Title: Maintenance of C sinks sustains enhanced C assimilation during long-term exposure to elevated [CO_2] in Mojave Desert shrubs

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Abstract

During the first few years of elevated atmospheric [CO_2] treatment at the Nevada Desert FACE Facility, photosynthetic down-regulation was observed in desert shrubs grown under elevated [CO_2], especially under relatively wet environmental conditions. Nonetheless, those plants maintained increased A_{sat} (photosynthetic performance at saturating light but treatment [CO_2]) under wet conditions but to a much lesser extent under dry conditions. To determine if plants continued to down-regulate during long-term exposure to elevated [CO_2], responses of photosynthesis to elevated [CO_2] was examined in two dominant Mojave Desert shrubs, the evergreen *Larrea tridentata* and the drought-deciduous *Ambrosia dumosa*, during the eighth full growing season of elevated [CO_2] treatment at the NDFF. A comprehensive suite of physiological processes were collected. Furthermore, we used C labeling of air to assess carbon allocation and partitioning as measures of C sink activity. Results show that elevated [CO_2] enhanced photosynthetic performance and plant water status in *Larrea*, especially during periods of environmental stress, but not in *Ambrosia*. $\delta^{13}C$ analyses indicate that *Larrea* under elevated [CO_2] allocated a greater proportion of newly assimilated C to C sinks than *Ambrosia*. Maintenance by *Larrea* of C sinks during the dry season partially explained the reduced [CO_2] effect on leaf carbohydrate content during summer, which in turn lessened carbohydrate build-up and feedback inhibition of photosynthesis. $\delta^{13}C$ results also showed that in a year when plant growth reached the highest rates in 5 years, 4% (*Larrea*) and 7% (*Ambrosia*) of C in newly emerging organs was remobilized from C that was assimilated and stored for at least 2 years prior to the current study. Thus after eight years of continuous exposure to elevated [CO_2], both desert perennials maintained photosynthetic capacity under elevated [CO_2] We conclude that C storage, remobilization, and partitioning influence responsiveness of these desert shrubs during long-term exposure to elevated [CO_2].

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Keywords: *Ambrosia dumosa*, C allocation/partitioning, Free-air CO_2 enrichment (FACE), *Larrea tridentata*, photosynthetic down regulation

Introduction

Initial increases in net assimilation rates of vascular plants exposed to elevated atmospheric [CO_2] may not be sustained over long time periods because of "a hierarchy of increasingly complex processes controlling the production and allocation of end-products" (Lemon 1983). For example, plants photosynthetically acclimate (*i.e.* down-regulate) to growth in elevated [CO_2] through changes in the photosynthetic apparatus, including lower light- and CO_2-saturated photosynthesis (A_{max}) as well as lower V_{cmax} (maximum Rubisco carboxylation) and J_{max} (maximum electron transport) (Tissue et al. 1993, 2001). A second process that may impact the long-term enhancement of photosynthesis under elevated [CO_2] is reduced N availability, such as through re-allocation of N within the plant to meet other growth needs (Theobald et al. 1998, Zhu et al. 2009) or reduced N cycling in the ecosystem (Zak et al. 2000). Support for this process comes from meta-analyses of both FACE (free-air carbon dioxide enrichment) and OTC (open-top chamber) studies: some plants, but particularly woody species, that exhibit photosynthetic acclimation also exhibit declines in leaf N and Rubisco content (Long et al. 2004, Ainsworth and Long 2005). These trends also have been replicated in comparative gas exchange studies across several FACE sites (Ellsworth et al. 2004). Another process that may impact the long-term enhancement of photosynthesis under elevated [CO_2] is feedback inhibition of photosynthesis by carbohydrate accumulation (Moore et al. 1999, Jifon and Wolfe 2002). Meta-analyses also support this process: many studies describe increased leaf starch or soluble sugars at elevated [CO_2] (Long et al. 2004, Ainsworth and Long 2005). Thus over the long term, elevated CO_2 effects on leaf C assimilation may be conditioned by an ecosystem's ability to provide adequate N through changes in N cycling and by a plant's ability to develop new sinks (*e.g.* new vegetative or reproductive structures) or to expand the storage capacity or growth rate of existing sinks like shoots and roots (Lewis et al. 2002, Aranjuelo et al. 2009). Clearly, these processes are not mutually exclusive. Nonetheless, understanding this "hierarchy of increasingly complex processes" is critical to realistically predict long-term ecosystem C assimilation from the atmosphere.

The Nevada Desert FACE Facility (NDFF) is an ideal system to investigate how plants control photosynthesis during long-term exposure to elevated [CO_2]. Nitrogen cycling at the NDFF rapidly changed after initiation of elevated [CO_2] treatments (Billings et al. 2002), increasing N availability (Billings et al. 2004) largely through shifts in soil microbial activity (Jin and Evans 2007, 2010). Thus, mechanisms that cause photosynthetic changes in plants at the NDFF should be influenced more by within-plant processes, such as by N re-allocation within leaves or among tissues or by the size and activity of C sinks, than by N availability through ecosystem N cycling. In the first five years of the NDFF experiment, alterations in physiological processes due to elevated [CO_2] were observed in annual and perennial plants in the NDFF. For example, water was severely limiting in most years such that differences in photosynthetic rates between perennial plants growing in elevated versus ambient [CO_2] were minimal, with significant differences in photosynthesis only observed in years or seasons with adequate or above-average rainfall (Naumburg et al. 2003). Similarly, photosynthetic down-regulation – reductions in A_{max} (maximum net photosynthesis at saturating photosynthetic photon flux density (PPFD) and [CO_2]), V_{cmax} and J_{max} – was observed only in wet years for plants grown in elevated [CO_2] (Huxman et al. 1998, Hamerlynck et al. 2000b). However, these previous studies, as well as those from other FACE experiments, did not examine the relative importance of different processes, including leaf N and carbohydrates and carbon management (allocation and partitioning), in regulating photosynthetic performance during long-term elevated [CO_2].

Another advantage of the NDFF and other FACE experiments is that use of a ^{13}C depleted fossil fuel source to achieve elevated [CO_2] treatments introduces C isotope tracers into the system. These $^{13}C/^{12}C$ tracers provide an essential tool to study carbon management in plants (Körner et al. 2005, von Felten et al. 2007, Aranjuelo et al. 2008a, 2009). Labelling with $^{13}C/^{12}C$ as tracers and characterization of the distribution of labelled compounds into different plant organs has provided novel and relevant information in studies determining the flow of C through plants grown in elevated [CO_2] (Aranjuelo et al. 2009). C allocation and partitioning can be studied further by analyzing the isotopic composition of soluble sugars, especially sucrose, glucose, and fructose

(Körner et al. 2005, Kodama et al. 2010), which are anticipated to change under elevated [CO_2] (Aranjuelo et al. 2009).

In this study, a comprehensive suite of physiological process and C balance data were collected from plants during the eighth full growing season of continuous exposure to elevated [CO_2] and used to examine the regulation of photosynthetic performance during long-term exposure to elevated [CO_2] at the NDFF. Photosynthetic responses and carbon allocation/partitioning patterns were measured for the two dominant shrub species of the Mojave Desert, the evergreen *Larrea tridentata* and the drought deciduous *Ambrosia dumosa*. Measurements were made throughout the growing season: from cooler, wetter periods of peak growth in spring to hotter, drier periods of pronounced water stress in summer. Specifically, we examined leaf gas exchange, leaf pigments, leaf N, and leaf soluble sugars and starch to determine the extent that photosynthetic performance was enhanced during long-term exposure to elevated [CO_2] and to test specific mechanisms that may cause reduced photosynthetic performance. Because photosynthetic performance is affected by leaf-level and plant-level C allocation, photoassimilate allocation and partitioning also were studied through the use of $^{13}C/^{12}C$ labeling. We hypothesized that these desert species would down-regulate photosynthesis under elevated [CO_2] during the moist, early portions of the growing season and that down-regulation would be accompanied by altered leaf pigmentation, decreased leaf N, and increased leaf starch and soluble sugars. Furthermore, we hypothesized that reduced capacity of plants to allocate C away from leaves also would limit photosynthetic performance under elevated [CO_2].

Materials and Methods

Field site and C-labeling procedures

The Nevada Desert FACE Facility (NDFF) is located within the Nevada Test Site (36°39'N, 122°55'W, 960 m altitude). Three plots (23 m diameter; 415 m^2) had the full FACE apparatus (stand-pipes and blowers) and continuously exposed plants to elevated

$[CO_2]$ (target of 550 μmol mol^{-1}; averaged over 2005, actual treatment was 521 μmol mol^{-1}) and three plots had the FACE apparatus but blow air onto the plots at ambient $[CO_2]$ (measured during the 2005 growing season as 380 μmol mol^{-1}). The NDFF operated continuously (24 h per day, 365 d per year), with conditional shut-downs occurring only when air temperature dropped below 4°C or when wind speed exceeded 7 m s^{-1}. The 2005 growing season was the eighth full year of operation for the NDFF. The ecosystem within each plot was not disturbed during installation of the FACE apparatus and represents the same functioning ecosystem as the surrounding landscape in the northern Mojave Desert. The facility, vegetation, and soils are fully described in Jordan et al. (1999).

Elevated $[CO_2]$ was provided by supplementing ambient air with pure CO_2 to achieve the desired CO_2 concentration. Prior to February 10, 2003, the pure CO_2 (supplied by BOC Gases; Murray Hill, NJ, USA) was from a geologic source and had a CO_2 isotopic composition ($\delta^{13}C$) of -5.4 ‰, which diluted ambient air $\delta^{13}C$ (-8.0 ‰) to $\delta^{13}C$ of air above the elevated $[CO_2]$ plots of -7.3 ‰ (Naumburg et al. 2003). On February 10, 2003, we switched the source of pure CO_2 to fossil fuels, which had a more ^{13}C depleted $\delta^{13}C$ (-32.0 ‰), resulting in a $\delta^{13}C$ of CO_2 in air of -18.2 $\pm$ 1.9 ‰ for elevated $[CO_2]$ plots (Schaeffer 2005).

Plant material and sampling

The evergreen shrub *Larrea tridentata* (creosote bush) and the drought-deciduous shrub *Ambrosia dumosa* (white bursage) were selected for study. New leaves on *Larrea* at the NDFF emerge in late April or early May, with the majority of new growth occurring between mid-May and mid-June (Housman et al. 2006). Individual leaves (leaflets) live approximately 18 months (Sharifi et al. 1988). *Ambrosia* initiates a leaf canopy in early spring and then loses all its leaves during the hot, dry summer months and remains deciduous until the next year (Ackerman et al. 1980).

For *Larrea* and *Ambrosia*, leaves, shoots and roots that emerged during the current year were harvested for C isotopic composition ($\delta^{13}C$) and N in early morning. Leaves and

shoots were harvested monthly from April until July for both species and until August for *Larrea*. No data were presented for *Ambrosia* in August because those plants had entered their physiological dormancy period. In both cases, root sampling occurred only during April-June because new root formation did not occur in July or August for either species. Root samples were collected from root boxes located at the base of each shrub species (Clark et al. 2010). On each sampling date, harvests were from two plants per species in each of the three elevated and the three ambient [CO_2] plots.

Samples for xylem water potential, sugar content, and pigment analysis were all taken at pre-dawn, when plants were under minimal daily water stress. Starting approximately 1.5 hours before sunrise on each sampling date, two terminal shoot (stem + leaves) samples were removed from each of two study plants per plot in each of the three elevated and three ambient [CO_2] plots. Samples were placed in plastic bags, stored in a cooler and moved to a field lab adjacent to the research plots where they were prepared and analyzed. Pigment samples (approximately 4-6 leaves) were removed from branches and quickly placed in liquid nitrogen. Plant water potential of the other intact shoot sample was determined using a Scholander-type pressure chamber (Soil Moisture Stress Inc., Santa Barbara, CA, U.S.A.). Sugar analyses required significantly larger samples; therefore, all the remaining leaves from water potential determinations were pooled. Leaves for sugar analyses were removed from the stems in the dark and placed in liquid nitrogen after harvest.

Photosynthetic measurements

Photosynthetic gas exchange was measured with a LI-6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA) equipped with a CO_2 control module and a red-blue light emitting diode light source (Model 6400-02B). For gas exchange measurements, we sampled five plants from one elevated [CO_2] ring and five plants from one ambient [CO_2] ring. Sampling from additional FACE rings was not logistically feasible in this study due to the requirement that plants be accessed from a pivoting walkway, so we maximized sample size within individual large plots. The two selected plots were paired plots (*i.e.* same watershed position) in the overall experimental site, and

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so had highly similar surface and soil characteristics. Also, previous studies confirmed that plot-based variation in plant physiological parameters was relatively low for both evergreen and deciduous shrubs. For the evergreen *Larrea*, using 18 different dates between 1998 and 2004 in which we had plot-replicate data from *ACi* curves (n = 36 with 18 dates times the two [CO_2] treatments), we found the following coefficients of variation (CV's): V_{cmax} plant-to-plant = 22.0, plot-to-plot = 18.2; J_{max} plant-to-plant = 34.2, plot-to-plot = 25.7. Therefore, whereas individual plant variation was large, variation was consistently lower between sampling plots than between individual plants. Furthermore, these photosynthetic parameters were not consistently higher in one plot than another for either ambient or elevated [CO_2] treatments. Sampling two plots also allowed paired-in-time measurements at ambient and elevated [CO_2] to occur at highly similar temperature and VPD conditions during the day. This requirement could not have been met if we sampled six separate plots (FACE rings) with a single plant in each plot due to the time required to move between plots.

Photosynthetic CO_2 response curves (AC_i) were determined by measuring the response of photosynthesis (A) to varying intercellular CO_2 concentration (C_i). External [CO_2] (C_a) was supplied in 8 steps, increasing from 120 to 1500 μmol [CO_2] mol^{-1} air, with irradiance (Q) maintained at a saturating value of 1500 μmol m^{-2} s^{-1}. Measurements were initiated after g_s reached steady state and then recorded automatically at each C_a set point when photosynthesis had equilibrated, which was typically less than 2 min. Foliage temperature during AC_i curves was maintained at ambient air temperature using thermoelectric coolers. Leaf-to-air vapor pressure deficit was generally between 1.5 and 3.0 kPa, reflecting ambient conditions. Because both *Larrea* and *Ambrosia* have small, microphyllous leaves, more than one leaf (leaflets in *Larrea*) was inserted into the gas exchange cuvette. After the AC_i curve was generated, all material inside the cuvette was collected and leaf area was determined using a flatbed scanner and analyzed with software from Scion Imaging (Scion Corporation, Frederick, MD, USA). Leaves were subsequently dried at 60°C for at least 2 days and then weighed.

AC_i data were analyzed using the photosynthetic biochemical model of Farquhar et al. (1980) to estimate two biochemical parameters potentially limiting to photosynthesis:

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205 V_{cmax} (maximum carboxylation rate of Rubisco) and J_{max} (maximum electron transport
206 rate), which were temperature corrected to 25°C (Bernacchi et al. 2001). We used the
207 Michaelis-Menten constants of Rubisco described in Harley et al. (1992) and used by
208 Wullschleger (1993), where K_c (Michaelis-Menten constant for RuBP carboxylation) =
209 16 Pa, K_o (Michaelis-Menten constant for oxygenation) = 37961 kPa, and τ (specificity
210 factor for Rubisco; Jordan and Ogren 1984) = 2823, for both species. Net photosynthesis
211 at saturating Q (A_{sat}) was taken directly from the AC_i curves at each growth [CO_2].
212 Previous experiments have shown that for these species, mid-morning A_{sat} is a good
213 estimate of diurnal integrated [CO_2] assimilation (A_{day}; Naumburg et al. 2003). Net
214 photosynthesis at saturating [CO_2] and saturating Q (A_{max}) was also determined from the
215 AC_i curves. The relative stomatal limitation to photosynthesis (L_s) was calculated using
216 the method of Farquhar and Sharkey (1982) as described in Tissue et al. (2005) using
217 CO_2 concentrations of 550 μmol mol^{-1} and 380 μmol mol^{-1} for elevated and ambient
218 [CO_2], respectively.

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219 *Biochemical analyses*

220 For sugar extraction, plant samples were lyophilized and then ground to a fine powder
221 (<10 μm). About 50 mg of the fine powder was suspended in 1 mL of distilled water in
222 an Eppendorf tube (Eppendorf Scientific, Hamburg, Germany), mixed, and then
223 centrifuged at 12,000 g for 5 minutes at 5 ºC. After centrifugation, the supernatant was
224 used for total soluble sugar quantification, whereas the pellet was stored at -80ºC for the
225 starch analyses. Supernatant fraction was heat denatured at 100 ºC for 3 minutes and
226 precipitated by centrifugation at 12,000 g during 5 minutes at 5 ºC. The non-precipitated
227 phase then was used for sugar content analysis (Nogués et al. 2004). Starch samples were
228 purified and quantified through the elimination of the chlorophyllous pigments using
229 ethanol, followed by the starch solubilization step with HCl and its flocculation using
230 methanol (Duranceau et al. 1999).

231 Purification of soluble sugar samples used a solid phase extraction pre-column (Oasis
232 MCX 3cc, Waters). Sugar contents were analyzed using a Waters 600 high performance
233 liquid chromatograph (Waters Millipore Corp., Milford, MA, USA). The HPLC

refractive index detector (Waters 2414) was set at 37 ºC. Samples were eluted from the columns at 85 ºC (Aminex HPX-87P and Aminex HPX-87C connected in series, 300 mm x 7.8 mm; BioRad) with water at 0.6 mL min^{-1} flow rate and 45 min retention time. Sucrose, glucose, and fructose were collected and transferred to tin capsules for isotope analysis. The use of the purification pre-columns, together with the two Aminex columns connected in series enabled the separation of sugars (sucrose, glucose and fructose), avoiding possible contamination problems raised by Richter et al. (2009). Furthermore, as an additional precaution, initial and final phases of peaks were discarded when collecting the peaks. Although there is no specific technique to measure purified starch $\delta^{13}C$, we utilised a protocol (Richter et al. 2009) to analyze $\delta^{13}C$ of the HCl-hydrolysable C (HCl-C), which is mainly composed of starch; subsequently, HCl-C was our surrogate for starch C isotopic composition. $\delta^{13}C$ of individual sugars and HCl-C was analyzed by isotope ratio mass spectrometry (Delta C, Finnigan Mat, Bremen, Germany) as described by Nogués et al. (2008).

Leaf pigment samples were collected at pre-dawn, immediately frozen in liquid N and stored in an ultra-low freezer (-85 °C) prior to lyophilization. Before HPLC analysis, the dry mass of each sample was determined and approximately 10 mg dry mass of leaf material was used for pigment extraction. Samples were ground to a fine pulp in the dark in ice-cold 80% acetone (v/v) with an addition of $MgCO_3$ (spatula tip) using a tissue grinder (Kontes Duall K885450-0021, Kontes, Vineland, NJ, USA). Following extraction, chlorophyll and carotenoid content and composition were determined by HPLC using the method of Gilmore and Yamamoto (1991), as modified by Adams and Demmig-Adams (1992).

Plant C and N content and C isotopic composition in total organic matter and air

Leaf, stem, and root samples were used for C and N content and for carbon isotope composition analyses. Six 1.5 mg replicates were analyzed for each sample. Determinations were conducted at the Serveis Cientifico-Tecnics, University of Barcelona using an elemental analyzer (EA1108, Series 1, Carbo Erba Instrumentazione, Milan, Italy) coupled to an isotope ratio mass spectrometer (Delta C, Finnigan, Mat.,

263 Bremen Germany) operating in continuous flow mode. $^{13}C/^{12}C$ ratios were expressed in δ
264 notation:

265
$$\delta^{13}C(‰) = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right]$$
266

267 where R_{sample} refers to plant material and $R_{standard}$ to Pee Dee Belemmnite (PDB) calcium
268 carbonate.

269

270 Carbon isotope discrimination (Δ) was calculated as described by Farquhar et al**.** (1989):
271
$$\Delta = \frac{\delta_a - \delta_p}{\delta_p + 1}$$
272 where δ_a and δ_p denote air ($\delta^{13}C_a$) and plant ($\delta^{13}C_p$) isotopic composition, respectively.

273

274 The $^{13}C/^{12}C$ ratios (*R*) of air samples were determined at the University of Arkansas
275 (USA). Air $\delta^{13}C$ was determined through a trace gas condensing device (PreCon,
276 Finnigan MAT, Bremen, Germany) coupled to a Finnigan Delta+ mass spectrometer. Air
277 samples from all the treatment plots were collected by connecting a 100 mL air sampling
278 flask (Kimble Kontes, Vineland, NJ, USA) to the outlet stream of an infrared gas
279 analyzer (LiCor 6262, LiCor inc., Lincoln, NE, USA) located in a shed next to each plot.
280 On each sampling date, two samples were collected from each plot and three samples of
281 CO_2 were taken directly from the exhaust vent of the liquid CO_2 supply tank. Samples
282 were analyzed at the University of Arkansas Stable Isotope Facility.

283 *New carbon in carbohydrates and total organic matter*

284 The proportion of "new" carbon (C_{new}) in specific carbohydrate pools and in total organic
285 matter (TOM) represents the proportion of C present that was derived from C fixed

during the labeling period (2003-2005). C_{new} of samples was calculated as described by Nogués et al. (2004):

$$C_{new} \approx \frac{\delta^{13}C_E - \delta^{13}C_A}{\delta^{13}C_L - \delta^{13}C_A} x100$$

where $\delta^{13}C_E$ and $\delta^{13}C_A$ refers to the carbon isotopic compositions of plants grown at elevated and ambient [CO_2], respectively. $\delta^{13}C_L$ refers to the theoretical maximum isotopic composition of leaves, which is given by:

$$\delta^{13}C_L \approx \delta^{13}C_{air} - \Delta$$

A similar relationship was used to calculate the proportion of new carbon in carbohydrates.

Statistical analyses

All data were log transformed prior to analyses. Because individual plants were repeatedly measured over time, a repeated measures analysis of variance (RM ANOVA) was used to determine the effects of elevated [CO_2] on physiology, leaf chemistry, and C isotopic determinations of *Larrea* and *Ambrosia*. The sample dates, converted to Julian date, were used as the within-subject factor, whereas [CO_2] treatment was used as the between-subject factor. These analyses has 1 degree of freedom *(*df) for [CO_2] effect and 8 *dfs* for the error term (variability). A factorial analysis was also conducted with these data, and results were similar to those obtained by the RM ANOVA; therefore we used the more conservative RM ANOVA.. Because *Larrea* is an evergreen and *Ambrosia* is drought-deciduous, the RM ANOVA's were performed separately for each species. There were eight measurement dates for *Larrea*, and four measurement dates for *Ambrosia*.

Prior to running the RM ANOVA, a principal components analysis was conducted for each species separately to determine the nature and strength of the correlations between parameters. After these analyses showed strong correlations among the physiological data, missing data (5% of total physiology data) were estimated with the maximum likelihood estimation function utilizing a multivariate approach. A discriminant function analysis (DFA) was used for each species to determine those variables which best described differences between plants grown in elevated and ambient [CO_2]. All multivariate analyses were conducted using MatLab (V.7.1, SP 3; The Mathworks Inc., Natick, MA, USA).

The RM ANOVA's were performed using the general linear model function in SYSTAT (V12, Systat Software Inc., Chicago, IL, USA). Values were considered significantly different if probabilities (*P*) were < 0.05.

Results

Environmental conditions

Temperatures during the 2005 growing season were typical of the Mojave Desert, with the highest average monthly temperature occurring in July (Fig. 1A). The hydrologic year (1 Oct to 30 Sep) for the Mojave Desert had above-average precipitation, with significant amounts of rainfall occurring between October and March, followed by a dry summer (Fig. 1B). Significant rainfall in the fall and mid-winter resulted in high soil moisture content, with 0-50 cm soil moisture content consistently above 10% through most of the spring (Fig. 1C). There were no plot (i.e., [CO_2]) differences in soil moisture, as has been consistently observed at the NDFF (Nowak et al. 2004). Of note, however, was that the average minimum temperature did not rise above freezing until late April (Fig. 1A); subsequently, the spring growing season was characterized by high soil moisture but frequent freezing temperatures at night. The driest part of the year corresponded with the hottest; although the summer was interrupted by several significant rainfall events (Fig. 1B), integrated 0-20 and 0-50 cm soil moisture never exceeded 5% during the summer months (Fig. 1C).

Physiology (A_{sat}, g_s, WUE, Ψ_{stem})

Elevated [CO_2] significantly increased A_{sat} (i.e. light-saturated photosynthesis measured at growth [CO_2]) in *Larrea* (Fig. 2A), whereas elevated [CO_2] had no significant effect on A_{sat} in *Ambrosia* (Fig. 2B). *Ambrosia* exhibited a mean growing season A_{sat} of 19.8 µmol m^{-2} s^{-1}, while during the same time period A_{sat} was 9.4 µmol m^{-2} s^{-1} in *Larrea*.

Stomatal conductance (g_s) was not affected by elevated [CO_2] for either species (Fig 2C, D). Significant date-by-species effects ($p < 0.05$) were observed such that g_s for both species increased in March and May, with the greatest increase in *Ambrosia* in late April (Fig. 2D), when g_s was 0.39 mmol m^{-2} s^{-1} compared to 0.10 mmol m^{-2} s^{-1} for *Larrea*. Beginning in June, g_s in *Larrea* decreased over time (Fig. 2C), whereas *Ambrosia* became physiologically dormant for the remainder of the year.

A_{sat}/g_s (intrinsic WUE) was significantly higher in elevated [CO_2] in *Larrea* (Fig. 2E) but not in *Ambrosia* (Fig. 2F). Overall, elevated [CO_2] increased WUE by 37% in *Larrea* over the spring growing season (March to May) and by 46% over the entire year (March to October). A significant date-by-[CO_2] effect for both species ($p < 0.05$) indicated that WUE responses differed over time. *Ambrosia* exhibited a significant increase in WUE in elevated [CO_2] plants in March, but no response thereafter (Fig 2F). In contrast, *Larrea* exhibited higher WUE in elevated [CO_2] plants during the entire growing season, but the greatest increase in WUE was at the end of the growing season (Fig 2E), when soil moisture was low.

Stem water potential (Ψ_{stem}) was significantly higher in elevated [CO_2] in *Larrea* (Fig. 2G) but not in *Ambrosia* (Fig. 2H). In the early growing season, Ψ_{stem} was relatively high and was not affected by elevated [CO_2] in either species, but later in the growing season, Ψ_{stem} declined at a slower rate in *Larrea* at elevated [CO_2] compared to ambient [CO_2]. For *Ambrosia*, Ψ_{stem} was significantly higher at elevated [CO_2] compared to ambient [CO_2] just before the plant became physiologically dormant in late May (Fig. 2H), whereas for *Larrea*, Ψ_{stem} was higher in elevated [CO_2] compared to ambient [CO_2] from July onward (Fig. 2G). During the most active growing season (spring), *Larrea* had

a significantly lower average Ψ_{stem} (-2.3 MPa) than *Ambrosia* (-1.8 MPa) from mid-March until the end of May ($p < 0.001$).

Photosynthetic capacity (A_{max}, V_{cmax}, J_{max}, L_s)

Elevated [CO_2] had no effect on A_{max} (i.e. maximum photosynthesis measured at both saturating light and [CO_2] levels) in either species (Fig. 3A,B). In *Ambrosia*, average A_{max} throughout the study was 30 µmol m^{-2} s^{-1}, whereas in *Larrea* it was 23 µmol m^{-2} s^{-1}. For both species, A_{max} increased during the growing season until May, after which A_{max} declined for *Larrea*, while *Ambrosia* became physiologically dormant. Elevated [CO_2] had no effect on V_{cmax} (Fig. 3C,D) or J_{max} (Fig. 3E,F) in either species. For both species, J_{max} increased during the growing season until May, after which J_{max} declined for *Larrea*, while *Ambrosia* became physiologically dormant. Elevated [CO_2] significantly decreased the relative stomatal limitation to photosynthesis (L_s) for both species (Fig. 3G,H). For *Larrea* from early March until mid-October, mean L_s was 39% in ambient [CO_2] and 29% in elevated [CO_2], whereas for *Ambrosia* from mid-March until late May, mean L_s was 28% and 16% in ambient and elevated [CO_2], respectively.

Leaf pigment, N, C/N and carbohydrate concentration

Ambrosia chlorophyll *a*+*b* levels were 57% lower in elevated [CO_2] during the spring growing season (Fig. 4B), but there was no [CO_2] effect on chlorophyll *a*+*b* levels in *Larrea* from March until mid-October (Fig. 4A). Elevated [CO_2] resulted in a greater [(Z+A)/(chl *a*+*b*)] (*i.e.* the ratio of xanthophyll cycle pigments to chlorophyll *a* and *b*) in late summer in *Ambrosia* only (Fig. 4C,D). The xanthophyll pool conversion state [(Z+A)/(V+A+Z)] was significantly higher in elevated [CO_2] for *Ambrosia* but not in *Larrea* (Fig. 4E,F). During the growing season, [(Z+A)/(chl *a*+*b*)] and [(Z+A)/(V+A+Z)] declined in both species (Fig. 4C,D,E,F)

In *Larrea*, elevated [CO_2] significantly decreased leaf N content ($P<0.01$) during the first half of the growing season, but [CO_2] treatment effects on leaf N were significant only during May for *Ambrosia* (Table 1). Significant effects of elevated [CO_2] on C/N ratios in *Larrea* were limited to increased C/N ratios during May-June. During the rest of the

experiment, no significant differences were observed for *Larrea* (Table 1). In the case of *Ambrosia*, with the exception of May (when C/N was higher in elevated [CO_2]), no [CO_2] effect on C/N was observed.

In *Larrea*, the elevated [CO_2] effect on leaf sucrose, glucose and fructose concentration was affected by sampling date ($P< 0.01$; $P= 0.02$; $P= 0.09$, respectively; Table 1). *Larrea* grown under elevated [CO_2] had higher sucrose levels only during June, glucose levels were increased during two sampling dates (April and June), and for fructose, the increase extended from May until July. With the exception of April, starch content increased in leaves exposed to elevated [CO_2] (Table 1). In *Ambrosia*, [CO_2] treatment effects on sucrose, glucose and fructose also were mediated by sampling date ($P< 0.01$ for each sugar, respectively; Table 2). During April, although fructose content increased under elevated [CO_2], glucose was not affected and sucrose content diminished. During May, the concentration of the three soluble sugars increased under elevated [CO_2]. However during June, growth in elevated [CO_2] increased sucrose content, whereas glucose and fructose in *Ambrosia* were diminished (Table 1). In July, glucose levels increased in *Ambrosia* exposed to elevated [CO_2], but no [CO_2] effect was observed in sucrose and glucose levels. Elevated [CO_2] increased starch concentration during May-June in both species, and additionally in August in *Larrea* (Table 1).

Proportion of new C in TOM and leaf soluble sugars

The proportion of newly fixed carbon (C_{new}) in different plant organs was similar throughout the study (Table 2), with one exception. C_{new} was significantly lower in *Larrea* leaves during July and August (Table 2) compared to earlier sampling dates ($P< 0.01$). On average, 4 and 7% of C present in the current year's total organic matter (TOM) of *Larrea* and *Ambrosia*, respectively, came from CO_2 that was assimilated before February 10, 2003 (i.e. >2 years prior to the current year), when the pure CO_2 for the elevated CO_2 treatment was switched from a geologic to a fossil fuel source. No significant variation in C_{new} was observed in shoots and roots of either species (Table 2).

The proportion of C_{new} in sucrose, glucose and fructose of *Larrea* leaves (Table 2) exposed to elevated [CO_2] varied depending on time ($P< 0.01$ for each sugar,

respectively). For *Larrea*, the greatest C_{new} occurred during June, with C_{new} declining during July and August. For *Ambrosia*, the proportion of C_{new} in sugars of elevated $[CO_2]$ plants maintained similar levels from April through June, although C_{new} in fructose levels declined in July (Table 2). C_{new} in HCl-hydrolysable C fraction (HCl-C), which is mainly composed of starch, showed that in *Larrea*, ~96% was formed by recently assimilated C, and no significant differences were observed throughout the study. In the case of *Ambrosia*, average C_{new} was ~89% and reached the largest values during May.

Multivariate analyses

In the principal components analysis (PCA; Fig. 5A,C), we observed species differences and correlations between the various physiological parameters. First, *Ambrosia* had stronger stomatal control of photosynthesis than did *Larrea* (r = 0.93 and 0.84, respectively). Also, g_s was more strongly correlated with J_{max} and V_{cmax} in *Ambrosia* (r = 0.79 and 0.55, respectively) compared to *Larrea* (r = 0.50 and 0.17, respectively). In *Larrea*, xylem water potential showed a stronger negative correlation with photosynthetic rates than in *Ambrosia* (r = 0.43 and 0.09, respectively). Finally, as xylem water potential seasonally declined in both species, sugar levels increased.

In the discriminant function analysis (DFA; Fig. 5B,D), we observed trends in various functional parameters in elevated *versus* ambient $[CO_2]$. WUE increased at elevated $[CO_2]$ in both species, particularly in *Larrea*, and L_s decreased in both species. We also observed differential effects of elevated $[CO_2]$ on several other functional parameters with this analysis: (1) sugar levels (fructose and glucose) decreased in *Larrea*, while fructose, glucose and sucrose all increased in *Ambrosia*; (2) chlorophyll $a + b$ decreased in *Ambrosia* but not in *Larrea*; and (3) xanthophyll cycle pigments increased in *Ambrosia* but not in *Larrea*.

Discussion

Regulation of photosynthetic performance under elevated $[CO_2]$

Photosynthetic down-regulation (typically indicated by reductions in A_{max}, V_{cmax}, and J_{max}) during the eighth growing season of long-term exposure to elevated [CO_2] at the Nevada Desert FACE Facility was not observed in either *Larrea tridentata* or *Ambrosia dumosa* (Figs. 3A-F). These results differ from earlier studies at the NDFF in that photosynthetic down-regulation was previously observed in *Larrea* (Huxman et al. 1998, Hamerlynck et al. 2000b) and a drought-deciduous shrub *Lycium andersonii* (Hamerlynck et al. 2002) in the first two years of elevated [CO_2] exposure at the NDFF, especially during the cool, moist early spring when plants are not generally water stressed. Based upon our results and those of Naumburg et al. (2004), the desert perennials *Larrea* and *Ambrosia* appear to have photosynthetically equilibrated to elevated [CO2] and maintained biochemical capacity over the long-term.

Although neither *Larrea tridentata* nor *Ambrosia dumosa* show evidence for photosynthetic down-regulation, only *Larrea* had increased photosynthetic performance (i.e. A_{sat}, light-saturated A at growth [CO_2]) during continuous, long-term exposure to elevated [CO_2] (Fig. 2A). A_{sat} for *Ambrosia* was not significantly different between [CO_2] treatments throughout the entire growing season (Fig. 2B). These results for *Larrea* are similar to earlier studies at the NDFF but differ for *Ambrosia*: earlier, both species had increased photosynthetic performance under elevated [CO_2] (Naumberg et al. 2003, Ellsworth et al. 2004, Housman et al. 2006), although elevated [CO_2] effects were greatly reduced during dry portions of the year or during years with below-average precipitation. Below, we first examine processes that may not account for how photosynthetic performance of *Larrea* may differ from that of *Ambrosia* under elevated [CO_2], and then examine those that may.

The difference in photosynthetic performance between the two species under elevated [CO_2] was not due to partial stomatal closure (Fig. 2C, D), reduced carboxylation activity (V_{cmax}, Fig. 3C, D), nor to reduced electron transport (J_{max}, Fig. 3E, F). In all cases, these processes were not significantly different between ambient and elevated [CO_2] treatments. In addition, both species also had reduced L_s under elevated [CO_2], as has been commonly observed in long-term field studies (Tissue et al. 2001). Although treatment effects on leaf N and carbohydrate concentrations differed between *Larrea* and

Ambrosia during the growing season (Table 1), the direction of these differences was not consistent with the observed treatment effects on photosynthetic performance. *Larrea* plants under elevated [CO_2] had more consistent decreases in leaf N versus *Ambrosia* plants (Table 1). Although greater decreases in leaf N for *Larrea* would be expected to result in greater decreases in photosynthetic performance because of the close relationship between leaf N and A_{sat} (Ellsworth et al. 2004), in fact *Larrea* had greater increases in A_{sat} under elevated [CO_2]. Sugar and starch concentrations under elevated [CO_2] were often significantly greater than those under ambient [CO_2] (Table 1), which indicated that both species had greater potential for feedback inhibition of net assimilation by carbohydrate accumulation under elevated [CO_2]. Although exceptions do occur for both species (e.g., glucose in April for *Larrea* and sucrose in April for *Ambrosia*), these exceptions occur slightly more frequently for *Ambrosia*, suggesting photosynthetic performance of *Ambrosia* would have benefited more under elevated [CO_2] because of less frequent feedback inhibition. However, this prediction of greater performance of *Ambrosia* under elevated [CO_2] also is contrary to observations.

As with leaf N and carbohydrates, the effects of elevated [CO_2] on pigment characteristics (Fig. 4) differed substantially between the two shrub species, but these pigment differences also were not consistent with differences in photosynthetic performance. Pigments are functional components of the photosynthetic machinery, providing information about biochemical investment and stress in the photosystems. The evergreen *Larrea* did not adjust pigment allocation in response to elevated [CO_2], as was documented for the evergreen tree loblolly pine after 8 years in FACE (Logan et al., 2009). In the deciduous *Ambrosia*, plants growing under elevated [CO_2] reduced chlorophyll *a* and *b* throughout the growing season, suggesting that less light absorbing and processing capabilities may be part of the reason why A_{sat} under elevated [CO_2] was not as high as expected for that species. However, desert plants typically are not light limited (Smith et al. 1997), and thus lower chlorophyll under elevated [CO_2] may at best be only a minor contribution towards lower than expected A_{sat} under elevated [CO_2] in *Ambrosia*. Generally, photoinhibition is a greater concern in high-light environments (Hymus et al. 1999, Aranjuelo et al. 2008b), but the pigment data indicate that increased protective pigment concentrations only occurred in *Ambrosia*. Photoprotection was

presumably employed to the level necessary during exposure to excess light each day, as violaxanthin was converted to zeaxanthin and the latter employed in thermal energy dissipation to avoid photodamage (Adams et al. 2006, Demmig-Adams and Adams 2006). Nonetheless, these differences were reduced during the peak growing season, when high photoprotection may be more important as sink activity increases (Adams et al. 2006) and into the summer dry season as drought-induced photoinhibition becomes more frequent. Thus, the greater ability to avoid photoinhibition in *Ambrosia* was not sufficient to improve photosynthetic performance under elevated [CO_2] over that under ambient [CO_2].

The lack of increased photosynthetic performance in *Ambrosia* after long-term exposure to elevated [CO_2] may reflect differences between *Larrea* and *Ambrosia* in allocation to C sinks and utilization of stored C pools. *Larrea* had greater percentages of newly fixed C in the current year's growth of leaves, shoots, and roots under elevated [CO_2] than *Ambrosia* (Table 2), suggesting *Larrea* maintained sufficient C sinks and hence enabled greater photosynthetic performance. Furthermore, the greatest enhancement of photosynthetic performance under elevated [CO_2] occurred in summer for *Larrea* (Fig. 2A), when carbohydrate concentrations were most similar between elevated and ambient [CO_2] treatments. Other studies have related photosynthetic performance under elevated [CO_2] to the ability of plants to develop new C sinks or expand the existing ones (Ceulemans, 1997) and suggested that down-regulation was the consequence of an insufficient sink plant capacity (Morgan et al. 2001, Ainsworth et al. 2004, Aranjuelo et al., 2009). Furthermore, when plants exposed to elevated CO_2 exhibited limited capacity to increase C sink strength, plants decreased their photosynthetic activity to balance C source activity and sink capacity (Thomas and Strain 1991).

Improved plant water relations in elevated [CO_2], indicated by higher WUE and higher Ψ_{stem} (Fig. 2), also helped maintain A_{sat} in *Larrea* during the driest part of the summer. In contrast, growth in elevated [CO_2] did not improve WUE or plant water relations in the drought-deciduous *Ambrosia*. During drought periods, plants may partially alleviate water stress by accumulating osmolytes (*e.g.* sugars) to increase cellular water uptake. In *Ambrosia*, sugars and starch were higher in elevated [CO_2], but there was no

commensurate increase in Ψ_{stem} in elevated [CO_2] plants during the hotter, drier period of the growing season. In *Larrea*, sucrose, glucose and fructose content increased in elevated [CO_2] during spring whereas few significant differences were detected during summer (July-August), suggesting that changes in soluble sugars were probably not significant contributors to higher Ψ_{stem} in elevated [CO_2] plants. However, soluble sugars were generally much higher in *Larrea* than *Ambrosia* during all periods of the growing season. Therefore, the maintenance of physiological activity in *Larrea* into the hottest and driest periods of the growing season, when *Ambrosia* drops its leaves and becomes inactive, may be partially attributed to greater access to osmolytes in *Larrea* (Smith et al. 1997).

Long-term C storage and C allocation patterns

Modification of atmospheric $\delta^{13}C$ in concert with experimental CO_2 exposure enabled the characterization of C allocation and partitioning of *Larrea* and *Ambrosia* under varying seasonal growth conditions. In *Larrea* and *Ambrosia* plants grown under elevated [CO_2], organs developed during the experimental period were partly (4% and 7 %, respectively) constructed from "old" C (*i.e.* C that was assimilated prior to the beginning of the labeling period two years earlier; February 10, 2003) when the source of CO_2 for elevated plots was switched to fossil-fuel-derived CO_2. Thus, most C utilized in plant growth was derived from "new" C in *Larrea* (96%) and *Ambrosia* (93%). Similar results were described by Körner et al. (2005), where after two years of labeling, 82-89 % of C present in newly formed shoots and leaves (respectively) for *Quercus*, *Fagus*, *Acer*, *Carpinus*, and *Tilia* trees grown under elevated [CO_2] was from C assimilated during the last two years. von Felten et al. (2007) also reported that after two years of C labeling and exposure to elevated [CO_2], 46 % and 42 % of C present in new *Larix decidua* and *Pinus uncinatus* (respectively) shoots was C fixed prior to the labeling period. As observed in other slow-growing plants, after a long term $^{12}CO_2$ enriched labeling period (Aranjuelo et al. 2009), our results suggest that in a high growth year, both species remobilized stored C to develop new biomass. Long-term storage and remobilization of C reserves has been observed in other woody species growing in ambient [CO_2] conditions (Lacointe et al. 1993).

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572 Both species have main roots (Wallace et al. 1974) that play essential roles in C storage
573 during stressful growth conditions (Chaves et al., 1995). As recently observed by
574 Franklin et al. (2009) and Crous et al. (2010) in long-term FACE experiments, larger
575 investment of C resources in root development will affect leaf N and photosynthetic
576 activity in elevated [CO_2] environments. For Mojave Desert shrubs such as *Larrea* and
577 *Ambrosia*, roots represent a large proportion of plant biomass and consequently an
578 important C storage organ (Wallace et al. 1974). Unfortunately, the long-term nature of
579 the Nevada Desert FACE experiment precluded harvesting main roots, and thus we were
580 not able to verify mobilization of stored C in the main roots of *Larrea* and *Ambrosia*.

581 The low variation in $\delta^{13}C$ and C_{new} for newly-formed organs of *Larrea* and of *Ambrosia*
582 throughout the study revealed that export of C to other organs also was fairly constant.
583 However for *Larrea*, water stress and elevated temperature (mainly July and August)
584 decreased C_{new} in leaves, which suggests that greater amounts of "old" C were allocated
585 to new leaf growth during the summer dry season. Regardless of seasonality, shoots and
586 roots had constant C_{new}, which suggests that these organs were effective sinks for C.

587 When analyzing seasonal fluctuations, it should be noted that C labeling, and
588 consequently leaf % of C_{new} in soluble sugars, will be affected by: (1) plant assimilation
589 rate; (2) respiration; and (3) translocation to other organs (shoots and roots) (Aranjuelo et
590 al. 2009). No significant photosynthetic decrease was observed under elevated [CO_2]
591 during June and July, although diminished photosynthesis and lower soluble sugar
592 content during August could have contributed to the decrease in C_{new} for fructose in
593 *Larrea*. We also note that in addition to recently formed photoassimilates, C in sucrose,
594 glucose, and fructose can be derived through sugar formation during degradation of
595 starch reserves (Farrar et al. 2000), and thus variations in starch $\delta^{13}C$ also could affect
596 $\delta^{13}C$ of sucrose (Tcherkez et al. 2003). However, C_{new} in HCl-hydrolyzable C fraction
597 (mainly as starch; Richter et al. 2009) was constant in *Larrea*, and thus this fraction was
598 apparently not involved in the decrease in C_{new} of soluble sugars. For *Ambrosia*,
599 diminishment in C_{new} during July also suggests a remobilization of pre-labelled C from
600 storage organs.

Conclusions

This study was conducted during the eighth full growing season of [CO_2] treatment at the NDFF, thereby providing insight into the long-term physiological responses of two perennial shrubs, *Larrea tridentata* and *Ambrosia dumosa*, to elevated [CO_2]. In the evergreen shrub *Larrea*, plants under elevated [CO_2] enhanced photosynthetic performance (A_{sat}), maintained C sinks, and improved plant water status (higher WUE and Ψ_{stem}), especially during periods of environmental stress in the later part of the growing season. In contrast, the drought-deciduous shrub *Ambrosia* did not increase A_{sat}, WUE, or Ψ_{stem} under elevated [CO_2]. Surprisingly, we found that g_s and photosynthetic capacity (A_{max}, V_{cmax}, J_{max}) were not affected by elevated [CO_2] in either species. Although increases in photoprotective pigments were observed in *Ambrosia* under elevated [CO_2], photoprotection was not sufficient to increase photosynthetic performance in *Ambrosia*. On average, 96% and 93% of C present in new growth and soluble sugars of *Larrea* and *Ambrosia*, respectively, was recently assimilated C, which implies that in this year (2005) when plant growth was strongly increased, plants mobilized stored C to fulfill new organ formation requirements. Furthermore, *Larrea* utilized a greater fraction of new C to grow new organs and sustained these sinks for longer during the growing season than *Ambrosia*, indicating that maintenance of C sinks by *Larrea* helps that shrub maintain increased photosynthetic performance during long-term exposure to elevated [CO_2] at the Nevada Desert FACE Facility. Thus, although the early biochemical adjustments that we observed at the FACE site (*i.e.* down-regulation of photosynthesis) have abated under longer-term exposure to elevated [CO_2], these physiological characteristics of *Larrea* should significantly enhance carbon gain under elevated [CO_2] on an annual basis over the long-term.

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Table 1. Elevated CO_2 exposure (ambient CO_2 *versus* elevated CO_2) effect in terms of N (%), C/N ratio, sucrose (mg g^{-1}DM), glucose (mg g^{-1}DM), fructose (mg g^{-1}DM), and starch (mg g^{-1}DM)of *Larrea tridentata* and *Ambrosia dumosa* leaves. Parameters that differed significantly due to [CO_2] were highlighted in bold. Each value represents the mean ± standard deviation.

Larrea tridentata	April		May		June		July		August	
	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
N	**2.1±0.1**	**1.8±0.1**	**1.9±0.1**	**1.6±0.1**	**2.2±0.1**	**1.8±0.2**	1.9±0.2	1.7±0.2	2.0±0.05	1.9±0.2
C/N	23.8±0.2	25.6±1.8	**26.3±0.9**	**30.5±2.9**	**23.3±0.0**	**27.2±2.0**	29.7±5.4	25.9±1.7	25.0±1.0	27.1±1.0
Sucrose	13.3±0.7	10.3±0.6	16.2±1.3	18.8±0.1	**18.8±0.3**	**25.2±0.2**	4.7±0.6	4.8±0.02	0.9±0.2	1.8±0.0
Glucose	**10.4±0.9**	**7.2±0.8**	11.7±1.4	9.7±0.1	**5.4±0.2**	**10.4±0.0**	2.3±0.0	1.2±0.0	1.6±0.0	1.4±0.0
Fructose	9.2±0.5	11.2±1.0	**8.1±1.1**	**15.9±0.1**	**6.4±0.3**	**13.3±0.24**	**2.5±0.0**	**5.4±0.1**	1.7±0.0	3.0±0.1
Starch	223.6±17.8	225.4±19.4	**109.6±21.6**	**165.8±23.2**	**83.7±7.2**	**112.7±11.7**	70.7±8.9	92.1±11.9	**24.7±4.7**	**64.6±6.8**

Ambrosia dumosa	April		May		June		July	
	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
N	4.6±0.0	3.6±0.1	**3.2±0.2**	**2.2±0.1**	2.4±0.1	2.4±0.1	1.8±0.1	1.7±0.1
C/N	9.5±0.1	11.7±0.4	**13.5±0.6**	**20.4±2.7**	20.7±1.4	18.1±1.1	23.9±0.9	24.6±0.8
Sucrose	**23.3±1.0**	**15.4±0.9**	**22.9±0.8**	**48.9±1.1**	**14.4±0.2**	**23.7±0.6**	4.9±0.1	13.4±0.0
Glucose	5.6±0.7	7.3±0.1	**6.1±0.0**	**9.8±0.8**	**4.4±0.1**	**2.1±0.0**	**2.8±0.2**	**4.9±0.1**
Fructose	**3.6±0.1**	**8.7±0.3**	**5.9±0.3**	**13.8±0.1**	**7.8±0.1**	**2.8±0.1**	1.5±0.3	3.4±0.0
Starch	44.9±5.9	58.9±9.1	**57.5±4.6**	**98.6±15.0**	**56.8±3.2**	**78.4±8.7**	39.2±3.8	46.1±6.1

862 **Table 2.** Elevated CO_2 exposure effect in terms of % of new C (C_{new}) in leaf shoot, root total organic matter (TOM) and C_{new} in leaf
863 sucrose, glucose, fructose and starch (HCl-C) for *Larrea tridentata* and *Ambrosia dumosa*. Each value represents the mean ± standard
864 deviation.

Larrea tridentata	**April**	**May**	**June**	**July**	**August**
Leaf C_{new}	96.21±0.82	96.17±0.3	97.04±0.17	93.04±0.23	94.71±0.24
Shoot C_{new}	96.97±0.36	96.53±0.15	96.50±1.49	95.89±1.24	95.92±0.11
Root C_{new}	92.15±0.50	92.41±0.17	93.78±0.13	No sample	No sample
Sucrose C_{new}	94.00±0.45	94.95±0.77	96.14±	92.21±0.17	93.68±0.01
Glucose C_{new}	93.89±0.25	95.79±0.35	95.91±0.42	87.79±0.23	89.90±0.14
Fructose C_{new}	92.76±0.21	93.08±0.25	94.58±0.16	90.39±0.11	87.80±0.16
Starch (HCl-C) C_{new}	95.57±0.24	95.79±0.11	96.07±0.09	95.81±1.02	95.82±0.95

865

Ambrosia dumosa	**April**	**May**	**June**	**July**
Leaf C_{new}	92.89±0.7	92.94±0.16	95±0.67	94.50±0.69
Shoot C_{new}	93.11±0.20	93.03±0.37	94.13±0.23	93.29±0.30
Root C_{new}	92.63±0.20	91.77±0.18	90.81±0.51	No sample
Sucrose C_{new}	93.60±0.41	95.11±0.53	94.69±0.37	92.970.28
Glucose C_{new}	92.01±0.12	92.66±0.14	92.01±0.23	90.28±0.07
Fructose C_{new}	90.23±0.54	93.27±0.13	94.02±0.03	88.40±0.26
Starch (HCl-C) C_{new}	86.52±0.30	92.00±0.53	88.25±0.32	84.78±0.83

866

867

Figure Legends

Fig. 1 Average monthly maximum and minimum temperature (A), daily precipitation (B), and volumetric soil water content at 0-30 and 0-50 cm depths (C) during 2005 at the Nevada Desert FACE Facility. There were no soil moisture differences between ambient and elevated [CO_2] plots for either depth.

Fig. 2 Plant physiological performance at ambient (filled symbols; 380 μmol mol^{-1}) *versus* elevated (open symbols; 550 μmol mol^{-1}) atmospheric [CO_2] measured as: (A,B) A_{sat} (light-saturated net assimilation rate, A_{net}); (C,D) stomatal conductance (g_s); (E,F) Water-Use Efficiency (WUE) calculated as A_{sat}/g_s; and (G,H) pre-dawn water potential (Ψ) for *Larrea tridentata* (left panels) and *Ambrosia dumosa* (right panels). Vertical bars represent ± one standard deviation.

Fig. 3 Mechanistic photosynthesis at ambient *versus* elevated [CO_2] in *Larrea tridentata* and *Ambrosia dumosa* measured as: (A,B) maximum (CO_2-saturated) assimilation rate (A_{max}); (C,D) maximum carboxylation rate of Rubisco (V_{cmax}); (E,F) maximum electron transport rate (J_{max}) and (G,H) relative stomatal limitation (L_s). All symbols are as in Fig. 2.

Fig. 4. Photosynthetic pigments at ambient *versus* elevated [CO_2] in *Larrea tridentata* and *Ambrosia dumosa* measured as: (A,B) chlorophyll *a*+*b*; (C,D) the ratio of xanthophyll cycle pigments to chlorophyll *a*+*b*; and (E,F) the xanthophyll pool conversion state ((Z+A)/(V+A+Z)) All symbols are as in Fig. 2.

Fig. 5. Results of principal components analysis (PCA) displayed as vector correlations among variables for *Larrea tridentata* (A) and *Ambrosia dumosa* (C) for the first and second principal components (PC1 and PC2). The length and angle between a pair of vectors is an indication of the strength and nature, repectively, of their correlations. Results of discriminant function analysis (DFA) in vector format show the direction of responses of *Larrea* (B) and *Ambrosia* (D) to ambient and elevated [CO_2] treatments. The direction of the vector is an indication of whether an increase or decrease was observed in a particular variable, with vectors to the right indicating a positive response

to elevated [CO_2], and the length of the vector is an indication of the strength of the response. Plant variables used in this analysis: A_{max}; A_{sat}; g_s; L_s; WUE; V_{cmax}; J_{max}; F_vF_m; plant Ψ; Chl *a* + *b*; [Z+A]/[V+A+Z]; [Z+A]/[Chl *a* + *b*] (plant variables as described in previous figure legends).

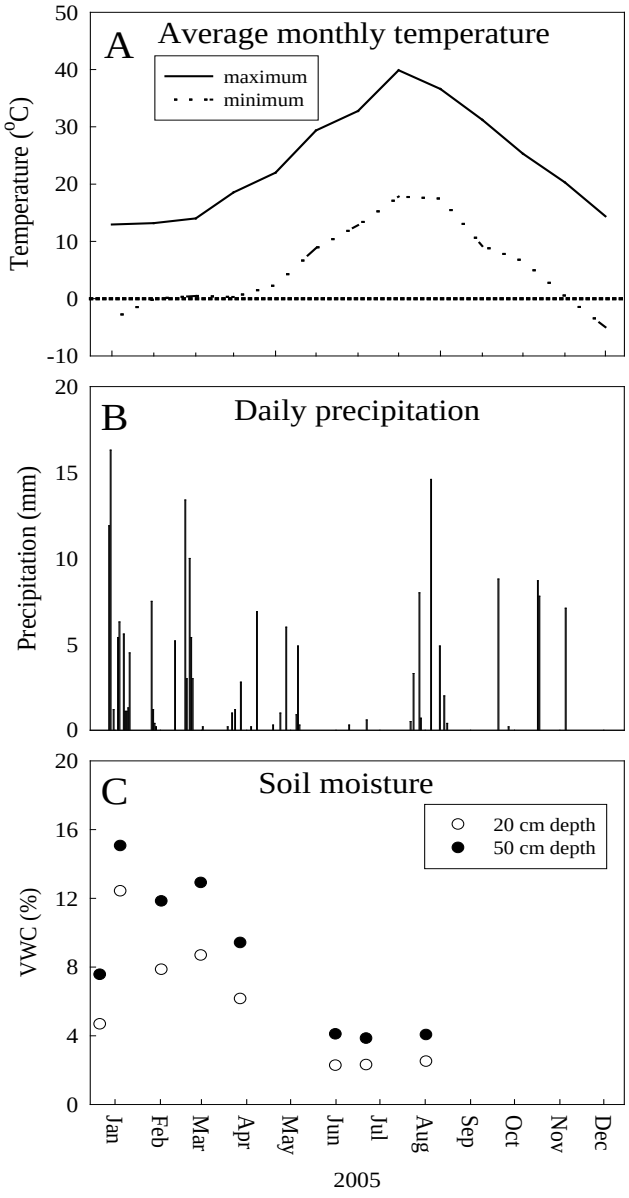
A
Average monthly temperature
maximum
minimum
Temperature (°C)
50
40
30
20
10
0
-10
B
Daily precipitation
Precipitation (mm)
20
15
10
5
0
C
Soil moisture
20 cm depth
50 cm depth
VWC (%)
20
16
12
8
4
0
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
2005

Figure 1.

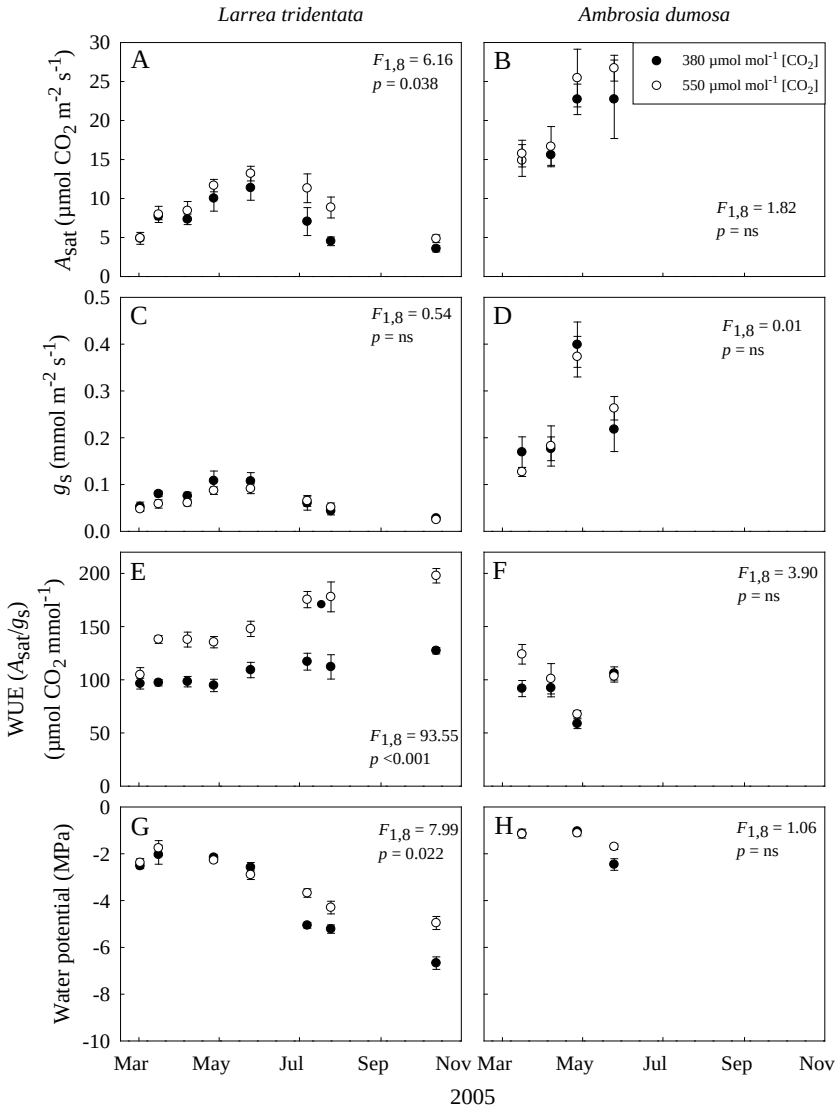
Larrea tridentata
Ambrosia dumosa
A
B
C
D
E
F
G
H
$F_{1,8}$ = 6.16
p = 0.038
$F_{1,8}$ = 1.82
p = ns
$F_{1,8}$ = 0.54
p = ns
$F_{1,8}$ = 0.01
p = ns
$F_{1,8}$ = 93.55
p <0.001
$F_{1,8}$ = 3.90
p = ns
$F_{1,8}$ = 7.99
p = 0.022
$F_{1,8}$ = 1.06
p = ns
380 µmol mol^{-1} [CO_2]
550 µmol mol^{-1} [CO_2]
A_{sat} (µmol CO_2 m^{-2} s^{-1})
g_s (mmol m^{-2} s^{-1})
WUE (A_{sat}/g_s) (µmol CO_2 mmol^{-1})
Water potential (MPa)
Mar
May
Jul
Sep
Nov
2005

Figure 2.

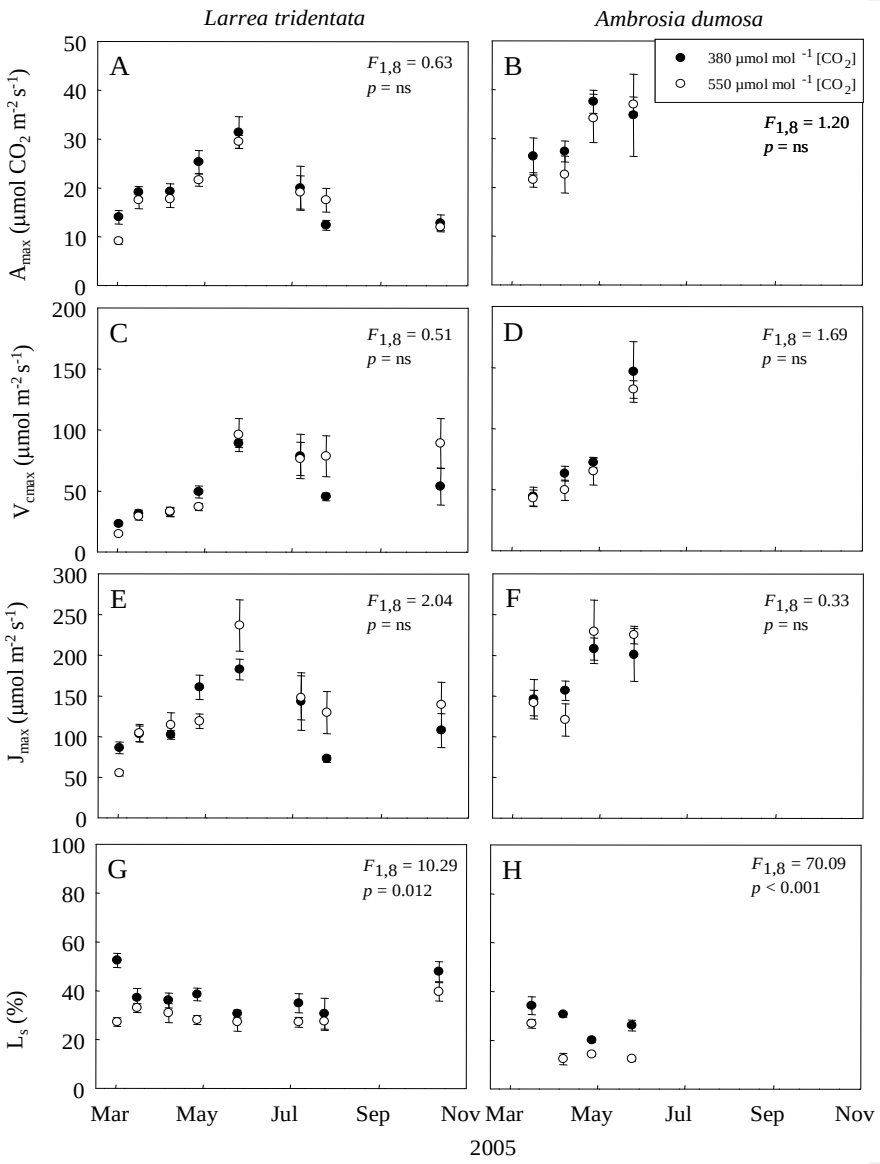
Larrea tridentata
Ambrosia dumosa
A
B
C
D
E
F
G
H
380 µmol mol -1 [CO2]
550 µmol mol -1 [CO2]
F1,8 = 0.63
p = ns
F1,8 = 1.20
p = ns
F1,8 = 0.51
p = ns
F1,8 = 1.69
p = ns
F1,8 = 2.04
p = ns
F1,8 = 0.33
p = ns
F1,8 = 10.29
p = 0.012
F1,8 = 70.09
p < 0.001
Amax (µmol CO2 m-2 s-1)
Vcmax (µmol m-2 s-1)
Jmax (µmol m-2 s-1)
Ls (%)
Mar
May
Jul
Sep
Nov
2005

Figure 3.

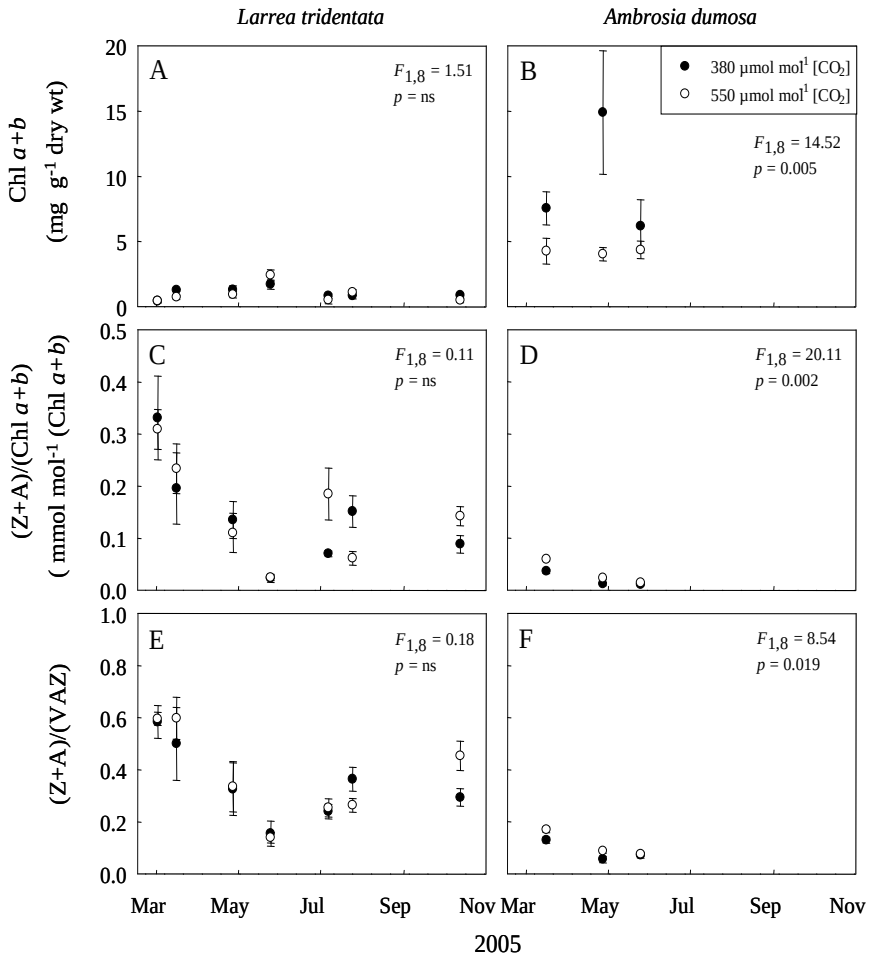
Larrea tridentata
Ambrosia dumosa
A
B
C
D
E
F
380 µmol mol1 [CO2]
550 µmol mol1 [CO2]
F1,8 = 1.51
p = ns
F1,8 = 14.52
p = 0.005
F1,8 = 0.11
p = ns
F1,8 = 20.11
p = 0.002
F1,8 = 0.18
p = ns
F1,8 = 8.54
p = 0.019
Chl a+b
(mg g-1 dry wt)
(Z+A)/(Chl a+b)
(mmol mol-1 (Chl a+b)
(Z+A)/(VAZ)
Mar
May
Jul
Sep
Nov
2005

Figure 4.

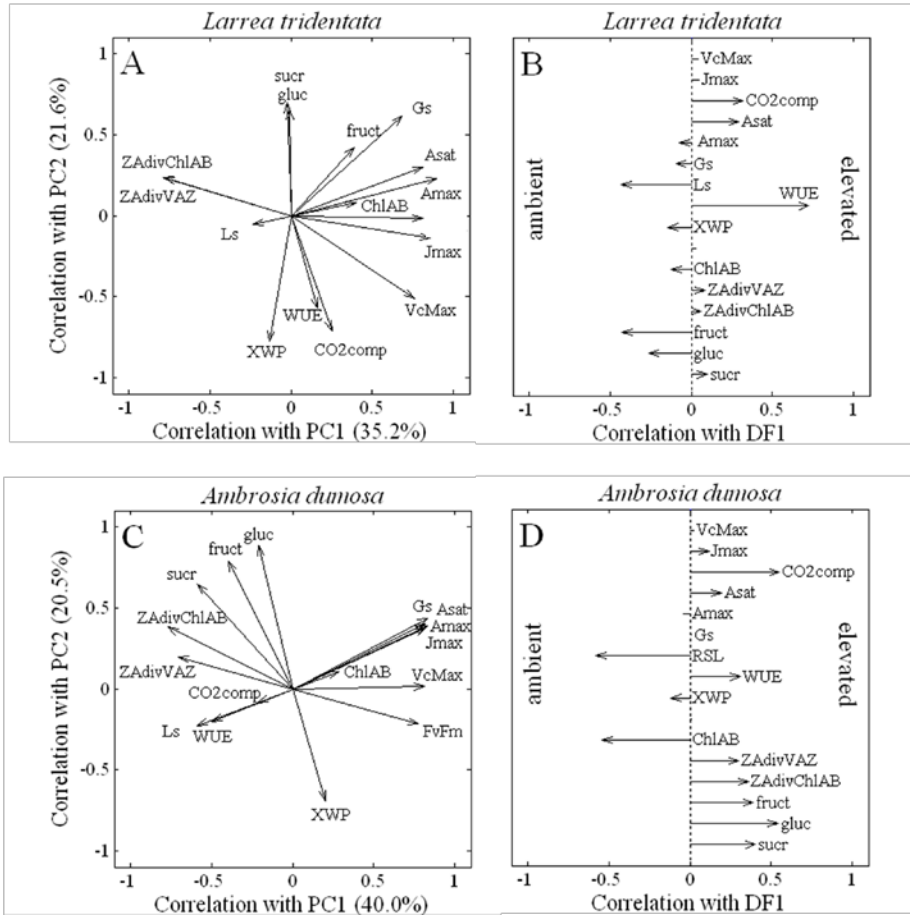
Larrea tridentata
A
sucr
gluc
Gs
fruct
Asat
ZAdivChlAB
ZAdivVAZ
Amax
ChlAB
Ls
Jmax
VcMax
WUE
XWP
CO2comp
Correlation with PC2 (21.6%)
Correlation with PC1 (35.2%)
Larrea tridentata
B
VcMax
Jmax
CO2comp
Asat
Amax
Gs
Ls
WUE
XWP
ChlAB
ZAdivVAZ
ZAdivChlAB
fruct
gluc
sucr
ambient
elevated
Correlation with DF1
Ambrosia dumosa
C
gluc
fruct
sucr
Gs
Asat
ZAdivChlAB
Amax
Jmax
ZAdivVAZ
ChlAB
VcMax
CO2comp
Ls
WUE
FvFm
XWP
Correlation with PC2 (20.5%)
Correlation with PC1 (40.0%)
Ambrosia dumosa
D
VcMax
Jmax
CO2comp
Asat
Amax
Gs
RSL
WUE
XWP
ChlAB
ZAdivVAZ
ZAdivChlAB
fruct
gluc
sucr
ambient
elevated
Correlation with DF1

Figure 5.