

Title: Maintenance of C sinks sustains enhanced C assimilation during long-term exposure to elevated [CO₂] in Mojave Desert shrubs

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1 **Abstract**

2 During the first few years of elevated atmospheric [CO₂] treatment at the Nevada Desert
3 FACE Facility, photosynthetic down-regulation was observed in desert shrubs grown
4 under elevated [CO₂], especially under relatively wet environmental conditions.
5 Nonetheless, those plants maintained increased A_{sat} (photosynthetic performance at
6 saturating light but treatment [CO₂]) under wet conditions but to a much lesser extent
7 under dry conditions. To determine if plants continued to down-regulate during long-term
8 exposure to elevated [CO₂], responses of photosynthesis to elevated [CO₂] was examined
9 in two dominant Mojave Desert shrubs, the evergreen *Larrea tridentata* and the drought-
10 deciduous *Ambrosia dumosa*, during the eighth full growing season of elevated [CO₂]
11 treatment at the NDDF. A comprehensive suite of physiological processes were
12 collected. Furthermore, we used C labeling of air to assess carbon allocation and
13 partitioning as measures of C sink activity. Results show that elevated [CO₂] enhanced
14 photosynthetic performance and plant water status in *Larrea*, especially during periods of
15 environmental stress, but not in *Ambrosia*. $\delta^{13}C$ analyses indicate that *Larrea* under
16 elevated [CO₂] allocated a greater proportion of newly assimilated C to C sinks than
17 *Ambrosia*. Maintenance by *Larrea* of C sinks during the dry season partially explained
18 the reduced [CO₂] effect on leaf carbohydrate content during summer, which in turn
19 lessened carbohydrate build-up and feedback inhibition of photosynthesis. $\delta^{13}C$ results
20 also showed that in a year when plant growth reached the highest rates in 5 years, 4%
21 (*Larrea*) and 7% (*Ambrosia*) of C in newly emerging organs was remobilized from C that
22 was assimilated and stored for at least 2 years prior to the current study. Thus after eight
23 years of continuous exposure to elevated [CO₂], both desert perennials maintained
24 photosynthetic capacity under elevated [CO₂] We conclude that C storage,
25 remobilization, and partitioning influence responsiveness of these desert shrubs during
26 long-term exposure to elevated [CO₂].

Comentario [*2]: REF. 1.3.

27

28 **Keywords:** *Ambrosia dumosa*, C allocation/partitioning, Free-air CO₂ enrichment
29 (FACE), *Larrea tridentata*, photosynthetic down regulation

30

31 **Introduction**

32 Initial increases in net assimilation rates of vascular plants exposed to elevated
33 atmospheric [CO₂] may not be sustained over long time periods because of “a hierarchy
34 of increasingly complex processes controlling the production and allocation of end-
35 products” (Lemon 1983). For example, plants photosynthetically acclimate (*i.e.* down-
36 regulate) to growth in elevated [CO₂] through changes in the photosynthetic apparatus,
37 including lower light- and CO₂-saturated photosynthesis (A_{max}) as well as lower V_{cmax}
38 (maximum Rubisco carboxylation) and J_{max} (maximum electron transport) (Tissue et al.
39 1993, 2001). A second process that may impact the long-term enhancement of
40 photosynthesis under elevated [CO₂] is reduced N availability, such as through re-
41 allocation of N within the plant to meet other growth needs (Theobald et al. 1998, Zhu et
42 al. 2009) or reduced N cycling in the ecosystem (Zak et al. 2000). Support for this
43 process comes from meta-analyses of both FACE (free-air carbon dioxide enrichment)
44 and OTC (open-top chamber) studies: some plants, but particularly woody species, that
45 exhibit photosynthetic acclimation also exhibit declines in leaf N and Rubisco content
46 (Long et al. 2004, Ainsworth and Long 2005). These trends also have been replicated in
47 comparative gas exchange studies across several FACE sites (Ellsworth et al. 2004).
48 Another process that may impact the long-term enhancement of photosynthesis under
49 elevated [CO₂] is feedback inhibition of photosynthesis by carbohydrate accumulation
50 (Moore et al. 1999, Jifon and Wolfe 2002). Meta-analyses also support this process:
51 many studies describe increased leaf starch or soluble sugars at elevated [CO₂] (Long et
52 al. 2004, Ainsworth and Long 2005). Thus over the long term, elevated CO₂ effects on
53 leaf C assimilation may be conditioned by an ecosystem’s ability to provide adequate N
54 through changes in N cycling and by a plant’s ability to develop new sinks (*e.g.* new
55 vegetative or reproductive structures) or to expand the storage capacity or growth rate of
56 existing sinks like shoots and roots (Lewis et al. 2002, Aranjuelo et al. 2009). Clearly,
57 these processes are not mutually exclusive. Nonetheless, understanding this “hierarchy
58 of increasingly complex processes” is critical to realistically predict long-term ecosystem
59 C assimilation from the atmosphere.

60 The Nevada Desert FACE Facility (NDFF) is an ideal system to investigate how plants
61 control photosynthesis during long-term exposure to elevated [CO₂]. Nitrogen cycling at
62 the NDFF rapidly changed after initiation of elevated [CO₂] treatments (Billings et al.
63 2002), increasing N availability (Billings et al. 2004) largely through shifts in soil
64 microbial activity (Jin and Evans 2007, 2010). Thus, mechanisms that cause
65 photosynthetic changes in plants at the NDFF should be influenced more by within-plant
66 processes, such as by N re-allocation within leaves or among tissues or by the size and
67 activity of C sinks, than by N availability through ecosystem N cycling. In the first five
68 years of the NDFF experiment, alterations in physiological processes due to elevated
69 [CO₂] were observed in annual and perennial plants in the NDFF. For example, water
70 was severely limiting in most years such that differences in photosynthetic rates between
71 perennial plants growing in elevated versus ambient [CO₂] were minimal, with
72 significant differences in photosynthesis only observed in years or seasons with adequate
73 or above-average rainfall (Naumburg et al. 2003). Similarly, photosynthetic down-
74 regulation – reductions in A_{\max} (maximum net photosynthesis at saturating photosynthetic
75 photon flux density (PPFD) and [CO₂]), V_{cmax} and J_{\max} – was observed only in wet years
76 for plants grown in elevated [CO₂] (Huxman et al. 1998, Hamerlynck et al. 2000b).
77 However, these previous studies, as well as those from other FACE experiments, did not
78 examine the relative importance of different processes, including leaf N and
79 carbohydrates and carbon management (allocation and partitioning), in regulating
80 photosynthetic performance during long-term elevated [CO₂].

81 Another advantage of the NDFF and other FACE experiments is that use of a ¹³C
82 depleted fossil fuel source to achieve elevated [CO₂] treatments introduces C isotope
83 tracers into the system. These ¹³C/¹²C tracers provide an essential tool to study carbon
84 management in plants (Körner et al. 2005, von Felten et al. 2007, Aranjuelo et al. 2008a,
85 2009). Labelling with ¹³C/¹²C as tracers and characterization of the distribution of
86 labelled compounds into different plant organs has provided novel and relevant
87 information in studies determining the flow of C through plants grown in elevated [CO₂]
88 (Aranjuelo et al. 2009). C allocation and partitioning can be studied further by analyzing
89 the isotopic composition of soluble sugars, especially sucrose, glucose, and fructose

90 (Körner et al. 2005, Kodama et al. 2010), which are anticipated to change under elevated
91 [CO₂] (Aranjuelo et al. 2009).

92 In this study, a comprehensive suite of physiological process and C balance data were
93 collected from plants during the eighth full growing season of continuous exposure to
94 elevated [CO₂] and used to examine the regulation of photosynthetic performance during
95 long-term exposure to elevated [CO₂] at the NDFF. Photosynthetic responses and carbon
96 allocation/partitioning patterns were measured for the two dominant shrub species of the
97 Mojave Desert, the evergreen *Larrea tridentata* and the drought deciduous *Ambrosia*
98 *dumosa*. Measurements were made throughout the growing season: from cooler, wetter
99 periods of peak growth in spring to hotter, drier periods of pronounced water stress in
100 summer. Specifically, we examined leaf gas exchange, leaf pigments, leaf N, and leaf
101 soluble sugars and starch to determine the extent that photosynthetic performance was
102 enhanced during long-term exposure to elevated [CO₂] and to test specific mechanisms
103 that may cause reduced photosynthetic performance. Because photosynthetic
104 performance is affected by leaf-level and plant-level C allocation, photoassimilate
105 allocation and partitioning also were studied through the use of ¹³C/¹²C labeling. We
106 hypothesized that these desert species would down-regulate photosynthesis under
107 elevated [CO₂] during the moist, early portions of the growing season and that down-
108 regulation would be accompanied by altered leaf pigmentation, decreased leaf N, and
109 increased leaf starch and soluble sugars. Furthermore, we hypothesized that reduced
110 capacity of plants to allocate C away from leaves also would limit photosynthetic
111 performance under elevated [CO₂].

112

113 **Materials and Methods**

114 *Field site and C-labeling procedures*

115 The Nevada Desert FACE Facility (NDFF) is located within the Nevada Test Site
116 (36°39'N, 122°55'W, 960 m altitude). Three plots (23 m diameter; 415 m²) had the full
117 FACE apparatus (stand-pipes and blowers) and continuously exposed plants to elevated

118 [CO₂] (target of 550 μmol mol⁻¹; averaged over 2005, actual treatment was 521 μmol
119 mol⁻¹) and three plots had the FACE apparatus but blow air onto the plots at ambient
120 [CO₂] (measured during the 2005 growing season as 380 μmol mol⁻¹). The NDFF
121 operated continuously (24 h per day, 365 d per year), with conditional shut-downs
122 occurring only when air temperature dropped below 4°C or when wind speed exceeded 7
123 m s⁻¹. The 2005 growing season was the eighth full year of operation for the NDFF. The
124 ecosystem within each plot was not disturbed during installation of the FACE apparatus
125 and represents the same functioning ecosystem as the surrounding landscape in the
126 northern Mojave Desert. The facility, vegetation, and soils are fully described in Jordan
127 et al. (1999).

128 Elevated [CO₂] was provided by supplementing ambient air with pure CO₂ to achieve the
129 desired CO₂ concentration. Prior to February 10, 2003, the pure CO₂ (supplied by BOC
130 Gases; Murray Hill, NJ, USA) was from a geologic source and had a CO₂ isotopic
131 composition (δ¹³C) of -5.4 ‰, which diluted ambient air δ¹³C (-8.0 ‰) to δ¹³C of air
132 above the elevated [CO₂] plots of -7.3 ‰ (Naumburg et al. 2003). On February 10, 2003,
133 we switched the source of pure CO₂ to fossil fuels, which had a more ¹³C depleted δ¹³C
134 (-32.0 ‰), resulting in a δ¹³C of CO₂ in air of -18.2 ± 1.9 ‰ for elevated [CO₂] plots
135 (Schaeffer 2005).

136 *Plant material and sampling*

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

144 For *Larrea* and *Ambrosia*, leaves, shoots and roots that emerged during the current year
145 were harvested for C isotopic composition (δ¹³C) and N in early morning. Leaves and

146 shoots were harvested monthly from April until July for both species and until August for
147 *Larrea*. No data were presented for *Ambrosia* in August because those plants had entered
148 their physiological dormancy period. In both cases, root sampling occurred only during
149 April-June because new root formation did not occur in July or August for either species.
150 Root samples were collected from root boxes located at the base of each shrub species
151 (Clark et al. 2010). On each sampling date, harvests were from two plants per species in
152 each of the three elevated and the three ambient [CO₂] plots.

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153 Samples for xylem water potential, sugar content, and pigment analysis were all taken at
154 pre-dawn, when plants were under minimal daily water stress. Starting approximately
155 1.5 hours before sunrise on each sampling date, two terminal shoot (stem + leaves)
156 samples were removed from each of two study plants per plot in each of the three
157 elevated and three ambient [CO₂] plots. Samples were placed in plastic bags, stored in a
158 cooler and moved to a field lab adjacent to the research plots where they were prepared
159 and analyzed. Pigment samples (approximately 4-6 leaves) were removed from branches
160 and quickly placed in liquid nitrogen. Plant water potential of the other intact shoot
161 sample was determined using a Scholander-type pressure chamber (Soil Moisture Stress
162 Inc., Santa Barbara, CA, U.S.A.). Sugar analyses required significantly larger samples;
163 therefore, all the remaining leaves from water potential determinations were pooled.
164 Leaves for sugar analyses were removed from the stems in the dark and placed in liquid
165 nitrogen after harvest.

Comentario [*8]: REF. 2. 7:

Comentario [BN9]: REF. 1.6

Comentario [*10]: REF. 1.7

166 *Photosynthetic measurements*

167 Photosynthetic gas exchange was measured with a LI-6400 portable photosynthesis
168 system (Li-Cor Inc., Lincoln, NE, USA) equipped with a CO₂ control module and a red-
169 blue light emitting diode light source (Model 6400-02B). For gas exchange
170 measurements, we sampled five plants from one elevated [CO₂] ring and five plants from
171 one ambient [CO₂] ring. Sampling from additional FACE rings was not logistically
172 feasible in this study due to the requirement that plants be accessed from a pivoting
173 walkway, so we maximized sample size within individual large plots. The two selected
174 plots were paired plots (*i.e.* same watershed position) in the overall experimental site, and

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175 so had highly similar surface and soil characteristics. Also, previous studies confirmed
176 that plot-based variation in plant physiological parameters was relatively low for both
177 evergreen and deciduous shrubs. For the evergreen *Larrea*, using 18 different dates
178 between 1998 and 2004 in which we had plot-replicate data from AC_i curves ($n = 36$ with
179 18 dates times the two $[CO_2]$ treatments), we found the following coefficients of
180 variation (CV's): V_{max} plant-to-plant = 22.0, plot-to-plot = 18.2; J_{max} plant-to-plant =
181 34.2, plot-to-plot = 25.7. Therefore, whereas individual plant variation was large,
182 variation was consistently lower between sampling plots than between individual plants.
183 Furthermore, these photosynthetic parameters were not consistently higher in one plot
184 than another for either ambient or elevated $[CO_2]$ treatments. Sampling two plots also
185 allowed paired-in-time measurements at ambient and elevated $[CO_2]$ to occur at highly
186 similar temperature and VPD conditions during the day. This requirement could not have
187 been met if we sampled six separate plots (FACE rings) with a single plant in each plot
188 due to the time required to move between plots.

189 Photosynthetic CO_2 response curves (AC_i) were determined by measuring the response
190 of photosynthesis (A) to varying intercellular CO_2 concentration (C_i). External $[CO_2]$
191 (C_a) was supplied in 8 steps, increasing from 120 to 1500 $\mu mol [CO_2] mol^{-1}$ air, with
192 irradiance (Q) maintained at a saturating value of 1500 $\mu mol m^{-2} s^{-1}$. Measurements were
193 initiated after g_s reached steady state and then recorded automatically at each C_a set point
194 when photosynthesis had equilibrated, which was typically less than 2 min. Foliage
195 temperature during AC_i curves was maintained at ambient air temperature using
196 thermoelectric coolers. Leaf-to-air vapor pressure deficit was generally between 1.5 and
197 3.0 kPa, reflecting ambient conditions. Because both *Larrea* and *Ambrosia* have small,
198 microphyllous leaves, more than one leaf (leaflets in *Larrea*) was inserted into the gas
199 exchange cuvette. After the AC_i curve was generated, all material inside the cuvette was
200 collected and leaf area was determined using a flatbed scanner and analyzed with
201 software from Scion Imaging (Scion Corporation, Frederick, MD, USA). Leaves were
202 subsequently dried at 60°C for at least 2 days and then weighed.

203 AC_i data were analyzed using the photosynthetic biochemical model of Farquhar et al.
204 (1980) to estimate two biochemical parameters potentially limiting to photosynthesis:

Comentario [*14]: REF. 2.10

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 $\mu\text{mol mol}^{-1}$ and 380 $\mu\text{mol mol}^{-1}$ for elevated and ambient
218 $[CO_2]$, respectively.

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 °C. After centrifugation, the supernatant was
224 used for total soluble sugar quantification, whereas the pellet was stored at -80°C for the
225 starch analyses. Supernatant fraction was heat denatured at 100 °C for 3 minutes and
226 precipitated by centrifugation at 12,000 g during 5 minutes at 5 °C. 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

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

248 Leaf pigment samples were collected at pre-dawn, immediately frozen in liquid N and
249 stored in an ultra-low freezer (-85 °C) prior to lyophilization. Before HPLC analysis, the
250 dry mass of each sample was determined and approximately 10 mg dry mass of leaf
251 material was used for pigment extraction. Samples were ground to a fine pulp in the dark
252 in ice-cold 80% acetone (v/v) with an addition of MgCO₃ (spatula tip) using a tissue
253 grinder (Kontes Duall K885450-0021, Kontes, Vineland, NJ, USA). Following
254 extraction, chlorophyll and carotenoid content and composition were determined by
255 HPLC using the method of Gilmore and Yamamoto (1991), as modified by Adams and
256 Demmig-Adams (1992).

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

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

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

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

266

267 where R_{sample} refers to plant material and R_{standard} to Pee Dee Belemnite (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 ($\delta^{13}\text{C}_a$) and plant ($\delta^{13}\text{C}_p$) isotopic composition, respectively.

273

274 The $^{13}\text{C}/^{12}\text{C}$ ratios (R) of air samples were determined at the University of Arkansas
275 (USA). Air $\delta^{13}\text{C}$ 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

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

288

$$C_{new} \approx \frac{\delta^{13}C_E - \delta^{13}C_A}{\delta^{13}C_L - \delta^{13}C_A} \times 100$$

289

290

291

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

295

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

296

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

299 *Statistical analyses*

300 All data were log transformed prior to analyses. Because individual plants were
301 repeatedly measured over time, a repeated measures analysis of variance (RM ANOVA)
302 was used to determine the effects of elevated [CO_2] on physiology, leaf chemistry, and C
303 isotopic determinations of *Larrea* and *Ambrosia*. The sample dates, converted to Julian
304 date, were used as the within-subject factor, whereas [CO_2] treatment was used as the
305 between-subject factor. These analyses has 1 degree of freedom (df) for [CO_2] effect and
306 8 *dfs* for the error term (variability). A factorial analysis was also conducted with these
307 data, and results were similar to those obtained by the RM ANOVA; therefore we used
308 the more conservative RM ANOVA.. Because *Larrea* is an evergreen and *Ambrosia* is
309 drought-deciduous, the RM ANOVA's were performed separately for each species. There
310 were eight measurement dates for *Larrea*, and four measurement dates for *Ambrosia*.

311 Prior to running the RM ANOVA, a principal components analysis was conducted for
312 each species separately to determine the nature and strength of the correlations between
313 parameters. After these analyses showed strong correlations among the physiological
314 data, missing data (5% of total physiology data) were estimated with the maximum
315 likelihood estimation function utilizing a multivariate approach. A discriminant function
316 analysis (DFA) was used for each species to determine those variables which best
317 described differences between plants grown in elevated and ambient [CO₂]. All
318 multivariate analyses were conducted using MatLab (V.7.1, SP 3; The Mathworks Inc.,
319 Natick, MA, USA).

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

323 **Results**

324 *Environmental conditions*

325 Temperatures during the 2005 growing season were typical of the Mojave Desert, with
326 the highest average monthly temperature occurring in July (Fig. 1A). The hydrologic
327 year (1 Oct to 30 Sep) for the Mojave Desert had above-average precipitation, with
328 significant amounts of rainfall occurring between October and March, followed by a dry
329 summer (Fig. 1B). Significant rainfall in the fall and mid-winter resulted in high soil
330 moisture content, with 0-50 cm soil moisture content consistently above 10% through
331 most of the spring (Fig. 1C). There were no plot (i.e., [CO₂]) differences in soil
332 moisture, as has been consistently observed at the NDFP (Nowak et al. 2004). Of note,
333 however, was that the average minimum temperature did not rise above freezing until late
334 April (Fig. 1A); subsequently, the spring growing season was characterized by high soil
335 moisture but frequent freezing temperatures at night. The driest part of the year
336 corresponded with the hottest; although the summer was interrupted by several
337 significant rainfall events (Fig. 1B), integrated 0-20 and 0-50 cm soil moisture never
338 exceeded 5% during the summer months (Fig. 1C).

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

340 Elevated $[\text{CO}_2]$ significantly increased A_{sat} (i.e. light-saturated photosynthesis measured
341 at growth $[\text{CO}_2]$) in *Larrea* (Fig. 2A), whereas elevated $[\text{CO}_2]$ had no significant effect
342 on A_{sat} in *Ambrosia* (Fig. 2B). *Ambrosia* exhibited a mean growing season A_{sat} of 19.8
343 $\mu\text{mol m}^{-2} \text{s}^{-1}$, while during the same time period A_{sat} was 9.4 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in *Larrea*.

344 Stomatal conductance (g_s) was not affected by elevated $[\text{CO}_2]$ for either species (Fig 2C,
345 D). Significant date-by-species effects ($p < 0.05$) were observed such that g_s for both
346 species increased in March and May, with the greatest increase in *Ambrosia* in late April
347 (Fig. 2D), when g_s was 0.39 $\text{mmol m}^{-2} \text{s}^{-1}$ compared to 0.10 $\text{mmol m}^{-2} \text{s}^{-1}$ for *Larrea*.
348 Beginning in June, g_s in *Larrea* decreased over time (Fig. 2C), whereas *Ambrosia*
349 became physiologically dormant for the remainder of the year.

350 A_{sat}/g_s (intrinsic *WUE*) was significantly higher in elevated $[\text{CO}_2]$ in *Larrea* (Fig. 2E) but
351 not in *Ambrosia* (Fig. 2F). Overall, elevated $[\text{CO}_2]$ increased *WUE* by 37% in *Larrea*
352 over the spring growing season (March to May) and by 46% over the entire year (March
353 to October). A significant date-by- $[\text{CO}_2]$ effect for both species ($p < 0.05$) indicated that
354 *WUE* responses differed over time. *Ambrosia* exhibited a significant increase in *WUE* in
355 elevated $[\text{CO}_2]$ plants in March, but no response thereafter (Fig 2F). In contrast, *Larrea*
356 exhibited higher *WUE* in elevated $[\text{CO}_2]$ plants during the entire growing season, but the
357 greatest increase in *WUE* was at the end of the growing season (Fig 2E), when soil
358 moisture was low.

359 Stem water potential (Ψ_{stem}) was significantly higher in elevated $[\text{CO}_2]$ in *Larrea* (Fig.
360 2G) but not in *Ambrosia* (Fig. 2H). In the early growing season, Ψ_{stem} was relatively
361 high and was not affected by elevated $[\text{CO}_2]$ in either species, but later in the growing
362 season, Ψ_{stem} declined at a slower rate in *Larrea* at elevated $[\text{CO}_2]$ compared to ambient
363 $[\text{CO}_2]$. For *Ambrosia*, Ψ_{stem} was significantly higher at elevated $[\text{CO}_2]$ compared to
364 ambient $[\text{CO}_2]$ just before the plant became physiologically dormant in late May (Fig.
365 2H), whereas for *Larrea*, Ψ_{stem} was higher in elevated $[\text{CO}_2]$ compared to ambient $[\text{CO}_2]$
366 from July onward (Fig. 2G). During the most active growing season (spring), *Larrea* had

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

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

370 Elevated $[\text{CO}_2]$ had no effect on A_{max} (i.e. maximum photosynthesis measured at both
371 saturating light and $[\text{CO}_2]$ levels) in either species (Fig. 3A,B). In *Ambrosia*, average
372 A_{max} throughout the study was $30 \mu\text{mol m}^{-2} \text{s}^{-1}$, whereas in *Larrea* it was $23 \mu\text{mol m}^{-2} \text{s}^{-1}$.
373 For both species, A_{max} increased during the growing season until May, after which A_{max}
374 declined for *Larrea*, while *Ambrosia* became physiologically dormant. Elevated $[\text{CO}_2]$
375 had no effect on V_{cmax} (Fig. 3C,D) or J_{max} (Fig. 3E,F) in either species. For both species,
376 J_{max} increased during the growing season until May, after which J_{max} declined for
377 *Larrea*, while *Ambrosia* became physiologically dormant. Elevated $[\text{CO}_2]$ significantly
378 decreased the relative stomatal limitation to photosynthesis (L_s) for both species (Fig.
379 3G,H). For *Larrea* from early March until mid-October, mean L_s was 39% in ambient
380 $[\text{CO}_2]$ and 29% in elevated $[\text{CO}_2]$, whereas for *Ambrosia* from mid-March until late May,
381 mean L_s was 28% and 16% in ambient and elevated $[\text{CO}_2]$, respectively.

382 *Leaf pigment, N, C/N and carbohydrate concentration*

383 *Ambrosia* chlorophyll $a+b$ levels were 57% lower in elevated $[\text{CO}_2]$ during the spring
384 growing season (Fig. 4B), but there was no $[\text{CO}_2]$ effect on chlorophyll $a+b$ levels in
385 *Larrea* from March until mid-October (Fig. 4A). Elevated $[\text{CO}_2]$ resulted in a greater
386 $[(Z+A)/(\text{chl } a+b)]$ (i.e. the ratio of xanthophyll cycle pigments to chlorophyll a and b) in
387 late summer in *Ambrosia* only (Fig. 4C,D). The xanthophyll pool conversion state
388 $[(Z+A)/(V+A+Z)]$ was significantly higher in elevated $[\text{CO}_2]$ for *Ambrosia* but not in
389 *Larrea* (Fig. 4E,F). During the growing season, $[(Z+A)/(\text{chl } a+b)]$ and
390 $[(Z+A)/(V+A+Z)]$ declined in both species (Fig. 4C,D,E,F)

391 In *Larrea*, elevated $[\text{CO}_2]$ significantly decreased leaf N content ($P < 0.01$) during the first
392 half of the growing season, but $[\text{CO}_2]$ treatment effects on leaf N were significant only
393 during May for *Ambrosia* (Table 1). Significant effects of elevated $[\text{CO}_2]$ on C/N ratios
394 in *Larrea* were limited to increased C/N ratios during May-June. During the rest of the

395 experiment, no significant differences were observed for *Larrea* (Table 1). In the case of
396 *Ambrosia*, with the exception of May (when C/N was higher in elevated [CO₂]), no
397 [CO₂] effect on C/N was observed.

398 In *Larrea*, the elevated [CO₂] effect on leaf sucrose, glucose and fructose concentration
399 was affected by sampling date (P< 0.01; P= 0.02; P= 0.09, respectively; Table 1). *Larrea*
400 grown under elevated [CO₂] had higher sucrose levels only during June, glucose levels
401 were increased during two sampling dates (April and June), and for fructose, the increase
402 extended from May until July. With the exception of April, starch content increased in
403 leaves exposed to elevated [CO₂] (Table 1). In *Ambrosia*, [CO₂] treatment effects on
404 sucrose, glucose and fructose also were mediated by sampling date (P< 0.01 for each
405 sugar, respectively; Table 2). During April, although fructose content increased under
406 elevated [CO₂], glucose was not affected and sucrose content diminished. During May,
407 the concentration of the three soluble sugars increased under elevated [CO₂]. However
408 during June, growth in elevated [CO₂] increased sucrose content, whereas glucose and
409 fructose in *Ambrosia* were diminished (Table 1). In July, glucose levels increased in
410 *Ambrosia* exposed to elevated [CO₂], but no [CO₂] effect was observed in sucrose and
411 glucose levels. Elevated [CO₂] increased starch concentration during May-June in both
412 species, and additionally in August in *Larrea* (Table 1).

413 *Proportion of new C in TOM and leaf soluble sugars*

414 The proportion of newly fixed carbon (C_{new}) in different plant organs was similar
415 throughout the study (Table 2), with one exception. C_{new} was significantly lower in
416 *Larrea* leaves during July and August (Table 2) compared to earlier sampling dates (P<
417 0.01). On average, 4 and 7% of C present in the current year's total organic matter
418 (TOM) of *Larrea* and *Ambrosia*, respectively, came from CO₂ that was assimilated
419 before February 10, 2003 (i.e. >2 years prior to the current year), when the pure CO₂ for
420 the elevated CO₂ treatment was switched from a geologic to a fossil fuel source. No
421 significant variation in C_{new} was observed in shoots and roots of either species (Table 2).

422 The proportion of C_{new} in sucrose, glucose and fructose of *Larrea* leaves (Table 2)
423 exposed to elevated [CO₂] varied depending on time (P< 0.01 for each sugar,

424 respectively). For *Larrea*, the greatest C_{new} occurred during June, with C_{new} declining
425 during July and August. For *Ambrosia*, the proportion of C_{new} in sugars of elevated
426 $[\text{CO}_2]$ plants maintained similar levels from April through June, although C_{new} in
427 fructose levels declined in July (Table 2). C_{new} in HCl-hydrolysable C fraction (HCl-C),
428 which is mainly composed of starch, showed that in *Larrea*, ~96% was formed by
429 recently assimilated C, and no significant differences were observed throughout the
430 study. In the case of *Ambrosia*, average C_{new} was ~89% and reached the largest values
431 during May.

432 *Multivariate analyses*

433 In the principal components analysis (PCA; Fig. 5A,C), we observed species differences
434 and correlations between the various physiological parameters. First, *Ambrosia* had
435 stronger stomatal control of photosynthesis than did *Larrea* ($r = 0.93$ and 0.84 ,
436 respectively). Also, g_s was more strongly correlated with J_{max} and V_{cmax} in *Ambrosia* (r
437 $= 0.79$ and 0.55 , respectively) compared to *Larrea* ($r = 0.50$ and 0.17 , respectively). In
438 *Larrea*, xylem water potential showed a stronger negative correlation with photosynthetic
439 rates than in *Ambrosia* ($r = 0.43$ and 0.09 , respectively). Finally, as xylem water potential
440 seasonally declined in both species, sugar levels increased.

441 In the discriminant function analysis (DFA; Fig. 5B,D), we observed trends in various
442 functional parameters in elevated *versus* ambient $[\text{CO}_2]$. WUE increased at elevated
443 $[\text{CO}_2]$ in both species, particularly in *Larrea*, and L_s decreased in both species. We also
444 observed differential effects of elevated $[\text{CO}_2]$ on several other functional parameters
445 with this analysis: (1) sugar levels (fructose and glucose) decreased in *Larrea*, while
446 fructose, glucose and sucrose all increased in *Ambrosia*; (2) chlorophyll $a + b$ decreased
447 in *Ambrosia* but not in *Larrea*; and (3) xanthophyll cycle pigments increased in *Ambrosia*
448 but not in *Larrea*.

449 **Discussion**

450 *Regulation of photosynthetic performance under elevated $[\text{CO}_2]$*

451 Photosynthetic down-regulation (typically indicated by reductions in A_{\max} , V_{cmax} , and
452 J_{\max}) during the eighth growing season of long-term exposure to elevated $[\text{CO}_2]$ at the
453 Nevada Desert FACE Facility was not observed in either *Larrea tridentata* or *Ambrosia*
454 *dumosa* (Figs. 3A-F). These results differ from earlier studies at the NDFF in that
455 photosynthetic down-regulation was previously observed in *Larrea* (Huxman et al. 1998,
456 Hamerlynck et al. 2000b) and a drought-deciduous shrub *Lycium andersonii*
457 (Hamerlynck et al. 2002) in the first two years of elevated $[\text{CO}_2]$ exposure at the NDFF,
458 especially during the cool, moist early spring when plants are not generally water
459 stressed. Based upon our results and those of Naumburg et al. (2004), the desert
460 perennials *Larrea* and *Ambrosia* appear to have photosynthetically equilibrated to
461 elevated $[\text{CO}_2]$ and maintained biochemical capacity over the long-term.

462 Although neither *Larrea tridentata* nor *Ambrosia dumosa* show evidence for
463 photosynthetic down-regulation, only *Larrea* had increased photosynthetic performance
464 (i.e. A_{sat} , light-saturated A at growth $[\text{CO}_2]$) during continuous, long-term exposure to
465 elevated $[\text{CO}_2]$ (Fig. 2A). A_{sat} for *Ambrosia* was not significantly different between
466 $[\text{CO}_2]$ treatments throughout the entire growing season (Fig. 2B). These results for
467 *Larrea* are similar to earlier studies at the NDFF but differ for *Ambrosia*: earlier, both
468 species had increased photosynthetic performance under elevated $[\text{CO}_2]$ (Naumburg et al.
469 2003, Ellsworth et al. 2004, Housman et al. 2006), although elevated $[\text{CO}_2]$ effects were
470 greatly reduced during dry portions of the year or during years with below-average
471 precipitation. Below, we first examine processes that may not account for how
472 photosynthetic performance of *Larrea* may differ from that of *Ambrosia* under elevated
473 $[\text{CO}_2]$, and then examine those that may.

474 The difference in photosynthetic performance between the two species under elevated
475 $[\text{CO}_2]$ was not due to partial stomatal closure (Fig. 2C, D), reduced carboxylation activity
476 (V_{cmax} , Fig. 3C, D), nor to reduced electron transport (J_{\max} , Fig. 3E, F). In all cases, these
477 processes were not significantly different between ambient and elevated $[\text{CO}_2]$
478 treatments. In addition, both species also had reduced L_s under elevated $[\text{CO}_2]$, as has
479 been commonly observed in long-term field studies (Tissue et al. 2001). Although
480 treatment effects on leaf N and carbohydrate concentrations differed between *Larrea* and

481 *Ambrosia* during the growing season (Table 1), the direction of these differences was not
482 consistent with the observed treatment effects on photosynthetic performance. *Larrea*
483 plants under elevated [CO₂] had more consistent decreases in leaf N versus *Ambrosia*
484 plants (Table 1). Although greater decreases in leaf N for *Larrea* would be expected to
485 result in greater decreases in photosynthetic performance because of the close
486 relationship between leaf N and A_{sat} (Ellsworth et al. 2004), in fact *Larrea* had greater
487 increases in A_{sat} under elevated [CO₂]. Sugar and starch concentrations under elevated
488 [CO₂] were often significantly greater than those under ambient [CO₂] (Table 1), which
489 indicated that both species had greater potential for feedback inhibition of net
490 assimilation by carbohydrate accumulation under elevated [CO₂]. Although exceptions
491 do occur for both species (e.g., glucose in April for *Larrea* and sucrose in April for
492 *Ambrosia*), these exceptions occur slightly more frequently for *Ambrosia*, suggesting
493 photosynthetic performance of *Ambrosia* would have benefited more under elevated
494 [CO₂] because of less frequent feedback inhibition. However, this prediction of greater
495 performance of *Ambrosia* under elevated [CO₂] also is contrary to observations.

496 As with leaf N and carbohydrates, the effects of elevated [CO₂] on pigment
497 characteristics (Fig. 4) differed substantially between the two shrub species, but these
498 pigment differences also were not consistent with differences in photosynthetic
499 performance. Pigments are functional components of the photosynthetic machinery,
500 providing information about biochemical investment and stress in the photosystems. The
501 evergreen *Larrea* did not adjust pigment allocation in response to elevated [CO₂], as was
502 documented for the evergreen tree loblolly pine after 8 years in FACE (Logan et al.,
503 2009). In the deciduous *Ambrosia*, plants growing under elevated [CO₂] reduced
504 chlorophyll *a* and *b* throughout the growing season, suggesting that less light absorbing
505 and processing capabilities may be part of the reason why A_{sat} under elevated [CO₂] was
506 not as high as expected for that species. However, desert plants typically are not light
507 limited (Smith et al. 1997), and thus lower chlorophyll under elevated [CO₂] may at best
508 be only a minor contribution towards lower than expected A_{sat} under elevated [CO₂] in
509 *Ambrosia*. Generally, photoinhibition is a greater concern in high-light environments
510 (Hymus et al. 1999, Aranjuelo et al. 2008b), but the pigment data indicate that increased
511 protective pigment concentrations only occurred in *Ambrosia*. Photoprotection was

512 presumably employed to the level necessary during exposure to excess light each day, as
513 violaxanthin was converted to zeaxanthin and the latter employed in thermal energy
514 dissipation to avoid photodamage (Adams et al. 2006, Demmig-Adams and Adams
515 2006). Nonetheless, these differences were reduced during the peak growing season,
516 when high photoprotection may be more important as sink activity increases (Adams et
517 al. 2006) and into the summer dry season as drought-induced photoinhibition becomes
518 more frequent. Thus, the greater ability to avoid photoinhibition in *Ambrosia* was not
519 sufficient to improve photosynthetic performance under elevated [CO₂] over that under
520 ambient [CO₂].

521 The lack of increased photosynthetic performance in *Ambrosia* after long-term exposure
522 to elevated [CO₂] may reflect differences between *Larrea* and *Ambrosia* in allocation to
523 C sinks and utilization of stored C pools. *Larrea* had greater percentages of newly fixed
524 C in the current year's growth of leaves, shoots, and roots under elevated [CO₂] than
525 *Ambrosia* (Table 2), suggesting *Larrea* maintained sufficient C sinks and hence enabled
526 greater photosynthetic performance. Furthermore, the greatest enhancement of
527 photosynthetic performance under elevated [CO₂] occurred in summer for *Larrea* (Fig.
528 2A), when carbohydrate concentrations were most similar between elevated and ambient
529 [CO₂] treatments. Other studies have related photosynthetic performance under elevated
530 [CO₂] to the ability of plants to develop new C sinks or expand the existing ones
531 (Ceulemans, 1997) and suggested that down-regulation was the consequence of an
532 insufficient sink plant capacity (Morgan et al. 2001, Ainsworth et al. 2004, Aranjuelo et
533 al., 2009). Furthermore, when plants exposed to elevated CO₂ exhibited limited capacity
534 to increase C sink strength, plants decreased their photosynthetic activity to balance C
535 source activity and sink capacity (Thomas and Strain 1991).

536 Improved plant water relations in elevated [CO₂], indicated by higher WUE and higher
537 Ψ_{stem} (Fig. 2), also helped maintain A_{sat} in *Larrea* during the driest part of the summer. In
538 contrast, growth in elevated [CO₂] did not improve WUE or plant water relations in the
539 drought-deciduous *Ambrosia*. During drought periods, plants may partially alleviate
540 water stress by accumulating osmolytes (*e.g.* sugars) to increase cellular water uptake. In
541 *Ambrosia*, sugars and starch were higher in elevated [CO₂], but there was no

542 commensurate increase in Ψ_{stem} in elevated $[\text{CO}_2]$ plants during the hotter, drier period of
543 the growing season. In *Larrea*, sucrose, glucose and fructose content increased in
544 elevated $[\text{CO}_2]$ during spring whereas few significant differences were detected during
545 summer (July-August), suggesting that changes in soluble sugars were probably not
546 significant contributors to higher Ψ_{stem} in elevated $[\text{CO}_2]$ plants. However, soluble
547 sugars were generally much higher in *Larrea* than *Ambrosia* during all periods of the
548 growing season. Therefore, the maintenance of physiological activity in *Larrea* into the
549 hottest and driest periods of the growing season, when *Ambrosia* drops its leaves and
550 becomes inactive, may be partially attributed to greater access to osmolytes in *Larrea*
551 (Smith et al. 1997).

552 *Long-term C storage and C allocation patterns*

553 Modification of atmospheric $\delta^{13}\text{C}$ in concert with experimental CO_2 exposure enabled the
554 characterization of C allocation and partitioning of *Larrea* and *Ambrosia* under varying
555 seasonal growth conditions. In *Larrea* and *Ambrosia* plants grown under elevated $[\text{CO}_2]$,
556 organs developed during the experimental period were partly (4% and 7 %, respectively)
557 constructed from “old” C (*i.e.* C that was assimilated prior to the beginning of the
558 labeling period two years earlier; February 10, 2003) when the source of CO_2 for
559 elevated plots was switched to fossil-fuel-derived CO_2 . Thus, most C utilized in plant
560 growth was derived from “new” C in *Larrea* (96%) and *Ambrosia* (93%). Similar results
561 were described by Körner et al. (2005), where after two years of labeling, 82-89 % of C
562 present in newly formed shoots and leaves (respectively) for *Quercus*, *Fagus*, *Acer*,
563 *Carpinus*, and *Tilia* trees grown under elevated $[\text{CO}_2]$ was from C assimilated during the
564 last two years. von Felten et al. (2007) also reported that after two years of C labeling and
565 exposure to elevated $[\text{CO}_2]$, 46 % and 42 % of C present in new *Larix decidua* and *Pinus*
566 *uncinatus* (respectively) shoots was C fixed prior to the labeling period. As observed in
567 other slow-growing plants, after a long term $^{12}\text{CO}_2$ enriched labeling period (Aranjuelo et
568 al. 2009), our results suggest that in a high growth year, both species remobilized stored
569 C to develop new biomass. Long-term storage and remobilization of C reserves has been
570 observed in other woody species growing in ambient $[\text{CO}_2]$ conditions (Lacointe et al.
571 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 [CO₂] 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 $\delta^{13}\text{C}$ 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 [CO₂]
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 $\delta^{13}\text{C}$ also could affect
596 $\delta^{13}\text{C}$ 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.

601 *Conclusions*

602 This study was conducted during the eighth full growing season of [CO₂] treatment at the
603 NDFF, thereby providing insight into the long-term physiological responses of two
604 perennial shrubs, *Larrea tridentata* and *Ambrosia dumosa*, to elevated [CO₂]. In the
605 evergreen shrub *Larrea*, plants under elevated [CO₂] enhanced photosynthetic
606 performance (A_{sat}), maintained C sinks, and improved plant water status (higher WUE
607 and Ψ_{stem}), especially during periods of environmental stress in the later part of the
608 growing season. In contrast, the drought-deciduous shrub *Ambrosia* did not increase A_{sat} ,
609 WUE, or Ψ_{stem} under elevated [CO₂]. Surprisingly, we found that g_s and photosynthetic
610 capacity (A_{max} , V_{cmax} , J_{max}) were not affected by elevated [CO₂] in either species.
611 Although increases in photoprotective pigments were observed in *Ambrosia* under
612 elevated [CO₂], photoprotection was not sufficient to increase photosynthetic
613 performance in *Ambrosia*. On average, 96% and 93% of C present in new growth and
614 soluble sugars of *Larrea* and *Ambrosia*, respectively, was recently assimilated C, which
615 implies that in this year (2005) when plant growth was strongly increased, plants
616 mobilized stored C to fulfill new organ formation requirements. Furthermore, *Larrea*
617 utilized a greater fraction of new C to grow new organs and sustained these sinks for
618 longer during the growing season than *Ambrosia*, indicating that maintenance of C sinks
619 by *Larrea* helps that shrub maintain increased photosynthetic performance during long-
620 term exposure to elevated [CO₂] at the Nevada Desert FACE Facility. Thus, although the
621 early biochemical adjustments that we observed at the FACE site (*i.e.* down-regulation of
622 photosynthesis) have abated under longer-term exposure to elevated [CO₂], these
623 physiological characteristics of *Larrea* should significantly enhance carbon gain under
624 elevated [CO₂] on an annual basis over the long-term.

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855

856 **Table 1.** Elevated CO₂ exposure (ambient CO₂ versus elevated CO₂) effect in terms of N (%), C/N ratio, sucrose (mg g⁻¹DM), glucose
 857 (mg g⁻¹DM), fructose (mg g⁻¹DM), and starch (mg g⁻¹DM) of *Larrea tridentata* and *Ambrosia dumosa* leaves. Parameters that differed
 858 significantly due to [CO₂] were highlighted in bold. Each value represents the mean ± standard deviation.

<i>Larrea tridentata</i>	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

859

<i>Ambrosia dumosa</i>	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

860

861

862 **Table 2.** Elevated CO₂ 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.

<i>Larrea tridentata</i>	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

<i>Ambrosia dumosa</i>	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	92.97±0.28
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

868 **Figure Legends**

869 Fig. 1 Average monthly maximum and minimum temperature (A), daily precipitation (B),
870 and volumetric soil water content at 0-30 and 0-50 cm depths (C) during 2005 at the
871 Nevada Desert FACE Facility. There were no soil moisture differences between ambient
872 and elevated [CO₂] plots for either depth.

873 Fig. 2 Plant physiological performance at ambient (filled symbols; 380 μmol mol⁻¹)
874 versus elevated (open symbols; 550 μmol mol⁻¹) atmospheric [CO₂] measured as: (A,B)
875 A_{sat} (light-saturated net assimilation rate, A_{net}); (C,D) stomatal conductance (g_s); (E,F)
876 Water-Use Efficiency (WUE) calculated as A_{sat}/g_s; and (G,H) pre-dawn water potential
877 (Ψ) for *Larrea tridentata* (left panels) and *Ambrosia dumosa* (right panels). Vertical bars
878 represent ± one standard deviation.

879 Fig. 3 Mechanistic photosynthesis at ambient versus elevated [CO₂] in *Larrea tridentata*
880 and *Ambrosia dumosa* measured as: (A,B) maximum (CO₂-saturated) assimilation rate
881 (A_{max}); (C,D) maximum carboxylation rate of Rubisco (V_{cmax}); (E,F) maximum electron
882 transport rate (J_{max}) and (G,H) relative stomatal limitation (L_s). All symbols are as in Fig.
883 2.

884 Fig. 4. Photosynthetic pigments at ambient versus elevated [CO₂] in *Larrea tridentata*
885 and *Ambrosia dumosa* measured as: (A,B) chlorophyll *a+b*; (C,D) the ratio of
886 xanthophyll cycle pigments to chlorophyll *a+b*; and (E,F) the xanthophyll pool
887 conversion state ((Z+A)/(V+A+Z)) All symbols are as in Fig. 2.

888 Fig. 5. Results of principal components analysis (PCA) displayed as vector correlations
889 among variables for *Larrea tridentata* (A) and *Ambrosia dumosa* (C) for the first and
890 second principal components (PC1 and PC2). The length and angle between a pair of
891 vectors is an indication of the strength and nature, respectively, of their correlations.
892 Results of discriminant function analysis (DFA) in vector format show the direction of
893 responses of *Larrea* (B) and *Ambrosia* (D) to ambient and elevated [CO₂] treatments.
894 The direction of the vector is an indication of whether an increase or decrease was
895 observed in a particular variable, with vectors to the right indicating a positive response

896 to elevated [CO₂], and the length of the vector is an indication of the strength of the
897 response. Plant variables used in this analysis: A_{\max} ; A_{sat} ; g_s ; L_s ; WUE; V_{cmax} ; J_{max} ;
898 $F_v F_m$; plant Ψ ; Chl $a + b$; $[Z+A]/[V+A+Z]$; $[Z+A]/[\text{Chl } a + b]$ (plant variables as
899 described in previous figure legends).

900

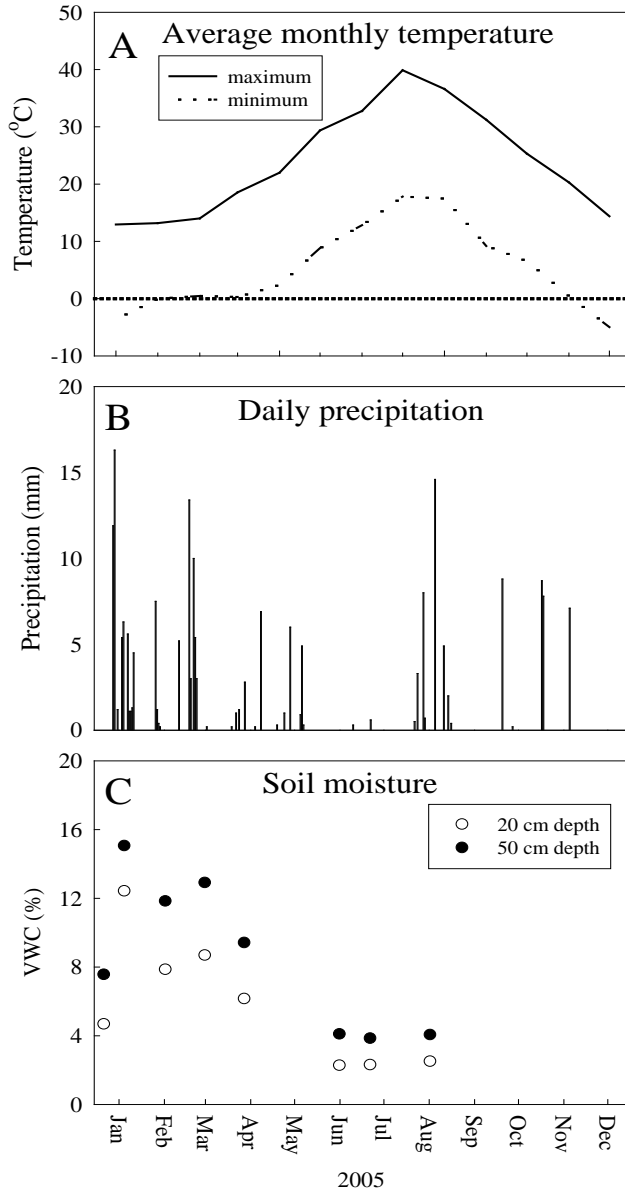


Figure 1.

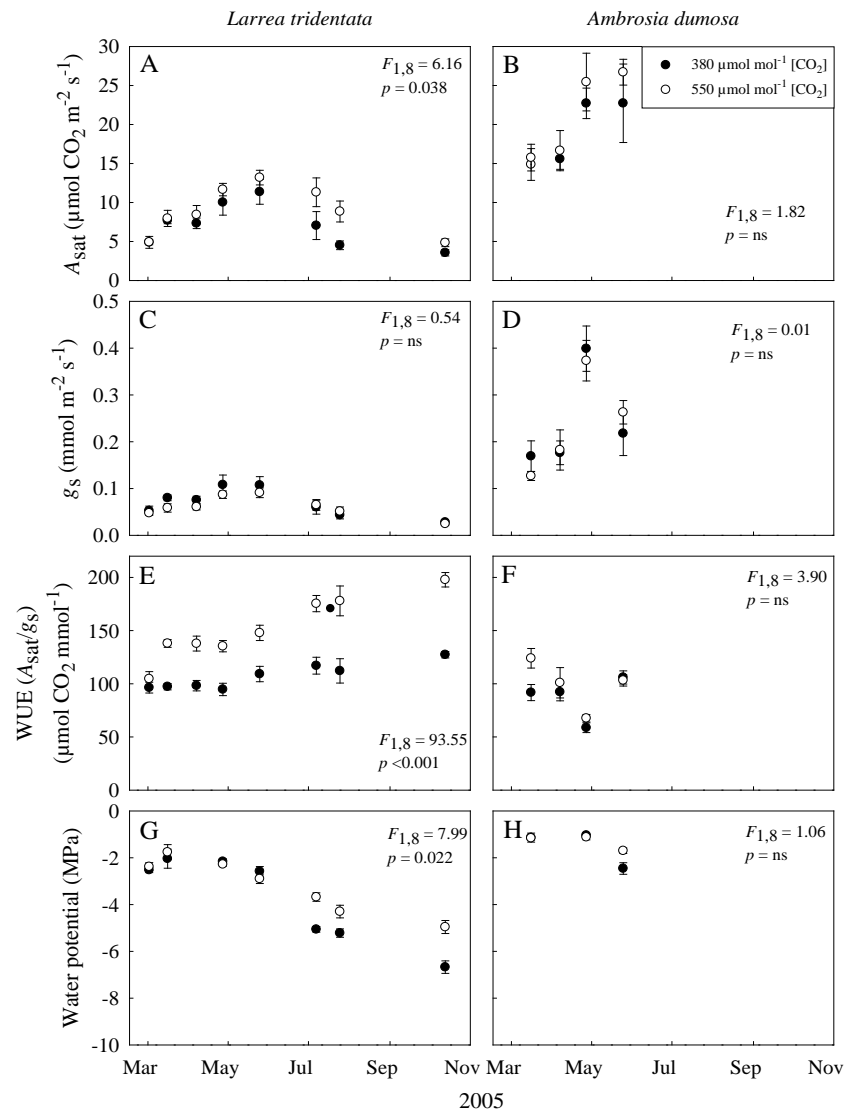


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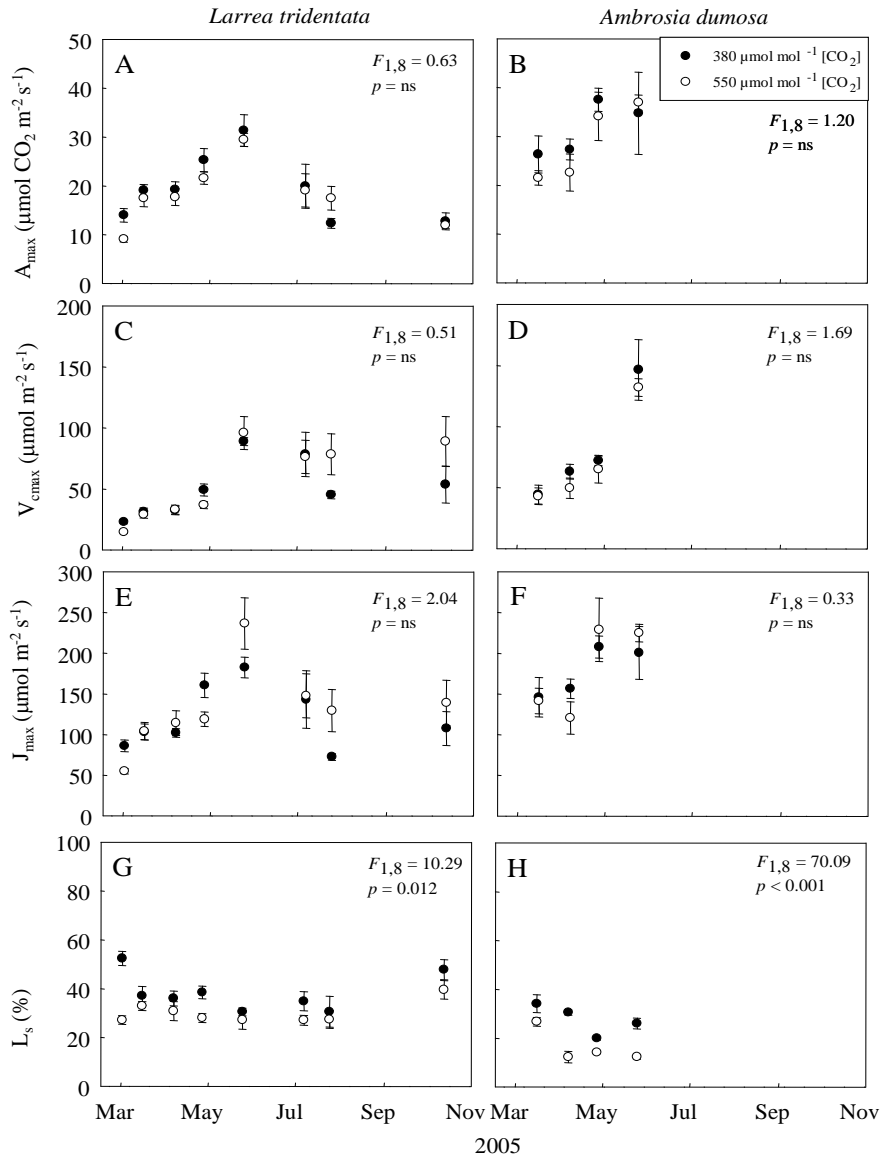


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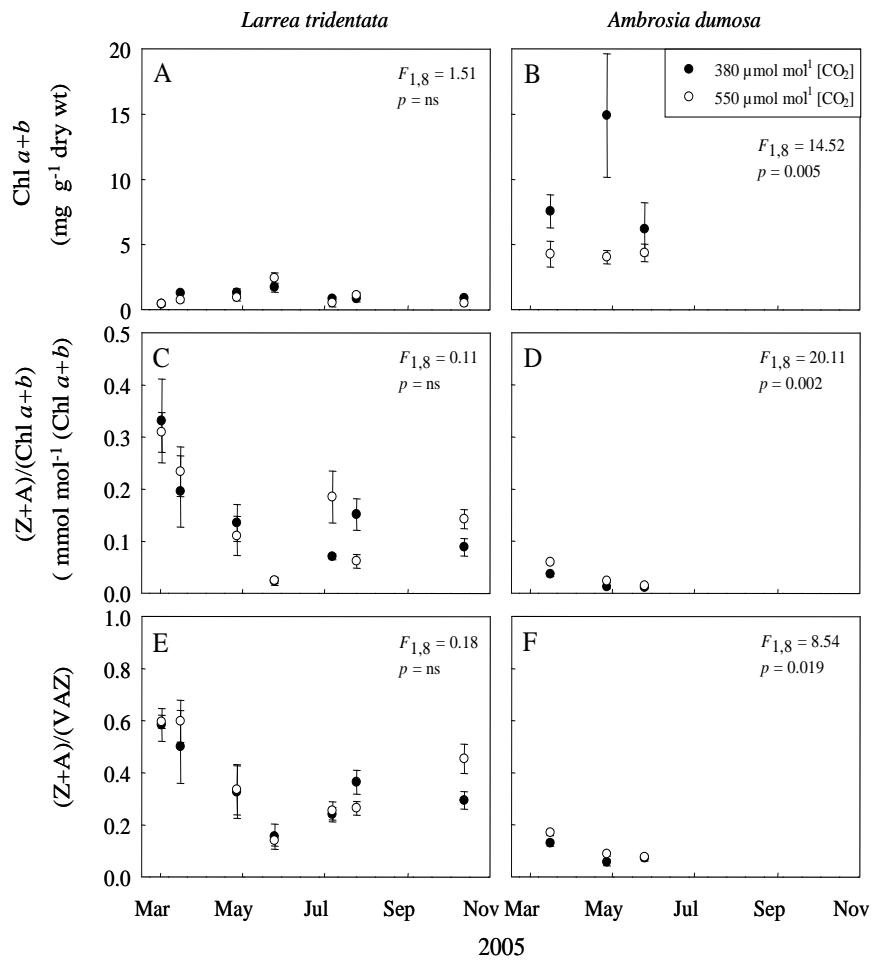


Figure 4.

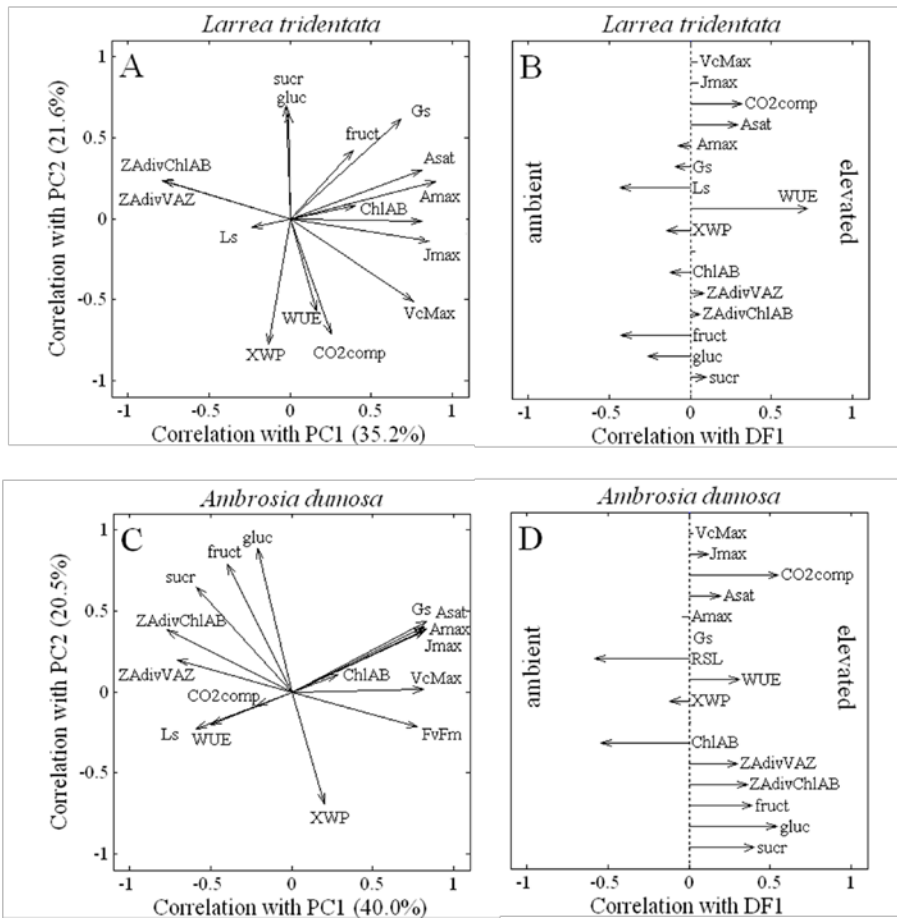


Figure 5.