nine plots, each 23 m in diameter (Fig. 1a), located within a relatively homogeneous piedmont surface. Ambient and elevated [CO_2] plots were spaced more than 100 m apart to reduce potential for CO_2 contamination of ambient plots. Elevated [CO_2] was distributed across the elevated plots with blowers and PVC pipe, as described in Jordan *et al.* (1999). Three plots received continuous exposure to elevated [CO_2] (550 μmol mol^{-1} target; 'elevated') except

Table 1 Total annual precipitation, mean annual temperature, mean minimum (December), and mean maximum (July) temperature at the NDFF (Nevada Desert FACE Facility) for each hydrologic year (1st October–30th September) over the 10 year experimental period (1997–2007). The experiment commenced April 1997 and continued through June 2007. Note: total annual precipitation for 2006–2007 only includes natural precipitation through June 2007, as well as an irrigation event on 21 March 2007

Hydrologic year	Total annual precipitation (mm)	Mean annual temperature (°C)	Mean low temperature (°C in December)	Mean high temperature (°C in July)
1996–1997	151	15.6	−1.6	36.8
1997–1998	328	13.3	−5.2	37.4
1998–1999	107	13.8	−6.6	35.4
1999–2000	98	16.5	−5.4	38.3
2000–2001	102	15.8	−5.9	36.5
2001–2002	47	15.9	−4.3	39.9
2002–2003	149	16.2	−4.6	40.1
2003–2004	123	15.5	−4.6	37.5
2004–2005	242	15.1	−3.6	39.9
2005–2006	113	16.1	−5.0	39.5
2006–2007	65*	na	−6.0	na
Average	146	15.4	−4.8	38.1

*Includes irrigation event on 3/21/2007.

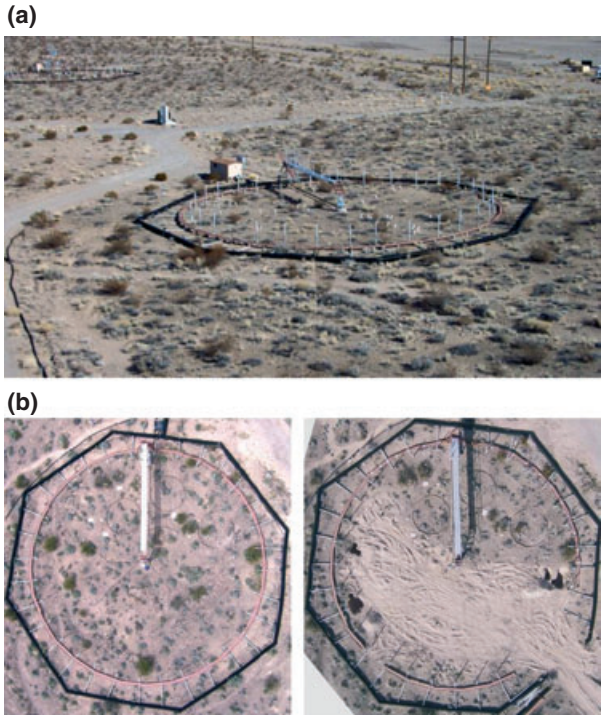

Fig. 1 (a) Photo of Nevada Desert FACE Facility (NDFF) showing experimental ring with center pivot and walkway for plot access, PVC blower pipes, and associated instrumentation. Photo courtesy of Stephen Zitzer. (b) Photo of experimental ring before (left) and during (right) the harvest in 2007. Plots were 25 m in diameter. Photos courtesy of Lynn Fenstermaker.

when air temperature fell below 4 °C or wind speed (5 min average) exceeded 7 m s^{-1}, resulting in an average [CO_2] of 513 μmol mol^{-1} over the entire experimental period. Three plots received ambient air ([CO_2] averaged 375 μmol mol^{-1} over the entire experimental period) that was applied to the plots using the same FACE technology ('ambient'), and three plots contained all infrastructure except for the blowers ('non-blower controls'). Suspended walkways with an attached aerial sampling platform were developed to eliminate walking in experimental plots and disturbing plants, biological soil crusts, and soils (Jordan *et al.*, 1999). CO_2 fumigation commenced in April 1997 and continued through June 2007. Due to low winter precipitation in 2006–2007, we irrigated all rings on 21 March 2007 (approximately 30 mm) to stimulate perennial green-up prior to terminating the experiment. All aboveground perennial biomass and a subset of belowground biomass were harvested at the end of this 10-year experiment.

Shoot harvest

Shoot biomass was collected in May and June 2007 by harvesting all individual perennial plants from approximately two-thirds of each experimental plot; the remaining one-third was reserved for subsequent studies (Fig. 1b). Although annual plant species are an important part of this ecosystem, their contributions to overall plant biomass are highly ephemeral; there were little to no annuals the year of this harvest and are thus not included. The harvested area for each ring was calculated from aerial photographs using image-processing software (ENVI; Exelis Visual Information Solutions, Boulder, CO, USA). Before harvesting each individual plant, we recorded the species, height (h), widest canopy dimension (c_1), and its perpendicular dimension (c_2). Woody plants were cut below the crown for ease of harvesting, and then were separated into shoot and root crown fractions with a bandsaw. Grasses and forbs were cut at ground level, excluding any roots. To reduce volume and drying time, individual plants were chipped in the field into 1–2 cm pieces and dried in the lab at 60 °C until the dry weight stabilized (usually 7–8 days).

Standing biomass was determined by summing shoot biomass for all individuals of each species. Canopy cover for each individual was calculated as the area of an ellipse: π*½c_1*½c_2. Because of the large number of sampled plants and to facilitate matching of shoot and root data, a barcode labeling system was used to track individual plants and their associated data and subsamples.

Eight species, which comprised >90% of the harvested perennial biomass, were selected for more detailed analyses. The species included seven shrubs and one perennial bunchgrass (Table 2). Plant height and canopy dimensions of five individuals of each species within each plot were measured, and plants were harvested as described above, but these individual plants were not chipped. Shoot biomass allocation of these individual plants was determined for each plant for all seven shrub species by separating tissues into vegetative (leaves + new green twigs), woody twigs (<8 mm diameter), and wood (>8 mm diameter). Total shoot biomass was calculated by summing vegetative, twigs, and woody tissue for these individuals.

We collected 10–20 leaves from each individual plant for evaluation of LAI, C and N content, and stable isotopic composition. LAI was determined on all species except for the leafless species, *Ephedra nevadensis*, by scanning this subsample of fresh leaves using Scion Image (NIH; Scion Corp., Frederick, MD, USA). Next, fresh leaf weight was recorded, leaves were oven dried (48 h at 60 °C), and dry weights were recorded. Whole plant leaf area was calculated as: whole plant leaf dry weight * (subsample leaf area/subsample leaf dry weight), and LAI was computed as whole plant leaf area/projected canopy area. For five of the eight species (*Ambrosia*, *Larrea*, the two *Lycium* species, and *Pleuraphis*), carbon and nitrogen content (and thus, C : N ratios) and isotope content ($\delta^{13}C$ and $\delta^{15}N$) were analyzed on all tissue types at the Stable Isotope Core Laboratory at Washington State University (Pullman, WA, USA). We also calculated carbon isotope discrimination ($\Delta^{13}C$) for leaves using $\Delta^{13}C = (\delta_a - \delta_T)/(1 + \delta_T)$, where δ_a = carbon isotope composition of the air ($-8.2‰$ for ambient and $-18.0‰$ for elevated [CO_2] plots), and δ_T is the carbon isotope composition of the tissue (Farquhar *et al*., 1989).

Table 2 Plant species, photosynthetic pathway, and functional type of the eight focal species at the Nevada Desert FACE (free-air carbon dioxide enrichment) Facility

Species	C_3/C_4	Functional type
Ambrosia dumosa	C_3	Drought-deciduous shrub
Lycium andersonii	C_3	Drought-deciduous shrub
Lycium pallidum	C_3	Drought-deciduous shrub
Psorothamnus fremontii	C_3	Drought-deciduous shrub; N-fixer
Krameria erecta	C_3	Winter-deciduous shrub
Larrea tridentata	C_3	Evergreen shrub
Ephedra nevadensis	C_3	Leafless shrub
Pleuraphis rigida	C_4	Bunchgrass; free-living N-fixers

Root harvest

After shoot samples were collected, collection of root samples began. Different destructive-harvest techniques were used to determine fine (≤ 2 mm root diameter), coarse (2–4 mm diameter), and woody (>4 mm diameter) root biomass in the top 1 m of soil. Fine roots were collected first, followed by coarse and woody roots. Root destructive harvests took approximately 4 months to complete.

To facilitate extrapolation of fine root biomass over the 1 m soil profile, we placed fine root sampling locations adjacent to minirhizotron tubes. Minirhizotron tubes were installed during 1997 near the beginning of the NDFF experiment, with tubes installed near individual *Ambrosia* and *Larrea* plants and in systematically located transects that sampled the entire plant community (Phillips *et al*., 2000). Minirhizotron measurements of root length growth, loss, and standing crop had been made for the last 4.5 years of the experiment (Ferguson & Nowak, 2011). For fine root biomass destructive harvests, a trench was dug parallel to each minirhizotron tube in the destructively harvested portion of each plot. At five depth locations along a minirhizotron tube, sample soil volumes (92 mm long × 44 mm wide × 44 mm deep; 0.18 l) were carefully removed immediately adjacent to the tube; in some cases, the presence of rocks near the tube reduced the number of depths that could be sampled. Soil samples were stored on ice in the field and then frozen until samples were hand-washed for fine roots. Fine roots were carefully cleaned to remove as much soil as possible, then dried at 60 °C and weighed. Subsamples of fine roots were ashed (Böhm, 1979) to determine ash-free dry biomass. Moreover, because approximately 48% of the soil volume was rocks and because fine root samples were collected along rock-free portions of minirhizotron tubes, we used percent rock volume for each plot to correct fine root biomass estimates for the volume of soil that was occupied by rocks.

We then coupled these corrected fine root biomass data with minirhizotron fine root length standing crop data to estimate the total amount of ash-free fine root biomass (kg m^{-2}) in a 1 m soil profile. Biomass data were collected at a relatively coarse scale such that the number of samples per tube varied from two to five (median = 5), and the position of each sample was not always consistent between tubes due to the presence of rocks. Thus, the number of biomass samples per tube was too few and irregularly placed to simply and reliably integrate total fine root biomass over the entire 1 m soil profile and obtain microsite-specific estimates of fine root biomass. Conversely, minirhizotron data were collected at a regularly spaced, fine-spatial resolution (i.e., every 4 cm, from 4 cm to 92 cm depth, for 23 sampling points per tube). Thus, we used minirhizotron data (i.e., standing crop of fine root length averaged across all sampling periods for each tube by depth combination) to help estimate depth profiles of root biomass for each specific microsite. The depth profile of minirhizotron root length data informed the depth profile of destructive-harvest root biomass data by simultaneously implementing models of each in a hierarchical Bayesian framework (Clark & Gelfand, 2006; Ogle & Barber, 2008). Within the Bayesian framework, our model of fine root depth

profiles assumed that $\log_e$ transformed fine root length varied as a function of depth according to a 3rd order polynomial. Fitted parameters for this polynomial were allowed to vary at the level of tube, with tube nested in microsite type crossed with plot, and plot was nested in CO_2 treatment. We assumed that this same polynomial function applied to the $\log_e$ transformed fine root biomass data, but data were rescaled by a parameter that converted fine root length to biomass. This scaling parameter was allowed to vary by tube to account for uncertainty in scaling of total fine root length to total fine root biomass. These simultaneous polynomial fits were used to estimate ash-free fine root biomass for depth intervals that were not sampled. These fine root biomass estimates for the missing depths were then added to the observed fine root biomass values to determine the complete, microsite-specific profile of total, ash-free fine root biomass (kg m^{-2}) integrated over the 1 m soil profile.

Two destructive harvest techniques were used to estimate coarse and woody root biomass to a soil depth of 1 m. One technique sieved all coarse and woody roots from excavated soil collected at specific microsites, and the other technique sieved roots from excavated soil collected from trenches placed along transects through the plot. The first technique provided detailed information about coarse and woody roots that were matched to shoot data via the sample barcodes. Six root microsites were sampled: four shrubs (*Ambrosia*, *Larrea*, and the two *Lycium* species), the bunchgrass (*Pleuraphis*), and the interspace between perennial plants. Five locations were sampled in each plot for each microsite, and plants that were used for coarse and woody root harvests generally were the same as those used for the detailed aboveground analyses described above. For each microsite, all soil to 1 m depth was excavated as shown in the shaded regions of Fig. S1. Coarse and woody roots were sieved from the soil, then dried and weighed. Because excavated soil included rocks, excavated roots were scaled directly to a kg per unit area basis. The second technique was designed to directly estimate coarse and woody root biomass of the entire plot. Three transects were randomly located along radii from each plot's center, and a trench was dug that was 0.5 m wide × 8.0 m long. All soil to 1 m depth was excavated, and coarse and woody roots were sieved from the soil. Roots were dried, weighed, and then scaled directly to a kg per unit area basis. We note that root biomass allocation results need to be interpreted carefully because the plants used for fine root destructive harvests were different from those used for coarse and woody destructive harvests. Thus, root allocation estimates are based on plot means rather than on individual plants because plant-level fine root data could not be linked to plant-level coarse/woody root data.

Subsamples of fine, coarse, and woody roots from all root destructive harvest techniques were analyzed for C and N content and for C and N stable isotopes at the Stable Isotope Core Laboratory at Washington State University. For woody roots, roots were split into small (4–10 mm diameter) and large (>10 mm diameter) woody roots prior to C and N analyses to account for the possibility that small woody roots (likely younger) may have incorporated the enriched ^{13}C signal into tissues, whereas the large (likely older) woody roots may primarily reflect conditions prior to the application of elevated $[CO_2]$.

Statistical analyses

All data were collected in a split-plot design where $[CO_2]$ treatment was applied to the whole plot and other effects, such as 'tissue', 'species' or 'microsite', were within each plot. Significance for each dependent variable was assessed in a mixed effects analysis of variance (ANOVA). In each model, plot nested within treatment and the interactions between plot and other terms in the model were included as random effects, and $[CO_2]$ treatment was included as a fixed effect, which was tested over plot. Tissue, species and/or microsite and their interactions with $[CO_2]$ treatment were included as fixed effects as appropriate for each dependent variable (as shown in Tables S1–S3), and they were tested over their respective interactions with plot. Data were transformed as needed to meet normal distribution and homogeneity of variance assumptions for ANOVAs, and means and standard errors (SE) were back-transformed so they could be represented on the original scale. Tukey *post-hoc* tests were used to determine pairwise differences for significant effects (α=0.05). Statistical analyses were performed in SAS v9.2 (PROC MIXED; SAS Institute 2002–2008, Cary, NC, USA).

The mixed effects ANOVAs for standing shoot biomass, vegetative cover, shoot biomass allocation, LAI, total biomass, and $\Delta^{13}C$ included fixed effects of $[CO_2]$ treatment, species, and their interaction. A compositional analysis (Pawlowsy-Glahn & Egozcue, 2006) would have been most appropriate for the allocation data, but the relatively small sample size did not support this method. Because there was no correlation between the allocation fractions and total biomass within a species (data not shown), total biomass was analyzed separately from the allocation data. The grass, *Pleuraphis*, was omitted from the biomass allocation analysis, and one shrub, *Ephedra*, was omitted from the LAI analysis because these variables do not apply to the species' growth form.

Root biomass was sampled at two spatial scales: by microsite and by plot transect. For microsite data, the mixed effects ANOVA included $[CO_2]$ treatment, microsite (*Ambrosia*, *Larrea*, both *Lycium* species, *Pleuraphis*, and interspace), tissue type (fine, coarse, woody), and all two-way and three-way interactions among them as fixed effects. Because fine root samples were collected from a different subset of plants as the coarse and woody root samples, individual plants were averaged together to estimate a mean value for each tissue type in each plot, and plot means were used in the ANOVAs. Furthermore, because of empty cells in the sampling design (fine roots were sampled for only two microsites, *Ambrosia* and *Larrea*; the bunchgrass *Pleuraphis* produces small but not large diameter woody roots), ANOVAs for two subsets of data were performed as needed to resolve significant interaction terms. These data subsets consisted of: (i) only the *Ambrosia* and *Larrea* microsites; and (ii) all microsites except *Pleuraphis*. For plot transect data, total root biomass and root mass ratio (i.e., the percentage of root biomass relative to total plant

biomass) were analyzed with mixed effects ANOVAs that included [CO_2] treatment, tissue type, and their interaction as fixed effects.

Carbon and nitrogen content, $\delta^{13}C$, $\delta^{15}N$, and C : N ratios were analyzed in a mixed effects ANOVA, conducted separately for shoots and for roots, that included [CO_2] treatment, species (except for *Pleuraphis)*, tissue type, and their two- and three-way interactions as fixed effects. Shoots had three tissue types (vegetative, twig, and wood), and roots had four tissue types (fine, coarse, small woody, and large woody). The bunchgrass, *Pleuraphis*, was analyzed separately in a mixed effects ANOVA with [CO_2] treatment as the lone fixed effect.

Results

Standing biomass

Long-term exposure to elevated [CO_2] had no significant effect on shoot standing biomass or on shoot allocation to vegetative, twig and woody tissues, and [CO_2] did not interact with species to significantly affect these responses (Table S1). In addition, [CO_2] had no significant main effect ($F_{2,6}$ = 0.61, P = 0.572) or interaction effect ($F_{16,48}$ = 1.06, P = 0.413) on canopy cover of the eight most common species at the end of the experiment (results not shown). Some species were more prevalent in the field site than others based on standing biomass, where *Larrea* comprised approximately 42% of total plot shoot biomass (Fig. 2, top panel). The two *Lycium* species were the next two most abundant shrubs, each comprising approximately 12% of shoot standing biomass. Species also varied in their allocation to vegetative, twig and woody tissues, but this did not depend on the [CO_2] level (Fig. 2, bottom panel). *Larrea* had the greatest allocation of shoot biomass to wood (50%), and *Larrea* and *Krameria* had the greatest allocation of shoot biomass to vegetative tissues (9% and 7%, respectively). The leafless shrub, *Ephedra*, had the smallest allocation to vegetative tissues (0.1%), although its allocation to wood (9.4%) was similar to the average of the five deciduous shrubs (9.5%).

Species differed in LAI, and we observed significantly higher LAI in elevated [CO_2] compared with ambient [CO_2] plots (Table S1, Fig. 3). Although the interaction between [CO_2] and species was not significant for LAI, increased LAI at elevated [CO_2] was much greater for *Ambrosia*, *Lycium andersonii*, and *Psorothamnus* than for *Larrea, Lycium pallidum* and *Krameria*.

Similar to shoot standing biomass, [CO_2] had no significant effect on root standing biomass, and [CO_2] did not significantly interact with microsite or tissue type, whether measured at six different microsites (Table S2a) or at the whole plot (Table S2b). Coarse and woody root biomass differed among microsites, although fine root biomass did not differ between the two microsites

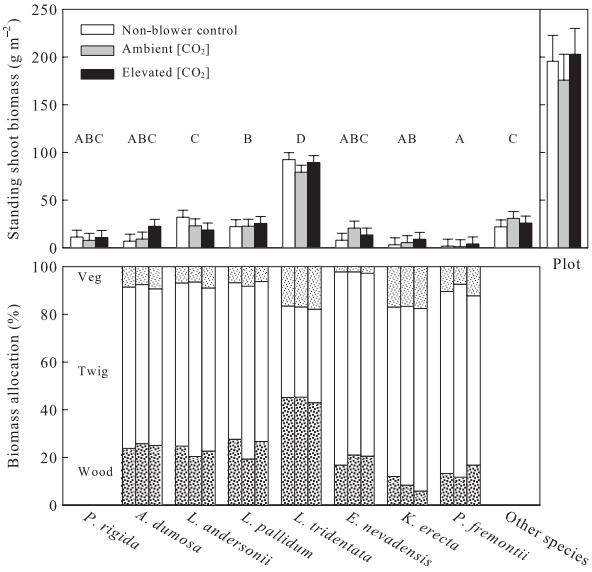

Fig. 2 Shoot biomass (top panel) for eight dominant species, all other species, and the total plot for nonblower controls, ambient [CO_2], and elevated [CO_2] plots at the Nevada Desert FACE (free-air carbon dioxide enrichment) Facility. Aboveground biomass allocation to vegetative tissue, twigs, and wood for seven dominant species is shown in the bottom panel; treatments from left to right are nonblower controls, ambient [CO_2], and elevated [CO_2]. Error bars represent ±1 SE; capital letters indicate significant differences across species when $P < 0.05$. Functional type for each species is shown in Table 1. 'Other species' include various functional groups; 'Plot' includes all species.

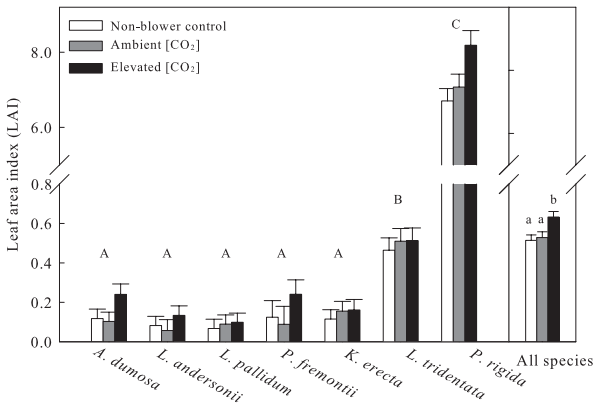

Fig. 3 Leaf area index (LAI) in non-blower controls, ambient [CO_2], and elevated [CO_2] plots at the Nevada Desert FACE (free-air carbon dioxide enrichment) Facility for seven dominant species (left panel) and all seven species combined (right panel). Error bars represent ±1 SE; capital letters indicate significant differences among species; lower case letters indicate significant differences across treatments when $P < 0.05$. Functional type for each species is shown in Table 2. 'All species' represents treatment effects across all seven species.

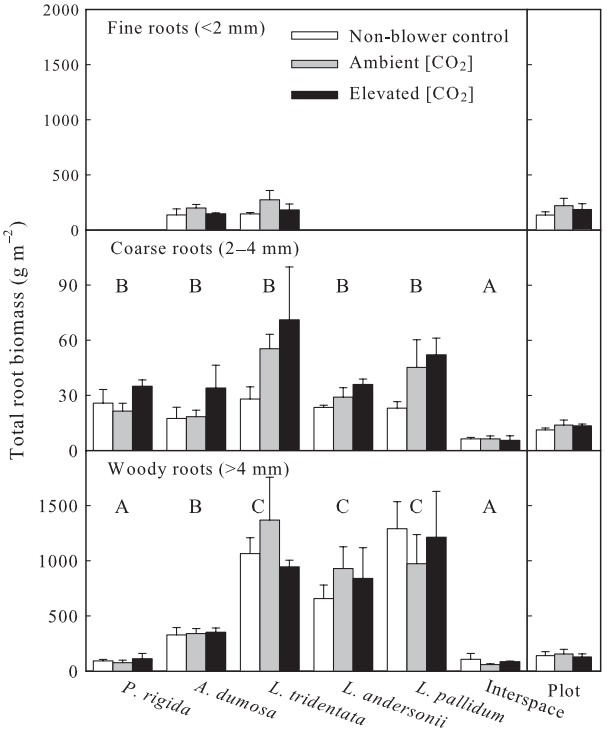

Fig. 4 Fine (<2 mm diameter, upper panels), coarse (2–4 mm diameter, middle panels), and woody (>4 mm diameter, lower panels) root biomass for six microsites and the whole plot for non-blower controls, ambient [CO_2], and elevated [CO_2] plots at the Nevada Desert FACE (free-air carbon dioxide enrichment) Facility. Error bars represent ±1 SE; capital letters indicate significant differences among species when $P < 0.05$. Functional type for each species is shown in Table 2. 'Interspace' is the large openings between perennial plants. 'Plot' values are measured independently of microsites and are representative of the whole plant community. Note that missing values occur for fine root samples because they were only collected from the *Ambrosia dumosa* and *Larrea tridentata* microsites.

(under *Ambrosia* and *Larrea* shrubs) (Fig. 4). The three shrubs that comprised approximately 70% of the plot's shoot biomass (the two *Lycium* species and *Larrea*) also had significantly greater woody root biomass than the other microsites. Although the interspace microsite did not have any perennial plants, desert shrubs extend their roots beyond the edges of their canopies; thus, roots were present in the interspace but in significantly lower amounts than most other microsites (Fig. 4).

Root biomass allocation to fine, coarse, and woody roots varied among microsites and the whole plot (Table S2). Although fine root standing biomass for *Ambrosia* and *Larrea* were not significantly different (Fig. 4, top left panel), a larger fraction of root standing biomass for *Ambrosia* was in fine roots (66%) than for *Larrea* (33%). In contrast, a larger fraction of root biomass for *Larrea* was in woody roots (62%) than for *Ambrosia* (29%). For the whole plot, fine roots were approximately 46% of root standing biomass, which was not significantly different than the approximately 49% for woody roots (Fig. 4, right panels). Moreover, total root biomass comprised the majority of total plant biomass (i.e., shoot plus root) for the plant community; root mass ratio was not significantly different among treatments ($F_{2,6} = 2.17$, $P = 0.195$) and averaged 58% ± 3% (SE) across all treatments.

Tissue C and N content

The [CO_2] treatment had no significant effect on C content of shoot tissues (Table S3a), but [CO_2] and the [CO_2] × tissue interaction were significant for root tissues (Table S3b). Carbon content of fine roots was greater in the nonblower control compared with ambient and elevated [CO_2] (Fig. S2, lower left panel), but the [CO_2] treatment did not significantly affect the C content of coarse or woody roots. We also analyzed plant C pools (i.e., biomass of each tissue type multiplied by its respective C content, then summed to get total shoot and total root C pools), but [CO_2] main effects and interaction terms were not significant (results not shown).

Significant differences in C content among species only occurred for some tissue types (Table S3, Fig. S2). For shoots, the two *Lycium* species generally had significantly lower C content than other species for vegetative and twig tissues, and a similar pattern also occurred for large diameter woody roots. Carbon content was significantly lower in vegetative compared with twig and woody tissue in the three drought-deciduous shrubs, whereas C content was not significantly different among *Larrea* tissue types. For root tissues, C content of fine roots was significantly greater than that of coarse roots, which in turn was significantly greater than both small and large diameter woody roots. C content of small diameter and large diameter woody roots were not significantly different from each other.

Percent N content showed similar patterns to C content, where species differed in N content, but [CO_2] did not significantly affect N content for both shoot and root tissues (Table S3, Fig. S3). The two *Lycium* species generally had tissue N content that was significantly different from other species, although, unlike C content, the two *Lycium* species had greater N content in their shoot and root tissues than the other species. The C_4 bunchgrass, *Pleuraphis*, consistently had the lowest tissue N contents, and the drought-deciduous shrub *Ambrosia* consistently had the second lowest tissue N contents. Shoot N content was significantly higher in vegetative tissue compared with twig and wood tissue for all four shrub species. For root N content, N content tended to decrease with increasing root diameter, but significant

differences among tissue types varied depending on the species. For *Ambrosia*, fine root N content was significantly greater than other root tissues, but coarse roots were not significantly different than woody roots. For *Larrea*, N content of fine and small diameter woody roots were significantly greater than that of large diameter woody roots. For *Lycium pallidum*, N content of coarse roots and of small diameter woody roots was significantly greater than that of large diameter woody roots. Although N content of tissue types for *L. andersonii* had a similar pattern, significant differences among tissue types did not occur for this species. In contrast, N content of coarse roots for *Pleuraphis* was significantly lower than that of small diameter woody roots.

Results for C : N ratios echoed C and N content results. The [CO_2] treatment had no significant effect on C : N ratios; however, significant differences occurred among species depending on tissue type (Table S3). Among all shoot and root tissues, the C_4 bunchgrass *Pleuraphis* had the greatest C : N ratio, and the two *Lycium* species had the lowest C : N ratio (results not shown). For twig and woody shoot tissue and for coarse and woody root tissues, C : N ratios for *Ambrosia* were significantly greater than the other three shrub species. However, C : N ratios of vegetative and fine root tissues were not significantly different between *Ambrosia* and *Larrea*. In addition, vegetative shoot tissue had a significantly lower C : N ratio than twig and woody shoot tissues. For roots, however, significant differences among tissue types only occurred for *Ambrosia* and *Pleuraphis*. In both species, the C : N ratio of coarse roots was significantly greater than that of small diameter woody roots. For *Ambrosia*, the C : N ratio of large diameter woody roots was not significantly different from that of coarse roots, and the C : N ratio of fine roots was not significantly different from that of small diameter woody roots.

Tissue C and N isotope composition

Tissue $\delta^{13}C$ was significantly different among CO_2 treatments, among species, and among tissue types for

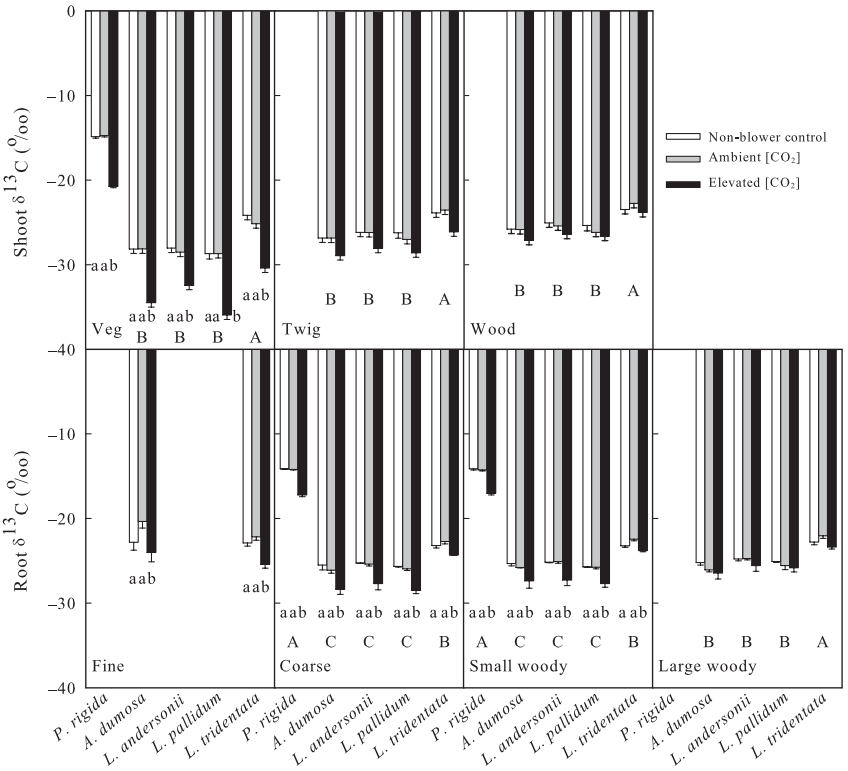

Fig. 5 Carbon stable isotope composition ($\delta^{13}C$) of shoot (top panels) and root (bottom panels) tissues for the five dominant species for non-blower controls, ambient [CO_2], and elevated [CO_2] plots at the Nevada Desert FACE (free-air carbon dioxide enrichment)Facility. Error bars represent ±1 SE; capital letters indicate significant differences among species when $P < 0.05$. Functional type for each species is shown in Table 2. Shoot tissues include vegetative (leaves), twig (<8 mm diameter), and wood (>8 mm diameter); root tissues include fine (<2 mm diameter), coarse (2–4 mm diameter), small woody (4–10 mm diameter), and large woody (>10 mm diameter) roots. Note that missing values are because (i) *Pleuraphis rigida* does not produce twig, woody shoots, or large diameter woody roots; and (ii) fine root samples were only collected from *Ambrosia dumosa* and *Larrea tridentata*.

both shoot and root tissues, and the effect of [CO_2] treatment depended on tissue type (Table S3). Vegetative shoots, fine roots, coarse roots, and small diameter woody roots in elevated [CO_2] consistently had significantly lower $\delta^{13}C$ than both ambient [CO_2] and nonblower control treatments for all five species (Fig. 5). This pattern of lower $\delta^{13}C$ for the elevated [CO_2] also occurred for twig and woody shoots and large diameter woody roots, but the differences among treatments were not significant. Differences in $\delta^{13}C$ among species were also generally consistent among shoot and root tissue types, as the C_4 bunchgrass *Pleuraphis* had significantly higher $\delta^{13}C$ than the four C_3 shrubs for all tissues types, and the evergreen shrub *Larrea* had significantly higher $\delta^{13}C$ than the three drought-deciduous shrubs for all tissue types except fine roots. Furthermore, $\delta^{13}C$ of vegetative shoots were consistently lower than that of twig and woody shoots; however, this difference was greatest under elevated [CO_2]. For root tissues, significant differences among tissue types only occurred for the drought-deciduous *Ambrosia*, where $\delta^{13}C$ of fine roots was significantly higher than that of the other root tissues, but none of the other root tissues were significantly different from each other.

The results for leaf carbon isotope discrimination ($\Delta^{13}C$) mirrored leaf $\delta^{13}C$, where $\Delta^{13}C$ depended upon treatment ($F_{2,6} = 46.64$, $P < 0.001$) and species ($F_{4,23} = 200.89$, $P < 0.001$), but the treatment x species interaction was not significant ($F_{8,23} = 0.95$, $P = 0.497$). Across all species, leaf $\Delta^{13}C$ for the elevated [CO_2] treatment averaged 13.2‰, which was significantly lower than that for both ambient (17.3‰) and nonblower control (17.0‰) treatments. Across all treatments, leaf $\Delta^{13}C$ for the C_4 bunchgrass *Pleuraphis* (5.4‰) was significantly lower than that for the evergreen shrub *Larrea* (15.5‰), which in turn was significantly lower than leaf $\Delta^{13}C$ for the drought-deciduous shrubs *Ambrosia* (19.4‰), *L. andersonii* (18.8‰), and *L. pallidum* (20.3‰). Differences in leaf $\Delta^{13}C$ among *Ambrosia* and the two *Lycium* species were not significant.

The [CO_2] treatment had no significant effect on $\delta^{15}N$ for any species or tissue type; however, tissue $\delta^{15}N$ varied greatly among species and tissue types (Table S3). The most consistent pattern among species was that $\delta^{15}N$ of woody shoots and roots for *Larrea* were

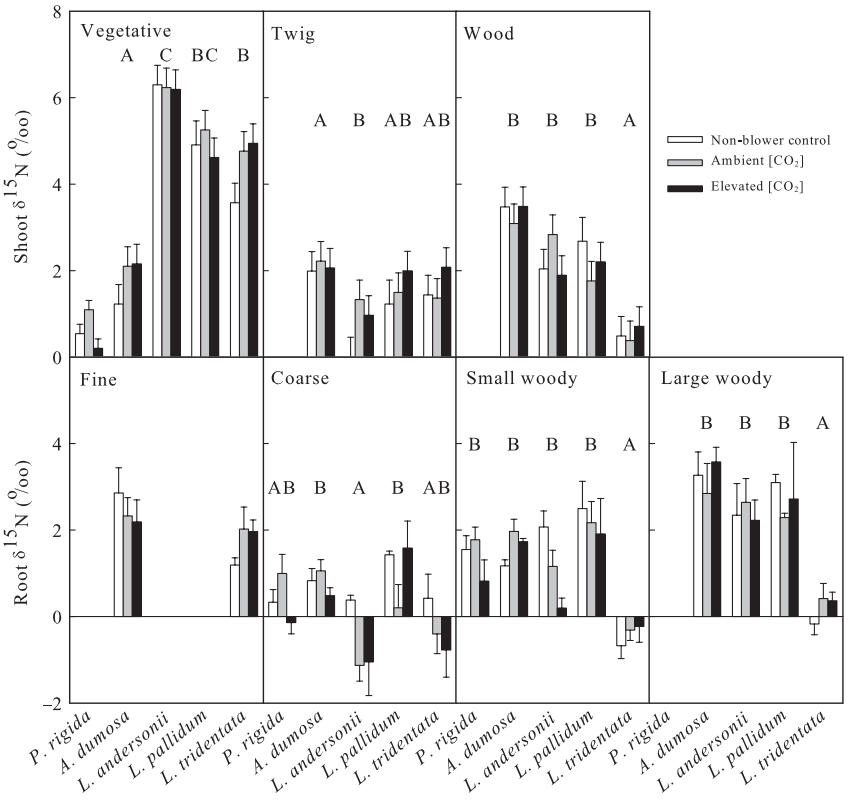

Fig. 6 Nitrogen stable isotope composition ($\delta^{15}N$) of shoot (top panels) and root (bottom panels) tissues for the five dominant species for non-blower controls, ambient [CO_2], and elevated [CO_2] at the Nevada Desert FACE (free-air carbon dioxide enrichment) Facility. See Fig. 5 for additional details.

significantly lower than those for other shrubs, although $\delta^{15}N$ of *Larrea* was intermediate to that of the other shrubs for nonwoody shoot and root tissues (Fig. 6). Vegetative shoots had significantly higher $\delta^{15}N$ than twig or woody shoots for *Larrea* and the two *Lycium* species, whereas $\delta^{15}N$ was highest in wood for *Ambrosia*. Among root tissues, fine roots of *Larrea* had significantly higher $\delta^{15}N$ than other root tissues, whereas $\delta^{15}N$ of *Ambrosia* fine roots was significantly higher than only that of coarse roots. In contrast, woody roots of the three drought-deciduous shrubs generally had significantly higher $\delta^{15}N$ than that of coarse roots. Finally, $\delta^{15}N$ was not significantly different between coarse and small diameter woody roots for *Pleuraphis*.

Discussion

Initial predictions about plant responses to elevated [CO_2] included increased photosynthesis and productivity, with C_3, leguminous, and herbaceous perennials having the greatest response (Nowak *et al*., 2004a). Indeed, meta-analyses have shown increased photosynthesis and productivity under elevated [CO_2] in the majority of grassland, forest, and cropland experiments (Ainsworth & Long, 2005). Furthermore, many predicted that plant responses in drier environments would be more pronounced than in other environments because of the known enhancement of plant WUE by elevated [CO_2] (Strain & Bazzaz, 1983; Melillo *et al*., 1993; Nowak *et al*., 2004a,b). Our leaf $\Delta^{13}C$ results clearly indicated substantial increases in leaf-level WUE across the major perennial life forms in the Mojave Desert under elevated [CO_2], but this response is a short-term effect (see below for more discussion). In contrast, both aboveground and belowground standing biomass and cover were not significantly affected by elevated [CO_2] at the end of our 10-year FACE experiment in the driest desert in North America. The lack of positive effects of elevated [CO_2] on standing crop consistently occurred both at the plot level and for all major plant functional types, and is in stark contrast to an initial prediction that deserts would substantially increase net primary productivity in response to elevated atmospheric CO_2 (Melillo *et al*., 1993).

We suspect that the lack of increased standing biomass is primarily influenced by precipitation/drought cycles, which limit the ability of desert plants to sustain increased biomass under elevated [CO_2] over long time periods. Enhancement of photosynthesis was only prevalent in years with above average rainfall under elevated [CO_2] (Huxman *et al*., 1998; Hamerlynck *et al*., 2000; Naumburg *et al*., 2003; Aranjuelo *et al*., 2011), but CO_2-enhanced growth was primarily confined to a single wet year that occurred at the beginning of the experiment (Hamerlynck *et al*., 2000; Housman *et al*., 2006). Furthermore, $\delta^{13}C$ data indicated that plants assimilated the applied [CO_2] into different tissues, including some woody tissues, but these positive responses did not result in long-term accumulation of additional biomass. The wettest year at the NDFF (1998) was followed by four consecutive dry years (including 1 year with <50 mm precipitation), and the FACE experiment was terminated in 2007, which was another extremely dry year. Our results suggest that the extended drought period caused biomass loss through canopy dieback (Miriti *et al*., 2007; McAuliffe & Hamerlynck, 2010), which negated any positive production responses to elevated [CO_2] that occurred in a wet year. Consequently, future effects of rising atmospheric CO_2 in desert ecosystems will depend on the episodic nature of future precipitation/drought cycles.

Because the Mojave Desert is composed of many stress-tolerant species, this ecosystem may be inherently less responsive to elevated [CO_2]. Lack of a productivity response to elevated [CO_2] has also been documented in other stressful ecosystems, such as alpine treeline (Handa *et al*., 2006, 2008) and glacial forefields (Inauen *et al*., 2012). Positive production responses to elevated [CO_2] in this water-limited ecosystem could be realized through increased WUE and thus increased soil moisture. Although previous evidence for increased WUE exists for *Larrea* and *Ambrosia* in earlier years during the experiment (Housman *et al*., 2006; Aranjuelo *et al*., 2011), as well as for the five species we examined at the time of harvest, soil moisture did not increase at the NDFF under elevated [CO_2] (Nowak *et al*., 2004b). Thus, many of the ecophysiological assumptions of the effects of elevated [CO_2] on Mojave Desert plants did not result in higher long-term production. Our results suggest that water availability regulates plant responses to elevated [CO_2] and that the high frequency of low precipitation years constrains long-term biomass responses to elevated [CO_2] in this arid system. Indeed, Weltzin *et al*. (2003) suggested that precipitation is a driving force in semiarid and arid environments and will dictate ecosystem responses to elevated [CO_2] or warming.

The lack of increased standing biomass at the end of the NDFF experiment also may be because the vegetation is a mature ecosystem that has reached steady state biomass and cover; such systems tend to have more moderate responses to elevated [CO_2] (Körner, 2006; Handa *et al*., 2008). Our FACE experiment differed from others in that we applied elevated [CO_2] to an intact ecosystem composed of long-lived shrubs that has been undisturbed by recent human activities. This starkly contrasts with many forest FACE sites, which

have young trees in rapidly growing stages of their life cycle and hence are accumulating biomass. Consistent with the concept that mature vegetation is less responsive to elevated [CO_2], trees in a mature deciduous forest had no stimulation in stem growth and litter production after 4 years of elevated [CO_2] treatment (Körner *et al*., 2005). Lack of elevated [CO_2] effects was also found in root production at treeline (Handa *et al*., 2008). Furthermore, mature vegetation in the Mojave Desert has low perennial cover (<20% at the NDFF) and low leaf area, which are ecosystem attributes that are directly related to long-term equilibrium with low rainfall (Smith *et al*., 1997). It is thus not surprising that elevated [CO_2], even over a full decade, had no quantitative cumulative effect on perennial plant cover or biomass at this site.

In addition, elevated [CO_2] did not alter biomass allocation to any of the aboveground or belowground tissues in the five species. Furthermore, elevated [CO_2] did not affect root mass ratio, that is, a measure of relative biomass allocation to roots vs. shoots. Several elevated [CO_2] studies have reported shifts in biomass allocation towards roots (Hättenschwiler & Körner, 1998; Norby *et al*., 2004; Inauen *et al*., 2012), whereas others did not report this trend (Handa *et al*., 2008; Bader *et al*., 2009). These variations in response suggest that shifts in biomass allocation are not necessarily dependent upon elevated [CO_2] affecting total growth. In comparison, Inauen *et al*. (2012) found no effect of elevated [CO_2] on the total biomass of nine species after 3 years of exposure but found biomass allocation shifts from shoots to roots in a glacier forefield.

Although biomass allocation was not affected by elevated [CO_2], LAI increased when considered across all species. LAI responses to elevated [CO_2] mostly range from neutral (Drake *et al*., 1997; Niklaus *et al*., 1998; Norby *et al*., 2003) to positive (Hartz-Rubin & Delucia, 2001; Hymus *et al*., 2002; Derner *et al*., 2003) and may vary seasonally (Hartz-Rubin & Delucia, 2001; Hymus *et al*., 2002). The increase in LAI was mainly driven by *Ambrosia*, *Psorothamnus*, and *Pleuraphis*, which are all different functional types. However, a meta-analysis of other studies suggested that only trees significantly increased LAI in response to elevated [CO_2] (Ainsworth & Long, 2005). Thus, our results add to the literature demonstrating LAI in both shrubs and a C_4 grass may increase with elevated [CO_2]. In addition, short-term responses such as greater LAI and higher WUE, coupled with the general observation of greater photosynthetic rates under elevated [CO_2], suggests potential for increased ecosystem C gain. Although increased ecosystem C gain is manifested as increased tree biomass in forested ecosystems, a similar manifestation of increased plant biomass does not appear to occur in the Mojave Desert, at least over the decade of this experiment.

We hypothesized that elevated [CO_2] would increase C and decrease N content in above- and belowground tissues, thereby increasing the C : N ratio. Surprisingly, the [CO_2] treatment did not affect C and N content of aboveground or belowground tissues, and this lack of an effect was consistent across all species or functional groups studied. Furthermore, C : N ratios were not increased by elevated [CO_2]. In contrast, a literature review across several ecosystems found that foliar C : N ratios increased for C_3 grasses, forbs, and woody species but not for C_4 grasses under elevated [CO_2] (Sardans *et al*., 2012). Our samples were collected on actively growing branches, so if any changes in C and N content were occurring during the experiment, our sampling should have detected the changes in new, younger leaves. In contrast to our current results, previous studies at this site found decreases in leaf N under elevated [CO_2]. During the first 3 years of the experiment, *Larrea* (in 1999) and *Lycium pallidum* (in 2000) had lower leaf N under elevated [CO_2]; however, leaf C was not affected in any year (Billings *et al*., 2003). Housman *et al.* (2006) also found decreased leaf N under elevated [CO_2] for *Ambrosia*, *Larrea*, and *Krameria*. Although Aranjuelo *et al.* (2011) found leaf N to decrease in *Larrea* and *Ambrosia*, these decreases only occurred in certain spring months. Nitrogen is often a secondary limiting factor in deserts (Smith & Nowak, 1990), and we suspect that the differences in how elevated [CO_2] affected leaf N during our experiment are due to seasonal and annual interactions between water and nitrogen availability.

$\delta^{13}C$ values decreased in vegetative tissue, fine, coarse, and small woody roots under elevated [CO_2], but the $\delta^{13}C$ of twigs, woody shoots, and large woody roots was not affected by elevated [CO_2]. This response was consistent across all five species examined and largely reflected the fossil fuel source of CO_2 that was used for the elevated [CO_2] plots during the last 4 years of the experiment. From initiation of the NDFF experiment on April 1997 to February 2003, elevated [CO_2] plots were fumigated with CO_2 derived from a geologic source whose $\delta^{13}C$ content was similar to the current atmosphere (Aranjuelo *et al*., 2011). On 10 February 2003, we changed to CO_2 from a fossil fuel source that was depleted in ^{13}C relative to current atmospheric CO_2. The carbon isotope signature of the fossil fuel CO_2 was clearly evident in younger tissues produced during the last 4 years of the experiment and was even detectable in some woody tissues that were largely produced prior to the use of fossil fuel derived CO_2. Species also consistently differed in $\delta^{13}C$. The C_4 grass, *Pleuraphis*, had the least negative $\delta^{13}C$ in each tissue type, as

typically occurs in plants with the C_4 photosynthetic pathway (O'Leary, 1981, 1988).

Carbon isotope discrimination ($\Delta^{13}C$) of leaves indicated greater leaf WUE under elevated [CO_2] across all growth forms in the Mojave Desert during the last growing season prior to harvest. Leaf $\Delta^{13}C$ is inversely proportional to leaf WUE integrated over the time period during which the leaf was produced, assuming a constant difference in water vapor pressure (Farquhar *et al*., 1989). Because we did not observe differences in leaf temperature between plants in ambient and elevated [CO_2] (Nowak *et al*., 2001), lower leaf $\Delta^{13}C$ observed under elevated [CO_2] indicates greater WUE than under ambient [CO_2]. Ainsworth & Long (2005) also reported greater leaf WUE under elevated [CO_2], although the increase was significant for C_3 species but not for the single C_4 species (sorghum) in their meta-analysis. In our study, the C_4 bunchgrass and both drought-deciduous and evergreen C_3 shrubs significantly decreased leaf $\Delta^{13}C$ from ambient to elevated [CO_2]. Furthermore, the absolute increment in leaf $\Delta^{13}C$ from ambient to elevated [CO_2] for the C_4 bunchgrass ($-3.8‰$) was only slightly smaller than that averaged over the four C_3 shrubs ($-4.1‰$). Thus, our results reinforce that inferences are difficult to generalize on how elevated [CO_2] affects species based on plant functional type (Nowak *et al*., 2004a).

$\delta^{15}N$ was substantially lower for *Pleuraphis* and *Ambrosia* compared with other species. Low $\delta^{15}N$ values for *Pleuraphis* may suggest the presence of free-living N-fixers in the rhizosphere of this species. However, $\delta^{15}N$ was not significantly different under elevated [CO_2]. In contrast, Billings *et al*. (2004) found elevated [CO_2] increased $\delta^{15}N$ values in foliage of *Larrea* and *Krameria* but not *Ambrosia*, the two *Lycium* species, and *Pleuraphis*. They interpreted these changes in $\delta^{15}N$ as an indication of changes in N cycling due to increased C input into the soil under elevated [CO_2], which provided C for microbial activity. Unfortunately, we are unable to determine if the lack of elevated [CO_2] effects on $\delta^{15}N$ at the end of our experiment is because soil microbes were no longer C limited, or because low precipitation in the final year limited N cycling similarly in both ambient and elevated [CO_2] treatments.

Results from the NDFF provide the first examination of how productivity of an intact desert ecosystem was affected by long-term exposure to elevated [CO_2]. In addition, our results provide invaluable measurements of community biomass in the understudied Mojave Desert, which is the driest of the North American deserts. For example, the species examined here represent a diversity of functional types and are important components of this desert ecosystem, yet little information is available about their belowground or aboveground productivity responses. It is useful to compare our results with measurements made in Rock Valley during the 1970s, which is <20 km from the NDFF and also in the *Larrea-Ambrosia* vegetation zone (Rundel & Gibson, 1996). Our aboveground biomass estimates for various species coincide with those of Rundel & Gibson (1996) with the exception of *Larrea*, where our aboveground biomass estimates are three times that found at Rock Valley in the 1970s (indeed, *Larrea* are of greater stature at the NDFF than at Rock Valley). Interestingly, our measurements of root biomass are also greater than those from Rock Valley (Wallace *et al*., 1980), which are presumably due to our greater soil excavation depths (1 m in our study vs. 0.3–0.5 m in Wallace *et al*., 1980) and our more detailed estimates of fine root standing crop. Although Mojave Desert plants extend roots much deeper than 1 m into the soil (Hartle *et al*., 2006), global studies of root distribution with soil depth indicate that 95% of root biomass is within the top 1.12 m of soil for desert ecosystems (Schenk & Jackson, 2002), which suggests that our root excavation recovered >90% of root biomass. Our more extensive measurements of root biomass also resulted in greater estimates of allocation to roots vs. shoots than earlier Mojave Desert studies: average root : shoot ratio across ten perennial species in Rock Valley was 0.91 (Rundel & Gibson, 1996), whereas root : shoot ratio for our study was 1.53. Utilizing information from Rock Valley and the NDFF provide the only long-term information on biomass trends in the northern Mojave Desert.

In summary, after 10 years of elevated [CO_2] in an intact Mojave Desert ecosystem, we found no differences in total plot biomass, aboveground and belowground biomass of the eight dominant species, or canopy cover. Carbon isotope discrimination results provided evidence for increased WUE and LAI averaged across all species increased under elevated [CO_2], but despite these results, elevated [CO_2] had no effect on biomass allocation, C and N content, and $\delta^{15}N$ values. These results are in contrast to previous predictions that enhanced WUE under elevated [CO_2] would significantly increase primary production. We propose that the lack of a long-term productivity response to elevated [CO_2] in this arid system is due to inter-annual water limitations, conservative life histories, and the long-term steady state of our Mojave Desert ecosystem. Increased production early in the experiment during an extremely wet year was not sustained during an intervening drought period. Thus, it appears that perennial plants in the Mojave Desert are not substantially carbon limited. Rather, other resource limitations (particularly water) appear more limiting in mature desert scrub communities. Further investigations into the fate of carbon via plant litter and soil organic matter are

warranted to broaden our understanding of whole ecosystem responses to elevated [CO_2] in this Mojave Desert ecosystem.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Schematic of soil volume excavations for coarse and woody root standing crops of shrub (left), bunchgrass (middle), and interspace (right) microsites. Shaded volumes of soil were excavated to estimate root biomass for each microsite. Asterisks represent center of target plant. For shrubs, the lightly-shaded 0.5 m wide × 1.0 m long × 1.0 m deep soil volume that included the target plant's center was excavated first, then the darker-shaded 0.5 m wide × 0.5 m long × 0.5 m deep soil volume was excavated.
Figure S2. Carbon content of shoot (top panels) and root (bottom panels) tissues for the five dominant species for non-blower controls, ambient [CO_2], and elevated [CO_2] plots at the Nevada Desert FACE Facility. See Fig. 5 for additional details.
Figure S3. Nitrogen content of shoot (top panels) and root (bottom panels) tissues for the five dominant species for non-blower controls, ambient [CO_2], and elevated [CO_2] plots at the Nevada Desert FACE Facility. See Fig. 5 for additional details.
Table S1. ANOVA results for shoot standing biomass, for shoot allocation to vegetative, twig, and woody tissues, and for leaf area index (LAI) for species that comprised >90% of shoot biomass. Degrees of freedom (DF) are reported as 'numerator, denominator'.
Table S2. ANOVA results for root standing biomass measured: (a) at six microsites in each plot; and (b) for transects in each plot that sampled the entire plant community. Degrees of freedom (DF) are expressed as 'numerator, denominator'.
Table S3. ANOVA results for C and N content, C : N ratio, and C and N stable isotope composition of (a) shoot tissues and (b) root tissues. Degrees of freedom (DF) are expressed as 'numerator, denominator'.