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**Effects of Elevated CO₂ on Root Function and Soil Respiration
in a Mojave Desert Ecosystem**

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SUMMARY

Key results from our research on responses of plants to elevated atmospheric CO₂ at the Nevada Desert FACE Facility (NDFF) and under controlled environment conditions include:

Root Structure and Function

Measurements of root physiological characteristics and of arbuscular mycorrhizae indicate that:

- Root respiration rates of two Mojave Desert shrubs, *Ambrosia dumosa* and *Larrea tridentata*, at the Nevada Desert FACE Facility (NDFF) over five growing seasons and *L. tridentata* seedlings in a greenhouse experiment were not significantly affected by elevated CO₂.
- The combination of elevated CO₂ and phosphorus reduction in greenhouse studies did not affect root growth or activity.
- Specific root length was not significantly affected by CO₂.
- Extraradical hyphae (ERH) of arbuscular mycorrhizal fungi (AMF) varied seasonally, with ERH significantly lower at the end of the year. However, percent root colonization was not affected by sample date. These seasonal patterns of AMF are likely due to greater shrub demands for nutrients early in the year rather than changes in soil moisture.
- AMF root colonization or ERH did not vary among soils beneath two shrubs, *Ambrosia dumosa* and *Larrea tridentata*, and their associated interspaces.
- Beneath *L. tridentata*, immunoreactive glomalin related soil protein (IRSP) concentrations were significantly greater than all other microsites, and IRSP declined significantly over the year in *L. tridentata* soils. Significantly higher IRSP concentrations in the spring under *L. tridentata* may be due to turnover of residual ERH produced in the preceding wet year.
- AMF root colonization, ERH lengths, and IRSP concentrations were not different between CO₂ treatments after 9 years of CO₂ treatment for samples collected in the spring and fall from three microsites: beneath *Larrea tridentata* and *Ambrosia dumosa* canopies and within shrub interspaces.

Thus, our results from both the greenhouse and field experiment suggest that root systems of these species have not altered carbon use for respiration processes on a root length basis, nor have they grown in size to compensate for any nutrient limitations imposed by carbon fertilization. Furthermore, neither species showed increased allocation to arbuscular mycorrhizae under elevated CO₂. AMF appear to explore all areas of the Mojave Desert equally even though nutrient availability varies considerably among microsites. Stimulated nitrogen cycling by other soil microbes in elevated CO₂ plots may eliminate the need for greater production of AMF by increasing nutrient availability in this system.

Root Growth

Minirhizotron measurements of root growth reveal that:

- Fine roots accumulate over long time periods in the Mojave Desert.
- Elevated CO₂ had transitory effects on the accumulation of fine roots, where standing crop of fine roots accumulated to a larger extent under elevated CO₂ for up to 5 months.

- The transitory greater accumulation of fine roots under elevated CO₂ was primarily due to lower rates of root loss through time.
- After a wet fall and winter time period, root loss under elevated CO₂ was increased to a greater extent than that under ambient CO₂, which resulted in the re-convergence of fine root standing crop between the elevated and ambient CO₂ treatments

Thus, our results from the minirhizotron studies indicate that are not allocating more of their carbon resources to fine root growth under elevated CO₂. Root loss is lower under elevated CO₂, likely because of changes in the C:N ratios of fine roots under elevated CO₂. Nonetheless, microbial degradation of fine roots occurs when sufficient soil moisture is available, and this degradation occurs over an extended time period.

Soil Respiration

Soil CO₂ effluxes (R_S) under elevated and ambient CO₂ conditions indicate that:

- R_S is spatially and temporally variable, with highest R_S during spring – early summer, and under plant canopies, especially in soil under *L. tridentata*.
- R_S responded rapidly and positively to rain.
- Elevated CO₂ increased soil respiration rates, inducing a mean increase of 42% at the ecosystem level over a two year period ($0.220 \pm 0.23 \text{ g C m}^{-2} \text{ d}^{-1}$ versus $0.155 \pm 0.20 \text{ g C m}^{-2} \text{ d}^{-1}$; $p = 0.0582$).

These results suggest that increases in C assimilation and sequestration due to elevated atmospheric CO₂ may be partially offset by increased CO₂ effluxes from increased soil respiration in a Mojave Desert ecosystem.

In summary, *A. dumosa* and *L. tridentata* root systems have not increased in size or activity, and increased shoot production observed under elevated CO₂ for these species does not appear to be constrained by the plant's belowground resources. Furthermore, greater soil respiration rates observed at the NDFF are not due to increased root activity, but rather stimulated microbial activity. Increased activity by carbon limited soil microbes with elevated CO₂ at the NDFF may provide adequate nutrients for these shrubs without further investment in root systems. Root exudation under high CO₂ may also be altered, and changes in the composition of root exudates could consequently influence the heterotrophic communities residing there. However, exudation and other aspects of root responses to elevated CO₂ remain uncertain for these Mojave Desert species and should be investigated in future studies to clarify potentially plant-mediated shifts in soil carbon fluxes.

INTRODUCTION

Increases in atmospheric CO₂ concentration during the last 250 years are unequivocal, and CO₂ will continue to increase at least for the next several decades (Houghton et al. 2001, Keeling & Whorf 2002). Arid ecosystems are some of the most important biomes globally on a land surface area basis, are increasing in area at an alarming pace (Dregne 1991), and have a strong coupling with regional climate (Asner & Heidebrecht 2005). These water-limited ecosystems also are predicted to be the most sensitive to elevated CO₂, in part because they are stressful environments where plant responses to elevated CO₂ may be amplified (Strain & Bazzaz 1983). Indeed, all C₃ species examined at the Nevada Desert FACE Facility (NDFF) have shown increased A_{net} under elevated CO₂ (Ellsworth et al. 2004, Naumburg et al. 2003, Nowak et al. 2004). Furthermore, increased shoot growth for individual species under elevated CO₂ was spectacular in a very wet year (Smith et al. 2000), although the response in low to average precipitation years has been smaller (Housman et al. 2006). Increases in perennial cover and biomass at the NDFF are consistent with long term trends in the Mojave Desert and elsewhere in the Southwest, indicating C sequestration in woody biomass (Potter et al. 2006).

Elevated CO₂ also increases belowground net primary production (BNPP), with average increases of 70%, 21%, and 11% for forests, bogs, and grasslands, respectively (Nowak et al. 2004). Although detailed studies of elevated CO₂ responses for desert root systems were virtually non-existent prior to our research, we anticipated that C sequestration may occur by desert root systems for several reasons. First, desert ecosystems exhibit increases in net photosynthesis and primary production at elevated CO₂. If large quantities of root litter enter the ecosystem at a time when most decomposers are inactive, significant quantities of carbon may be stored belowground in relatively recalcitrant forms. Indeed, a model-based analysis predicted that the arid/semiarid southwestern bioclimatic region had one of the highest rates of net carbon storage in the United States over the past century (Schimel et al. 2000). Second, root systems of desert plants are often extensive (Foxy et al. 1984, Hartle et al. 2006) with relatively large proportions of roots deep in the soil (Schenk & Jackson 2002). Thus, an understanding of belowground processes in desert ecosystems provides information on the potential for terrestrial carbon sequestration in desert ecosystems.

The Nevada Desert FACE Facility

The Nevada Desert FACE Facility (NDFF) is the only FACE experiment in an arid or semiarid ecosystem, which collectively account for over 40% of the earth's terrestrial surface area (Reynolds 2001). It is also one of only two operational FACE experiments in a natural, unmanaged ecosystem (Nowak et al. 2004). The experiment is composed of three 25-m diameter plots maintained at a CO₂ set point of 550 ppm, three plots with the FACE apparatus but maintained at ambient CO₂ (blower controls), and three plots without the FACE apparatus (non-blower controls) (Jordan et al. 1999). The vegetation of the NDFF is characteristic of the Mojave Desert and is dominated by the xerophytic shrubs *Ambrosia dumosa* (bur-sage) and *Larrea tridentata* (creosote bush). *Ambrosia* is a drought-deciduous and small (ca. 50 cm height) shrub, whereas *Larrea* is an evergreen shrub that reaches over 1 m in height. In addition to these shrub species, the site is characterized by abundant *Bromus madritensis* (red brome), an exotic annual grass that is rapidly invading the Mojave Desert, and a moderate cover of a C₄ perennial bunchgrass *Pleuraphis rigida*. The site also has well developed biological surface "crusts" (cryptogamic crusts) comprised of bacteria, algae, mosses and lichens. These crusts protect soils from wind erosion and are important in nutrient cycling by fixing atmospheric nitrogen (Evans & Ehleringer 1993). Pictures and additional information on the facilities and resources at the NDFF are found at our web site: www.unlv.edu/Climate_Change_Research/.

Elevated CO₂ fumigation at the NDFF began on 28 April 1997 and has continued 24 hours per day and 365 days per year, except when the operational thresholds of air temperature (below 3 C) or wind speed (typically greater than 6 m s⁻¹) are reached or exceeded. Because ambient CO₂ concentrations in desert ecosystems do not vary greatly during the day, fumigation occurs 24 hours per day to avoid artifactual changes in CO₂ gradients during the diurnal time period. Because frosts are uncommon in the Mojave Desert and because it contains evergreen species as well as soil biological crust and other plant

species that can respond to precipitation at any time of the year, we fumigate all year long.

This Final Report covers the period of support from DOE's Terrestrial Carbon Processes Program between our renewal in 2004 and termination of support in 2007.

MAJOR FINDINGS

Root Structure and Function: Root Physiological Characteristics

Elevated CO₂ – In both greenhouse and field experiments, elevated CO₂ did not significantly affect root respiration rates of either *Ambrosia dumosa* or *Larrea tridentata* (Tables 1, 2, & 3; Figs. 1 & 2) (Clark et al. in prep), two dominant shrubs in the Mojave Desert of western North America. Similar results have been reported for three Mojave Desert grasses, *Bromus madritdensis* ssp. *rubens*, *Achnatherum hymenoides*, and *Pleuraphis rigida* (Yoder et al. 2000). Although similar results have been observed in other ecosystems (George et al. 2003, Tjoelker et al. 1999), contrary results have also been reported (Janssens et al. 1998, Edwards & Norby 1999, BassiriRad et al. 1996), making broad statements on how elevated CO₂ affects root respiration difficult to infer. However, nitrogen contents of *A. dumosa* and *L. tridentata* roots sampled from the elevated CO₂ plots at the NDDFF are not significantly different from those from ambient CO₂ plots (Nowak et al. unpublished data), and *L. tridentata* root nitrogen was also not affected in our greenhouse experiment after 10 months of CO₂ treatment (Table 4). These similar nitrogen contents may help explain the lack of differences in root respiration, which is highly correlated with nitrogen

Table 1. ANOVA tables (A) and means and standard errors for parameters measured on 4-week old *Larrea tridentata* seedlings grown in a greenhouse under ambient and elevated.

A) ANOVA tables

Effect	Num df	Den df	P value
Plant weight			
CO ₂	1	26	0.010
Shoot weight			
CO ₂	1	27	0.049
Root weight			
CO ₂	1	24	0.253
Root type	1	24	0.120
Root type x CO ₂	1	24	0.728
R:S Ratio			
CO ₂	1	26	0.253
Root respiration			
CO ₂	1	16	0.713
Root type	1	37	0.083
Root type x CO ₂	1	37	0.484
Specific root length			
CO ₂	1	26	0.332
Root type	1	31	0.614
Root type x CO ₂	1	31	0.714

B) Means and standard errors

Parameter	Ambient CO ₂		Elevated CO ₂	
	Mean	SE	Mean	SE
Total plant biomass (mg)	6.717	0.715	9.634	0.773
Root:Shoot ratio	0.341	0.053	0.462	0.067
Lateral root biomass (mg)	0.591	0.195	1.236	0.322
Tap root biomass (mg)	1.091	0.202	1.751	0.266
Shoot biomass (mg)	5.110	0.536	6.785	0.685
Lateral root respiration (μmol O ₂ min ⁻¹ m ⁻¹)	-0.0303	0.0161	-0.0171	0.0038
Tap root respiration (μmol O ₂ min ⁻¹ m ⁻¹)	-0.0092	0.0028	-0.0116	0.0016
Lateral root specific root length (m g ⁻¹)	59.83	17.84	72.85	27.32
Tap root specific root length (m g ⁻¹)	63.62	13.01	39.93	4.69

Table 2. ANOVA table (A) and means and standard errors (B) for very fine root respiration of 10-month old *L. tridentata* seedlings grown in a greenhouse at ambient and elevated CO₂ with two levels of phosphorus addition.

A) ANOVA tables

Effect	Num df	Den df	P value
Root respiration			
CO ₂	1	36	0.407
Phosphorus	1	36	0.475
CO ₂ x Phosphorus	1	36	0.314

B) Means and standard errors for root respiration (μmol O₂ min⁻¹ m⁻¹)

CO ₂ treatment	Reduced P		Control P	
	Mean	SE	Mean	SE
Ambient	-0.0159	0.0018	-0.0139	0.0016
Elevated	-0.0120	0.0010	-0.0143	0.0018

Table 3. ANOVA tables for root respiration of *Ambrosia dumosa* and *Larrea tridentata* for: (A) roots of different size and age classes measured during 2001-2003; and (B) young, very fine roots measured during all 5 years of sampling (2001, 2002, 2003, 2005, and 2006).

A) All root size and age classes, but only 2001-2003 sample dates

Effect	<i>Ambrosia dumosa</i>			<i>Larrea tridentata</i>		
	Num df	Den df	P value	Num df	Den df	P value
CO ₂	1	4	0.998	1	4	0.540
Diameter	1	22	0.353	1	22	0.156
CO ₂ x Diameter	1	22	0.724	1	22	0.182
Age	1	22	0.278	1	22	0.648
Age x Diameter	1	22	0.832	1	22	0.641
CO ₂ x Age	1	22	0.841	1	22	0.425
Age x CO ₂ x Diameter	1	22	0.570	1	22	0.745

B) All sample dates, but only young, very fine roots

Effect	<i>Ambrosia dumosa</i>			<i>Larrea tridentata</i>		
	Num df	Den df	P value	Num df	Den df	P value
CO ₂	1	5	0.824	1	4	0.526
Sample date	11	97	<0.001	12	90	<0.001
CO ₂ x Sample Date	11	97	0.721	12	90	0.695

Figure 1. Respiration of *Ambrosia dumosa* and *Larrea tridentata* roots grown under field conditions in ambient and elevated CO₂ plots at the Nevada Desert FACE Facility. Means and standard error bars are shown for two root diameter classes (very fine <1.0 mm; fine 1-2 mm). None of the means shown within a species are significantly different from each other (see Table 6). Data were averaged over all age classes for roots sampled during 2001, 2002, and 2003.

Table 4. ANOVA tables for carbon and nitrogen content of 10-month *Larrea tridentata* seedlings grown in a greenhouse at ambient and elevated CO₂ with two levels of phosphorus additions.

Effect	Very fine roots			Fine roots			Stems			Leaves		
	Num	Den	P	Num	Den	P	Num	Den	P	Num	Den	P
	df	df	value	df	df	value	df	df	value	df	df	value
%C												
CO ₂	1	45	0.383	1	42	0.984	1	45	0.008	1	43	0.505
P	1	45	0.089	1	42	0.049	1	45	0.001	1	43	0.329
CO ₂ x P	1	45	0.896	1	42	0.015	1	45	0.241	1	43	0.411
%N												
CO ₂	1	43	0.789	1	42	0.653	1	45	0.034	1	45	<0.001
P	1	43	0.005	1	42	0.004	1	45	0.079	1	45	0.922
CO ₂ x P	1	43	0.604	1	42	0.344	1	45	0.221	1	45	0.945
C:N												
CO ₂	1	45	0.732	1	42	0.457	1	44	0.001	1	43	<0.001
P	1	45	0.029	1	42	0.009	1	44	0.002	1	43	0.985
CO ₂ x P	1	45	0.849	1	42	0.178	1	44	0.464	1	43	0.980

Figure 2. Respiration rates of (a) *Ambrosia dumosa* and (b) *Larrea tridentata* fine roots grown in ambient and elevated CO₂ field plots at the NDFF across all sampling dates. (c) Percent soil water content at 15 cm depth across sampling dates in ambient and elevated CO₂ treatments. Sample dates in 2005 with the same letter were not significantly different for fine root respiration within a species. Sample dates within a year were not significantly different in all other years sampled.

Table 5. ANOVA table for very fine root specific root length of 10-month old *L. tridentata* seedlings grown in a greenhouse at ambient and elevated CO₂ with two levels of phosphorus addition.

Effect	Num	Den	P
	df	df	value
CO ₂	1	41	0.864
Phosphorus	1	41	0.306
CO ₂ x Phosphorus	1	41	0.284

Table 6. ANOVA tables for specific root length of *Ambrosia dumosa* and *Larrea tridentata* for: (A) roots of different size and age classes measured during 2001-2003; and (B) young, very fine roots measured during all 5 years of sampling (2001, 2002, 2003, 2005, and 2006).

A) All root size and age classes, but only 2001-2003 sample dates

Effect	<i>Ambrosia dumosa</i>			<i>Larrea tridentata</i>		
	Num df	Den df	P value	Num df	Den df	P value
CO ₂	1	4	0.998	1	4	0.540
Diameter	1	22	0.353	1	22	0.156
CO ₂ x Diameter	1	22	0.724	1	22	0.182
Age	1	22	0.278	1	22	0.648
Age x Diameter	1	22	0.832	1	22	0.641
CO ₂ x Age	1	22	0.841	1	22	0.425
Age x CO ₂ x Diameter	1	22	0.570	1	22	0.745

B) All sample dates, but only young, very fine roots

Effect	<i>Ambrosia dumosa</i>			<i>Larrea tridentata</i>		
	Num df	Den df	P value	Num df	Den df	P value
CO ₂	1	5	0.824	1	4	0.526
Sample date	11	97	<0.001	12	90	<0.001
CO ₂ x Sample Date	11	97	0.721	12	90	0.695

concentrations in root tissue across ecosystems (Burton et al. 2002). For loblolly pine and sweetgum, root nitrogen was not been altered with elevated CO₂, nor was total root respiration (George et al. 2003).

When scaled up to the entire root system in our field study, our results indicate that root respiration rates of the entire root system were not greater with elevated CO₂. Minirhizotron images at the Nevada Desert FACE Facility (NDFF) do not show persistent changes in root length of *A. dumosa* or *L. tridentata* with elevated CO₂ treatment (this report, Phillips et al. 2006). Because we did not observe a change in specific root length (SRL) of either species (Tables 5 & 6; Figs. 3 & 4), belowground biomass for these two species is also unaffected by the high CO₂ treatment. Phillips et al. (2006) did not find a significant CO₂ x sampling date interaction effect on root length; therefore, the sampling date differences we report for SRL do not extrapolate as seasonal belowground biomass differences between CO₂ treatments. Although examining respiration rates of additional species at the NDFF would clarify the autotrophic respiration estimates, *A. dumosa* and *L. tridentata* constitute a large portion of the biomass in the Mojave Desert and

Figure 3. Specific root length (SRL) of *Ambrosia dumosa* and *Larrea tridentata* very fine and fine roots grown under field conditions in ambient and elevated CO₂ plots at the NDFF. Fine roots of both species had significantly lower SRL (see Table 8), but no other means differed within a species. Data were averaged over all age classes for roots sampled during 2001, 2002, and 2003.

Figure 4. Specific root lengths (SRL) of (a) *Ambrosia dumosa* and (b) *Larrea tridentata* fine roots in ambient and elevated CO₂ field plots at the NDFF across all sampling dates. (c) Percent soil water content at 15 cm depth across sampling dates in ambient and elevated CO₂ treatments. Although sample date significantly affected SRL, means of sample dates within a year were not significantly different from each other.

likely have the greatest contributions of roots to soil respiration. Thus, root respiration at the NDFF is unlikely the cause of increased soil or ecosystem respiration observed with elevated CO₂ (this report, Jasoni et al. 2005).

Contrary to the field results, *L. tridentata* root systems under elevated CO₂ were larger in 10-month old greenhouse seedlings (Table 7). However, the increase in root biomass after 10 months may be short-lived and restricted to the early developmental periods of seedlings. Obrist & Arnone (2003) noted that stimulation of root growth in *L. tridentata* with high CO₂ declined after 100 days until the final harvest at 178 days. Although our experiment ended after 10 months, we only harvested roots after 4 weeks and at the completion. Alterations in root production occurred sometime after initial establishment at 4-weeks, but may not have increased at a constant rate under elevated CO₂ throughout the rest of the study's duration. Greater root investments in early developmental stages with elevated CO₂ may be an attempt to help seedling establishment. Indeed, at the NDFF, early survival of *L. tridentata* seedlings was greater in the elevated CO₂ plots; however, this effect did not continue with severe drought (Housman et al. 2003).

C:N ratios were significantly greater in leaf and stem tissues of *L. tridentata* seedlings after 10-month exposure to elevated CO₂ treatment (Table 4). This effect has also been documented in other studies of this species (Billings et al. 2003, Housman et al. 2006) and is likely the result of increased carbon assimilation with elevated CO₂ (Ebbets et al. unpublished, Housman et al. 2006). In addition, lower aboveground nitrogen concentrations can be the result of decreased reliance on Rubisco or reduced transpiration rates, which, in turn, slows the bulk flow of soluble nitrate to roots for uptake (Conroy 1992). The C:N ratios of leaves may also change litter contributions to soil carbon and nitrogen pools (Weatherly et al. 2003) and consequently affect heterotrophic soil respiration.

Phosphorus availability – In greenhouse studies, root biomass and root:shoot ratios were lower in the reduced phosphorus treatment, but root respiration and SRL were unaffected by phosphorus reduction (Tables 2, 5, & 7). Phosphorus reductions also caused lower C:N ratios in roots and stems, but not in leaves (Table 4). When extrapolated to the entire root systems however, root respiration decreased indirectly with low phosphorus via a reduction in root system size. This lack of effect on individual root activity and a decrease in belowground biomass was unexpected, although similar results have been noted for *Brassica oleracea* (Singh & Blanke 2000). One possible explanation for the lack of increased root growth with reduced phosphorus is that rather than investing carbon into root structures, seedlings deprived of phosphorus may have been exuding organic acids in an attempt to overcome this limitation (e.g. Hoffland 1992). Alternatively, control phosphorus seedlings with larger root systems and root:shoot ratios may have been allocating carbon belowground to ensure establishment and to maximize uptake of nitrogen and water, which, when limiting, can overshadow phosphorus deficiencies for this species (Lajtha 1987). A third possibility is that plants cope with low phosphorus by reabsorbing phosphorus, which mature *L. tridentata* plants are quite efficient at under field conditions (Lajtha 1987), although our seedlings may have been too young to overcome phosphorus limitations with internal nutrient cycling. However, this experiment was conducted on seedlings in a controlled greenhouse setting, and any conclusions must be made considering these limitations to field applications.

We did not find any CO₂ x phosphorus interaction effects on *L. tridentata* seedlings, except for fine root carbon content, which was greater with phosphorus reduction only under ambient CO₂ (Table 4). Root respiration and system size were not affected by this interaction term. CO₂ enrichment does not

Table 7. ANOVA tables (A) and means and standard errors (B) for biomass and root:shoot ratios of 10-month old *Larrea tridentata* seedlings in ambient and elevated CO₂ treatments with two levels of phosphorus addition.

A) ANOVA tables				
Effect	Num df	Den df	P value	
Root biomass				
CO ₂	1	76	<0.001	
Phosphorus	1	76	0.018	
CO ₂ x Phosphorus	1	76	0.904	
Shoot biomass				
CO ₂	1	76	<0.001	
Phosphorus	1	76	0.839	
CO ₂ x Phosphorus	1	76	0.151	
Root:Shoot ratio				
CO ₂	1	76	0.002	
Phosphorus	1	76	0.007	
CO ₂ x Phosphorus	1	76	0.381	
B) Means and standard errors				
	Reduced P		Control P	
Ambient CO ₂	Mean	SE	Mean	SE
Root (mg)	1.65	0.27	2.08	0.23
Shoot (mg)	1.27	0.15	1.50	0.15
Root:Shoot	1.24	0.12	1.54	0.19
Elevated CO ₂				
Root (mg)	3.35	0.36	4.56	0.54
Shoot (mg)	2.27	0.20	2.01	0.15
Root:Shoot	1.47	0.10	2.47	0.35

affect PO_4^{3-} uptake of *L. tridentata* seedlings (BassiriRad et al. 1997), and our data suggest that elevated CO_2 does not help overcome additional phosphorus limitations. Furthermore, elevated CO_2 does not appear to further exaggerate belowground limitations either, and none of the plants from any treatment showed signs of extreme phosphorus deficiency.

Sample date – In the field experiment, both shrubs had reduced respiration rates at the end of the growing season in 2005 (Table 3, Figs. 2A, 2B). During these times, soil water content was much lower than at the beginning of the year (Fig 2C). In this year, we found a significant, positive relationship between soil moisture and root respiration (data not shown); however, the other years in our study had too few data points to reliably include in a regression analysis. The relationship between soil water and root activity is well documented in many systems (e.g. Burton et al. 1998); however, in the Mojave Desert, the direct effects of soil moisture on root respiration have yet to be addressed. Although we observed a decline in respiration rates of *L. tridentata* and *A. dumosa* with low soil moisture, the water availability at this site is highly confounded with temperature and phenological differences. To accurately determine the importance of soil moisture on root activity, a manipulative experiment should be implemented with varying water availability throughout the season.

Root diameter – Varying root diameters of *A. dumosa* and *L. tridentata* did not exhibit significantly different respiration rates (Table 3). The lack of an observed effect may be due to the low number of fine roots collected versus very fine roots. Finding fine roots was difficult in the root boxes, particularly in dry years and years of heavy harvesting, and to analyze CO_2 , age, and diameter effects, we compiled samples across sample dates to complete our data set. Because fine roots are typically responsible for nutrient uptake, we expected the respiration rates of smaller diameter roots to be greater than those of larger roots, and this pattern is often reported (Pregitzer et al. 1998, Desrochers et al. 2002). However, very fine and fine roots may both have been active in nutrient uptake and other high-energy processes, and we did not observe any coarse roots in the root boxes for comparison. Additionally, the classification of roots by diameter may not be the best way to categorize roots in a functional sense. When classified by branching order, clearer relationships between root form and function are reported (e.g. Pregitzer et al. 2002). Indeed, when we accounted for this variation in our 4-week old *L. tridentata* seedlings, we found a trend of greater lateral root respiration than tap roots (Table 1). Due to our field sampling technique, branching order was not always feasible to determine, particularly for fine roots, although the majority of harvested roots were of first branching order.

Root age – Root respiration rates were not significantly different between age classes for either *L. tridentata* or *A. dumosa* (Table 3). Root nutrient uptake is typically thought to decline with age (Eissenstat & Yanai 1997), and grape, apple and citrus roots have all exhibited a decline in root respiration with age (Volder et al. 2005, Bouma et al. 2001). Mojave Desert shrubs are slow growing species, but we expected to see a similar correlation after roots were approximately 30 days old (30 days was the approximate time interval between root samplings). However, we did not detect this difference. Because of root availability and collection feasibility, we defined root age as a categorical variable (young or old) rather than numerical (exact age). Defining root age as older or younger than 30 days may not have provided the proper resolution to detect root age effects, and knowing exact age may have provided more insight.

Root Structure and Function: Arbuscular Mycorrhizae

Overview – Symbiotic relationships between plant roots and arbuscular mycorrhizal fungi (AMF) are ubiquitous in terrestrial ecosystems (Smith & Read 1997). Recent reviews of the role of AMF in mediating ecosystem response to elevated CO_2 highlight a significant void in research addressing the contributions of AMF to terrestrial organic carbon cycling (Treseder & Allen 2000a). AMF have been shown to have many effects on plant physiology and plant community structure (Smith & Read 1997, van

der Heijden et al. 1998), which, in turn have indirect effects on soil carbon storage. AMF are also very important in the process of soil aggregate stabilization (Wright et al. 2000). Relatively labile forms of carbon are protected inside soil aggregates, which means AMF have another indirect influence on SOC storage (Miller & Kling 2000). However, the direct contributions of AMF-derived compounds are just beginning to be studied and have not yet been considered as a major fraction of the SOC in ecosystem models.

AMF could directly influence SOC dynamics through the growth and turnover of extraradical hyphae and by adding the stable glycoprotein, glomalin. Estimates of extraradical hyphal lengths in the field range widely (Rillig & Allen 1999). One of the highest estimates is 11 m cm^{-3} of soil for a prairie community for which a hyphal dry weight of less than 0.5 mg g^{-1} was calculated (Miller et al. 1995). Olsson et al. (1999) estimated AMF hyphal dry weight as $0.03\text{--}0.35 \text{ mg g}^{-1}$ and concluded that extraradical hyphal dry weight was the greatest soil microbial biomass component in a linseed field (around 20% of total soil microbial biomass). The turnover rate has been calculated to be very rapid (5-7 days) (Staddon et al. 2003). The turnover of extraradical hyphae is most likely governed by their diameter and cell wall thickness as well as by climatic and edaphic factors.

Extraradical hyphae have been shown to have indirect effects on SOC through stabilization of soil aggregates (Rillig & Field 2003, Rillig et al. 1999a, Rillig et al. 2001, Rillig et al. 2002). The physical dimensions of the extraradical hyphae allow them to penetrate through soil pores ($< 0.25 \text{ nm}$) such as those found between soil aggregates (Wright & Upadhyaya 1998). The mechanism by which extraradical hyphae aggregate soil has been described as a “sticky-string-bag” (Miller & Jastrow 1990) where the hyphae contribute to the entanglement of soil particles to form macroaggregate structures, the basic building blocks of soil structure (Jastrow et al. 1998).

AMF hyphae produce an iron-containing glycoproteinaceous substance called glomalin (Wright & Upadhyaya 1996), which has been shown to accumulate in soils in concentrations up to several mg cm^{-3} (Wright & Upadhyaya 1998, Rillig et al. 1999a). Glomalin pools in the soil are responsive, even in short-term measurements, to ecosystem changes such as elevated atmospheric CO_2 (Rillig & Allen 1999, Rillig et al. 2000). Whereas glomalin is linked to aggregate stability (Rillig et al. 2002), it also presents a potentially recalcitrant SOC pool (Rillig et al. 2001) for two reasons. First, the glomalin extraction process requires an unusually large input of energy compared to the other components of the SOC pool (Wright & Jansson 2001). Second, in a laboratory incubation of prairie soils, glomalin concentrations decreased by only 25% after 5 months while in the same incubation study, hyphae was decreased by 75% in that time (Steinberg & Rillig 2003).

The concentration of glomalin ranges from 2 to 15 mg g^{-1} of soil in temperate climates (Wright & Upadhyaya 1998), and over 60 mg cm^{-3} was found in a chronosequence of Hawaiian soils (Rillig et al. 2001). Glomalin is produced by extraradical hyphae of all members of AM genera, but not by other groups of soil fungi tested so far (Wright & Upadhyaya 1999, Wright et al. 2000). Glomalin is approximately 30% C by weight (Wright & Upadhyaya 1996, Wright & Upadhyaya 1999, Treseder & Allen 2000b), and has been shown to account for 3.2% of the total soil C in tropical soils (Lovelock et al. 2004), 5% of the total soil C in Hawaiian soils (Rillig et al. 2001), and has been calculated to account for up to 30% of the total organic carbon in undisturbed grasslands (Treseder & Allen 2000b). Radiocarbon dating of glomalin extracted indicates a residence time in soils of 6-42 years and is speculated to be even greater in soils where microbial activities are retarded by either low temperatures and/or low water availability. Because AMF get their entire C from photosynthate, the C found in glomalin is directly traced from atmospheric CO_2 . Our knowledge of glomalin in soils is extremely limited, and the protein has not yet been considered in ecological and biogeochemical studies, although the concentrations of glomalin in soil seem to be responsive to global change factors such as elevated CO_2 (Rillig & Allen 1999, Rillig et al. 1999a).

Few studies have examined the seasonality of AM extraradical hyphae in the field, and sampling intensity was generally very low. Kabir et al. (1998) examined the seasonal changes of intraradical and extraradical AM hyphae in agricultural soil over a growing period. Abundance of AM hyphae fluctuated significantly within a growing season, with the lowest ERH densities found in the spring. Seasonal

variation in AM root colonization followed plant development, increasing up to fruiting and decreasing thereafter. Staddon et al. (2003) found changes in extraradical hyphae over a growing season from April to November across almost an order of magnitude. Only one study has examined the seasonality of AMF extraradical hyphae and glomalin. Lutgen et al. (2003) measured the seasonal dynamics of AMF extraradical hyphae and glomalin concentration as well as soil moisture, percent root colonization, and root length in soils of intermountain grassland in western Montana over one growing season and found significant seasonal changes in total glomalin concentration (TG: 24.5% change) and AM hyphal length (107% change). From these studies, evidence exists for important seasonal patterns in AM extraradical hyphal length and for hyphal products such as glomalin.

One of the challenges to understanding and measuring the contribution of AMF to the SOC in desert ecosystems is the inherently patchy spatial distribution of resources in arid regions. Vegetation distribution creates an ‘island of fertility,’ or “resource island” that forms under shrub canopies in arid regions (Titus et al. 2002a). Moreover, host plants have a significant effect on AMF distribution by directly regulating carbon allocation to roots and by new root growth. Spatial distribution of SOC in deserts tends to be highly correlated with vegetation patterns and plant community dynamics (Breshears et al. 1998). In a study of the spatial heterogeneity of aggregate stability and soil carbon in a semiarid rangeland in New Mexico (Bird et al. 2002), the aggregate stability, organic C, and N, C: N ratios, and glomalin were all higher under plant canopy than in the interspaces.

Scenarios based on elevated CO₂ levels suggest that plants grown at elevated CO₂ will exhibit increased photosynthesis, plant biomass and plant water use efficiency. Plant growth in natural communities is mediated by the availability of soil resources, and any enhancement of plant growth should result in a concomitant increase in their demand for soil resources, further resulting in photoassimilate allocations to below ground structures (Staddon & Fitter 1998, Staddon et al. 2002). One possible outcome of predicted increase in plant growth and photoassimilate allocation response to elevated CO₂ is an increase in the reliance of plants on nutrients supplied by the mycorrhizal symbiosis (BassiriRad et al. 2001), with increased levels of mycorrhizal colonization and hyphal biomass production (Diaz et al. 1993).

Whereas increases in atmospheric CO₂ concentrations may not directly affect AMF in the soil, AMF could be affected indirectly through CO₂ effects on the host plant. AMF are likely important in the movement of C from plants to soil because AMF may: 1) use up to 20% of plant fixed C for their own growth and functioning (Jakobsen & Rosendahl 1990); and 2) improve C source-sink relationships and increase below ground C allocation through a stimulation of plant C assimilation (Wright et al. 1998). Experimental evidence indicates that hyphal P uptake is not affected by elevated CO₂, regardless of inoculum level (Gavito et al. 2002, Gamper et al. 2004). However, mycorrhizal plants have higher photosynthetic rates than nonmycorrhizal plants, and the higher photosynthetic capacity of mycorrhizal plants is maintained when plants are grown at elevated CO₂ for long periods of time (Wright et al. 1998, Gavito et al. 2002, Gamper et al. 2004). In all of these studies, the increased photosynthetic capacity has been attributed to enhanced source-sink activity in mycorrhizal plants.

Several field studies provide evidence for the persistence of stimulation of AMF root colonization and glomalin production after long-term exposure to elevated CO₂. Rillig et al. (2000) used a naturally occurring CO₂ gradient created by a CO₂ spring near grassland in New Zealand that has been active for at least several decades. The data reveals that AMF root colonization, ERH lengths, and glomalin concentration increased linearly with increased atmospheric CO₂. Because the CO₂ vent has been active for at least several decades, the findings of increased percent colonization, hyphal lengths, and glomalin concentration along the CO₂ gradient is a confirmation that previously reported short-term increases occur over longer time scales. Gamper et al. (2004) examined the effects of elevated atmospheric CO₂ on AMF colonization and spore production in two commercially grown forage plants *Lolium perenne* and *Trifolium repens*. Both plant species were grown for 7 years in permanent monoculture plots under elevated and ambient atmospheric CO₂ concentration and high and low N fertilization in the Swiss Free Air Carbon Dioxide Enrichment (FACE) facility. For both species and at both N levels, root colonization and spore production increased in response to elevated atmospheric CO₂ after 7 years of exposure.

Although a commonly observed response to elevated CO₂ is increased percent colonization and hyphal growth, reports also show no significant changes in root colonization or hyphal length (Gavito et al. 2002, Rillig et al. 2002).

In order to understand if and how elevated atmospheric CO₂ may affect AMF dynamics in Mojave Desert ecosystems, we conducted two studies. The first determined seasonal patterns of AMF in the Mojave Desert. The second focused on effects of elevated atmospheric CO₂. With extreme changes in climate and soil resource availability throughout the year, the Mojave Desert presents opportunities to examine possible shifts in allocation to these symbiotic fungi. How desert shrubs seasonally allocate resources to AMF could have large impacts on soil carbon through ERH production and the deposition of glomalin with ERH turnover. In addition, the Mojave Desert has a very heterogeneous landscape composed of shrubs with various life histories. These microsites impact nutrient availability (Titus et al. 2002a) and thus may affect shrub foraging. Indeed, in semiarid ecosystems, microsite has influenced AMF abundance (Bird et al. 2002; Rillig et al. 2003). To account for spatial variation in the Mojave Desert, we examined three important microsites: beneath *Ambrosia dumosa* (a drought deciduous shrub), beneath *Larrea tridentata* (an evergreen shrub), and within shrub interspaces.

Seasonal changes in AMF in the Mojave Desert –

Arbuscular mycorrhizal fungi (AMF) indicated a general trend of decline throughout the year (Fig. 5) (Clark et al. submitted). Although percent root colonization did not significantly vary across sample dates, ERH was greatest in the spring. Both fractions of immunoreactive glomalin related soil protein (IRSP) were significantly greater early in the year, but only beneath *L. tridentata* shrub canopies. Because soil moisture did not differ significantly among sample dates (except for a very small decline in *L. tridentata* interspace soils) (Table 8; Fig. 6), water availability is not likely to be a major factor that caused seasonal variation in AMF in 2006. In this desert ecosystem, we suspect that shrub nutrient demands drive AMF seasonality in a dry year. *A. dumosa*, a drought deciduous shrub, began to fruit during the May sampling date and had dropped its leaves by the August soil collection. Thus, dormancy likely reduced nutrient demands and the need for soil exploration. Although *L. tridentata* is an evergreen shrub, it also flowered and fruited during the May sampling date. These activities

Figure 5. Percent root colonization, extraradical hyphal length, and immunoreactive soil protein (IRSP) concentrations of *A. dumosa* and *L. tridentata* canopy and interspace soils across sample dates.

Figure 6. Soil water content and root length density of *A. dumosa* and *L. tridentata* canopy and interspace soils across sample dates.

impose large nutrient demands, and summer and fall investments in belowground foraging for *L. tridentata* were low after these events. Thus, our results may be due to a reduced need for belowground resources as the year progresses. Other

Table 8. ANOVA tables for soil water, root length, root AMF colonization, extraradical hyphae (ERH), easily extractable MAb32B11-immunoreactive soil protein (EE-IRSP), and immunoreactive soil protein (IRSP). Measurements of each factor were made over four sample dates that represented different seasons of the year for two different microsites (beneath shrub canopy, interspace) associated with each of two Mojave Desert shrub species (*Larrea tridentata*, *Ambrosia dumosa*).

Effect	Soil water			Root length intensity			Percent colonization			ERH			EE-IRSP			IRSP		
	Num	Den	p	Num	Den	p	Num	Den	p	Num	Den	p	Num	Den	p	Num	Den	p
	df	df	value	df	df	value	df	df	value	df	df	value	df	df	value	df	df	value
Species	1	2	0.675	1	2	0.844	1	2	0.512	1	2	0.909	1	2	0.007	1	2	0.002
Microsite	1	24	0.002	1	16	0.005	1	14	0.265	1	14	0.668	1	2618	<0.001	1	261	<0.001
Species x Microsite	1	24	0.378	1	16	0.464	1	14	0.821	1	14	0.202	1	261	<0.001	1	261	<0.001
Date	3	58	0.214	3	74	<0.001	3	14	0.396	3	14	<0.001	3	259	0.003	3	260	0.003
Species x Date	3	58	<0.001	3	74	0.847	3	14	0.699	3	14	0.185	3	259	0.131	3	260	<0.001
Microsite x Date	3	58	<0.001	3	88	0.534	3	14	0.935	3	14	0.376	3	260	<0.001	3	260	<0.001
Species x Microsite x Date	3	58	0.218	3	88	0.606	3	14	0.623	3	14	0.552	3	260	0.021	3	260	0.001

studies in this ecosystem observed greatest root colonization in the spring (Titus et al. 2002b) or in the fall after copious precipitation (Apple et al. 2005). Discrepancies among these studies stress the importance of including other aspects of AMF when studying these symbiotic organisms. In our study, the rate of root colonization throughout the year did not vary significantly (Fig. 5); however, by including additional measurements, we can infer that the actual length of root colonized decreased throughout the year because of the observed reduction in root length. Although our data and the two studies mentioned above (Apple et al. 2005, Titus et al. 2005b) do not show a consistent seasonal pattern in root colonization in the Mojave Desert, our results are the best indication of changes in colonized root length to date, and root length colonized appears to decline with reduced nutrient demand throughout the season.

ERH was greatest in the spring and lowest in the fall (Fig. 5), results that are also concordant with reduced demand for nutrients. Other studies also have found declines in ERH with seasonal progression and reduced plant nutrient requirements. For example, Kabir et al. (1997) observed a decrease in ERH in a corn monoculture after silking, and Querejeta et al. (2007) noted a large decline in hyphal abundance after senescence of grass in a *Bromus*-dominated grassland. However, some systems have demonstrated changes in ERH to be more related to changes in soil moisture (Staddon et al. 2003, Lutgen et al. 2003). Because soil water did not vary across sample dates in this study, we were unable to robustly test how AMF in this ecosystem respond to this factor.

Although ERH and root length were not significantly correlated (data not shown), both were lower later in the year. These shrubs and their associated interspaces do not appear to reallocate resources from one method of soil exploration to the other on different sample dates. In an Idaho fescue/bluebunch wheatgrass grassland, ERH and fine root length also had similar seasonal patterns, and the two factors in this study were positively correlated (Lutgen et al. 2003). Root length intensity and ERH lengths had similar seasonal patterns in an upland sheep pasture (Staddon et al. 2003). However, not every system exhibits similar root and ERH patterns. During drought in prairie and pasture sites, ERH length increased whereas root length remained unchanged (Miller et al. 1995). Ecosystems may have unique allocation strategies from AMF to roots (e.g. Rillig et al. 1999a, 1999b), but future studies should be conducted to determine whether these differences are due to plant or AMF species influences or abiotic factors.

The seasonal patterns of immunoreactive glomalin related soil protein (IRSP) in this study were surprising, given the recalcitrant nature of glomalin (Wright et al. 1996) and its slow turnover rates (Rillig et al. 2001, Harner et al. 2004). The sample date effect, however, was largely driven by the large decline in soil concentrations beneath *L. tridentata* canopies throughout the season (Fig. 5). The *A. dumosa* and interspace microsites examined did not have a significant seasonal change. Decomposition of ERH could be the main source of IRSP (Driver et al. 2005), but these differences in IRSP do not appear to be due to differences in substrate availability between microsites because microsite was not a significant effect on

ERH (Fig. 5). An unusually high precipitation year (240 mm versus average of 140 mm) prior to our experiment may have been responsible for initially high IRSP concentrations at the beginning of our study. If ERH abundance under *L. tridentata* canopies is indeed correlated with soil moisture (an effect we were unable to test due to insignificant difference in soil moisture, but has been observed in other systems (Lutgen et al. 2003, Staddon et al. 2003)), then decomposition of these additional hyphae before our measurements began could have resulted in the high IRSP concentrations reported for February and May.

The mechanisms driving these hypothesized dynamics are very microsite specific, as seasonal dynamics do not appear to occur under *A. dumosa* shrubs or in interspaces. The different life strategies of the two shrubs examined in this study may explain potentially greater production of ERH in the wet year of 2005 beneath *L. tridentata*. Evergreen species such as *L. tridentata* can readily take advantage of rain events occurring after the main growing season in the spring (Smith et al. 1997, Barker et al. 2006), and continuous carbon assimilation by *L. tridentata* may allow for immediate ERH production by associated AMF after rain events. While dormant and leafless, *A. dumosa*, a drought deciduous shrub, does not photosynthesize and greatly reduces nutrient demands that could be satisfied by ERH. Additionally, aboveground production of *L. tridentata* is much greater than *A. dumosa* (Housman et al. 2006), and enhanced reliance on AMF when water is available may be necessary to support this growth.

If ERH production was indeed greater beneath *L. tridentata* canopies in 2005, decomposition of these hyphae must also have been enhanced before 2006 sampling began to account for the lack of an observed microsite effect on ERH, but a significant difference in IRSP. Microbes residing within *L. tridentata* soils are carbon limited (Schaeffer et al. 2003), and decomposition of AMF hyphae and IRSP may help overcome this deficiency. However in interspace soils, microbes are also constrained by nitrogen availability (Schaeffer et al. 2003), which may limit ERH decomposition. Indeed, a chitin degradation enzyme (*N*-acetylglucosaminidase) has higher activities beneath *L. tridentata* than in interspaces (Jin & Evans 2007), and ERH are largely composed (~60%) of this structural polymer (Muzzarelli 1977). Nitrogen mineralization and microbial diversity of carbon substrate use is also greater under *L. tridentata*, especially early in the growing season (Jin & Evans 2007). These patterns in microbial activity suggest that ERH turnover in *L. tridentata* soils may be greater than within interspaces, and this effect may be most pronounced at the beginning of the year. Because IRSP is deposited with this turnover, concentrations may be greatest in February and May after possible high rates of ERH decomposition. Once in the soil, carbon limited microbes may readily attack this carbon rich (~20-30% C) glycoprotein (reviewed in Rillig & Allen 1999), causing concentrations to return to background levels by the end of the year.

Another study to examine the seasonality of IRSP found small differences in the total IRSP and the immunoreactive easily extractable IRSP (Lutgen et al. 2003). However, this study was conducted in an Idaho fescue/bluebunch wheatgrass grassland with much higher concentrations of IRSP (~125x) than the Mojave Desert. The high background levels may have prevented detection of small seasonal changes in other IRSP fractions (Lutgen et al. 2003). Our sites had very low concentrations, which possibly gave our experiment better resolution to detect seasonal changes. If put on the scale of the concentrations reported by Lutgen et al. (2003), our reported changes would likely not be significant either. We were able to detect seasonal differences in IRSP even though the rates of decline in the Mojave Desert were much lower than those reported for decomposition in soils from a Montana forest (Steinberg & Rillig 2003).

Effects of elevated CO₂ on AMF of Mojave Desert shrubs

Although shrub aboveground productivity is increased with elevated CO₂ at this site (Smith et al. 2000, Housman et al. 2006), allocation to roots (Phillips et al. 2006) and mycorrhizal biomass (Table 9, Fig. 7) (Clark et al. in prep) is not enhanced. Like studies of other host plants and ecosystems, we did not find a significant CO₂ effect on root colonization (Rillig et al. 1998, Sanders et al. 1998), ERH length (Rillig et al. 1999a, Klironomos et al. 1998), or IRSP concentrations (Rillig et al. 2001). *Ambrosia dumosa*, *Larrea tridentata*, and other Mojave Desert plants that explore interspace soils are thus likely not increasing their reliance on AMF with the additional fixed carbon under elevated CO₂. Nitrogen cycling in the soil at this site is stimulated by the elevated CO₂ treatment (Billings et al. 2004), and apparently, the increased nitrogen cycling under elevated CO₂ is sufficient to avoid further belowground limitations to shrub productivity. Differences in shrub $\delta^{15}\text{N}$ indicate microbial utilization of older substrates (Billings et al. 2002), which could be the result of stimulated microbial activity (Jin & Evans 2007). The microbial community in this Mojave Desert site is limited by carbon (Schaeffer et al. 2003). If carbon substrate has become more available with high CO₂ through increased litter inputs (Weatherly et al. 2003), root exudates, or other processes, these carbon sources could prime the microbial community. Although some nitrogen is immobilized with an increase in microbial biomass N (Jin & Evans 2007), resin available nitrogen has increased (Billings et al. 2004). When plants are exposed to high nutrient conditions, photosynthate is allocated to shoots and leaves rather than root systems and AMF (Marschner et al. 1996). Because mycorrhizae are conservatively estimated to consume 10-20% of photosynthetically fixed carbon (Jakobsen et al. 2002), further investment in this association would be uneconomical to the host if the plant's root systems can obtain a sufficient amount of nutrients.

We did not find significant differences in AMF root colonization among our microsites of interest (Table 9, Fig. 7). Titus et al. (2002b) also did not report significant differences between colonization of *A. dumosa* or *L. tridentata* roots, but Apple et al. (2005) did find greater colonization within *A. dumosa* roots than those of *L. tridentata*, although this difference was dependent on sampling date. However, as mentioned previously, percent colonization does not always represent differences in AMF soil

Table 9. ANOVA tables for root length intensity, root colonization, extraradical hyphae (ERH), easily extractable MAb32B11-immunoreactive soil protein (EE-IRSP), and immunoreactive soil protein (IRSP). Measurements were made during the ninth year of elevated CO₂ treatment at the Nevada Desert FACE Facility on two sample dates (one each in spring and fall) for three microsites (beneath *Larrea tridentata*, beneath *Ambrosia dumosa*, and in the shrub interspace).

Effect	Root length intensity			Root colonization			ERH			EE-IRSP			IRSP		
	Num	Den	P	Num	Den	P	Num	Den	P	Num	Den	P	Num	Den	P
	df	df	value	df	df	value	df	df	value	df	df	value	df	df	value
CO ₂	1	12	0.716	1	9	0.356	1	6	0.943	1	13	0.519	1	14	0.841
Date	1	108	0.003	1	9	0.016	1	6	0.218	1	43	0.659	1	42	0.405
Date x CO ₂	1	108	0.704	1	9	0.953	1	6	0.651	1	43	0.246	1	42	0.235
Microsite	2	12	0.906	2	50	0.698	2	10	0.328	2	13	0.387	2	13	0.131
CO ₂ x Microsite	2	12	0.532	2	50	0.370	2	10	0.894	2	13	0.130	2	13	0.637
Date x Microsite	2	108	0.814	2	50	0.730	2	8	0.874	2	43	0.115	2	42	0.747
Date x CO ₂ x Microsite	2	108	0.987	2	50	0.106	2	8	0.846	2	43	0.879	2	42	0.088

Figure 7. Root length intensity (A), percent root length colonized (B), extraradical hyphal (ERH) length (C), and MAb32B11-immunoreactive soil protein (IRSP) (D) means and standard errors in ambient and elevated CO₂ in the spring and fall of 2006 within three microsites (AD= *Ambrosia dumosa*, IS= shrub interspaces, and LT= *Larrea tridentata*). Neither the CO₂ main effect nor any CO₂ interaction terms were significant in the ANOVAs (Table 9).

exploration. Similar ERH and IRSP abundance beneath shrub canopies and within interspaces is surprising because of the heterogeneity of soil nutrients in this ecosystem. Nitrogen and phosphorus concentrations are significantly greater under shrubs (Titus et al. 2002a), but ERH did not explore these nutrient rich soils to a greater extent. However, neither AMF spore densities nor the mycorrhizal inoculum potentials of the three microsites that we examined differ significantly from one another (Titus et al. 2002a, 2002b). Root systems may be able to obtain sufficient amounts of nutrients beneath the canopy without further mycorrhizal exploration. Lower microbial presence within interspaces (Jin & Evans 2007) may reduce nutrient competition, and AMF may consequently exploit these areas to an equal extent as shrub microsites.

Our study was the first to examine Mojave Desert microsite effects on ERH or IRSP, and studies within other ecosystems are limited. In a semi-arid Mediterranean steppe, hyphal length was not greater beneath *Stipa tenacissima* than bare interspaces; however, ERH were not distinguished from saprophytic hyphae (Rillig et al. 2003). Interspaces in this steppe did have less IRSP (Rillig et al. 2003), and a similar pattern has been documented in the Chihuahuan Desert (Bird et al. 2002). Different ecosystems may have varying microsite influences on AMF, and these effects need to be explored further in other systems to better account for landscape level estimates of AMF presence as well as soil changes with plant community shifts.

We did observe a sampling date effect for percent root colonization ($p = 0.016$) with greater colonization in the spring sampling date. A similar pattern was observed in 1999 (a very dry year) at a nearby site for vesicle colonization, but the hyphal and arbuscular colonization was unchanged between spring and fall sampling dates (Titus 2002b). In 2001-2002, a study conducted 100 m away from the NDDF found root colonization increased throughout the spring with increased root growth, decreased with summer drought, and reached its highest levels in the fall following rain events (Apple et al. 2005). Studies in other ecosystems have also observed changes in root colonization over the growing season with precipitation, although the direction of responses is not consistent (Staddon et al. 2003, Lutgen et al. 2003). Our seasonal changes in colonization were also likely due to precipitation patterns with the majority of rain in the spring and very little precipitation in the fall. Soil moisture values were also greater in the spring sampling date (data not shown).

Although root standing crop was greater in the spring sampling date, we did not observe a sampling date effect on ERH in this mixed-shrub community (Fig. 7). Soil moisture was also greater in the spring, which likely influenced root production (Phillips et al. 2006). Despite drier conditions in the fall, ERH length did not decline as did root length. Perhaps when water is abundant, shrubs increase allocation to root growth. However, when dry conditions make investments in root production and maintenance costly, AMF appear to play a greater role in nutrient acquisition. Rather than producing or maintaining roots, plants in the Mojave Desert may benefit more from AMF when soils are dry. A similar pattern was observed in both a prairie and a pasture system during an unusual drought event (Miller et al. 1995).

The lack of a sampling date effect on IRSP concentrations (Fig. 7) is not surprising because of IRSP's recalcitrant nature. The only seasonal study examining IRSP to date observed only minimal seasonal changes in the Bradford assay-quantified total IRSP and the ELISA-quantified easily extractable fraction of IRSP in an Idaho fescue/bluebunch wheatgrass community (Lutgen et al. 2003). However, little research has been conducted on seasonal patterns, and conclusions are difficult to make with only two sampling points in this study.

Root Growth

Twenty-eight minirhizotron tubes were installed in 1997 in each ambient and elevated CO₂ plot to a depth of 1 m (Phillips et al. 2000). Tubes were installed under *Larrea* and *Ambrosia* as well as systematically along transects to sample the plant community. Root images were collected every 4 weeks from December 1997 to January 2001 and from January 2003 to the present. Using excavated tubes, we verified that fine root biomass density adjacent to tubes did not significantly differ from that between 5.4 and 54.0 cm away (Johnson et al. 2001).

Although elevated CO₂ increased root growth of *Larrea* in a controlled environment (Obrist & Arnone 2003), repeated measures ANOVAs did not show significant main effects of CO₂ for fine root length standing crop, production, and root loss under *Ambrosia*, *Larrea*, or the plant community (transect tubes) (Phillips et al. 2006, Ferguson et al. in prep, Figs. 8-10). (Note: We use the term “root loss” rather than “mortality” because we know when a root disappeared from the minirhizotron image, but we do not know when it became physiologically inactive; nonetheless, root loss is the appropriate data for tracking C balance.) Thus, overall sampling dates, elevated CO₂ did not affect fine root length production, loss, or standing crop. However, CO₂ x time interactions were significant for *Ambrosia* and for the community transects. For example, fine root length standing crop of *Ambrosia* under elevated CO₂ was significantly greater than that under ambient CO₂ during November 2004 – March 2005 (Fig 8). Although some of this difference in standing crop is due to significantly greater root production under elevated CO₂ during February–March 2005, the significantly lower rates of root loss under elevated CO₂ during October–December 2004 as well as during earlier time periods are also important. Surprisingly, this tendency for lower root loss under elevated CO₂ did a complete turn-about during March–August 2005, during which time standing crop for the two CO₂ treatments converged. We suspect that this rapid change in root loss under elevated CO₂ was driven by the very wet fall and spring of 2005, *i.e.* the presence of abundant soil water during the warm spring and summer months provided ideal conditions for decomposition. Statistical results for community transect minirhizotron measurements (Fig. 10) were similar to those for *Ambrosia*. Although the CO₂ x time interaction term was not significant for *Larrea* (Fig. 9), the general patterns observed for *Ambrosia* also occurred for *Larrea*.

A striking aspect of the minirhizotron data is the gradual accumulation of fine roots through time for both CO₂ treatments through March 2005 (Figs. 8-10). This increase is most apparent between January

Figure 8. Standing crop and cumulative precipitation (top), production (middle), and loss (bottom) of roots from minirhizotron images of *Ambrosia dumosa* plants under elevated (filled circles, solid lines) and ambient (open circles, dashed lines) CO₂ during Jan 1998 – Jan 2000 (left) and Jan 2003 – May 2007 (right) at the NDDF.

Figure 9. Standing crop and cumulative precipitation (top), production (middle), and loss (bottom) of roots from minirhizotron images of *Larrea tridentata* plants under elevated (filled circles, solid lines) and ambient (open circles, dashed lines) CO₂ during Jan 1998 – Jan 2000 (left) and Jan 2003 – May 2007 (right) at the NDDF.

2000, when the first set of minirhizotron image collections ended, and September 2003, approximately 9 months after when they were resumed. The accumulation of roots during 2003 was due largely to the very low (near zero) rate of root loss during 2003.

For all minirhizotron locations, root standing crop was relatively constant during 2004 with very little root production or loss until a series of rain storms occurred in fall 2004 through summer 2005. Root loss increased significantly more for ambient CO₂ during fall 2004, resulting in a bigger dip in root standing crop under ambient CO₂. Root production spiked during the winter and spring 2005, with greater production initially under elevated CO₂. However, root production under ambient CO₂ eventually matched or exceeded that of elevated CO₂ as the 2005 growing season progressed. Root loss also increased during summer and fall 2005, with loss under elevated CO₂ greater (and significantly greater on some dates) than ambient CO₂. Root production was low during the 2006 and 2007 growing seasons, but a low but sustained rate of root loss over this time period resulted in gradual declines in root standing crop to levels near those in January 2003.

Figure 10. Standing crop and cumulative precipitation (top), production (middle), and loss (bottom) of roots from minirhizotron images of community transect tubes placed systematically in plots with elevated (filled circles, solid lines) and ambient (open circles, dashed lines) CO₂ during Jan 1998 – Jan 2000 (left) and Jan 2003 – May 2007 (right) at the NDFF.

Soil Respiration

Our study of the CO₂-efflux characteristics of soil under elevated and ambient CO₂ environments suggests that soil respiration (R_s) is significantly higher in elevated CO₂ plots, and that this elevated efflux is persistent over time and location (DeSoyza et al. in prep). Furthermore, we show that the rate of R_s is limited by soil moisture and that in the presence of moisture, R_s increases temporarily, especially under elevated CO₂. Monthly mean data from elevated and ambient CO₂ plots showed a high degree of variability (Fig. 11), not surprising since these means incorporate diurnal fluctuations in R_s and variations due to the location of the measurement chambers under different canopy types. Overall however, a general pattern emerged of higher R_s during the cooler months decreasing toward the height of summer. While this may be a temperature response, it appears to be substantially affected by the general pattern of higher rainfall during the winter months. That rainfall has strong positive effects on R_s was clearly demonstrated by comparing cool and warm months with and without significant amounts of rainfall (Fig. 12). In the absence of rainfall, R_s rates were low and no discernible differences were seen in the R_s rates between elevated and ambient CO₂ plots during warm or cool months. However, during months with substantial rainfall, rates of R_s in both ambient and elevated CO₂ plots were significantly higher. This suggests that while air temperature may limit R_s to a small degree, the key limiting factor is the availability of moisture in the soil. Strikingly, the R_s of elevated plots seemed to respond more to the additional moisture than did the ambient plots. When moisture was added to the system we detected an immediate and large increase in R_s from all plot locations. This surge was of a high enough magnitude that CO₂ values far exceeded the ability of our system to accurately measure it. Given the rapidity and magnitude of this surge in R_s , we suggest that it is due to a combination of increased R_s and a physical displacement of CO₂ from soil pores by the water entering the soil. Note that this physical displacement

Figure 11. Mean monthly rates of soil respiration for soil in elevated (solid circles) and ambient CO_2 plots (open circles). Error bars represent the standard deviation of the mean.

effect of CO_2 stored in the soil is reversed as the soil dries and air refills the soil pores, and thus the net effect of emptying then re-filling soil pores with CO_2 on R_s is zero over time.

Given that during a particular month all plots received approximately the same amount of rainfall, the greater R_s in elevated CO_2 plots suggests that a larger pool of carbon exists in the soil in elevated plots, or that the population or quantity of microorganisms in the soil of elevated CO_2 plots is different, or both. A complementary explanation suggests that desert plants produce rapid root growth during periods of high soil moisture, resulting in increased respiration and thereby increased R_s from the soil. Although *L. tridentata* and other species growing under elevated CO_2 exhibited approximately double the growth of plants growing under ambient CO_2 conditions during a period of drought alleviation due to a pronounced El Nino cycle (Smith et al., 2000, Housman et al 2006), no concomitant increase in new root production occurred during this same period for evergreen or deciduous shrubs (Phillips et al., 2006). Thus, although C inputs to the soil from canopy litter fall may have increased, root growth or turnover may not be a significant factor affecting R_s . Furthermore, root respiration rates did not differ between plants grown under ambient or elevated CO_2 (this report).

Soil at the three canopy locations also had somewhat different soil R_s characteristics (Fig. 13). In general, soil under the canopy of the evergreen shrub *L. tridentata* in elevated CO_2 plots supported the highest rates of R_s . In the presence of added water, soil under *A. dumosa* and soil with no canopy generated the

Figure 12. Monthly mean composite of diurnal soil respiration for elevated (solid circles) and ambient (open circles) CO_2 plots. June 2003 and December 2004 were dry periods while July 2004 and February 2005 were relatively wet.

Figure 13. Mean monthly soil respiration rates for the three soil locations (under *L. tridentata*, under *A. dumosa*, and under no canopy) are shown for the two-year period.

highest rates of R_s , and those rates were significantly greater than those found under *L. tridentata* (Fig. 14). We suspect that *L. tridentata*, with its thicker canopy cover and greater quantities of organic litter under its canopy, litter which also tends to be somewhat hydrophobic, reduces water infiltration into the soil more effectively than does the more sparse canopy and litter accumulation under *A. dumosa*. Thus the greater increase in R_s found under *A. dumosa* and non-canopy areas immediately after rain are probably a response to the greater quantities of water entering the system in these areas. Conversely, after an initial period of high R_s , in the absence of continued rain, R_s rates quickly return to normal levels with soil under *L. tridentata* being marginally higher. We suggest that this could be due to a slower rate of water loss under the protected canopy and litter layers of *L. tridentata* together with water redistributed from stem-flow water stored in the soil.

Mounting evidence suggests that as atmospheric CO_2 levels continue to rise, many plant species will show increased photosynthetic carbon assimilation thereby sequestering C and potentially muting the adverse effects of increased atmospheric CO_2 . However, the effects of increased photosynthesis will only be truly beneficial if the assimilated carbon is sequestered in the long-term and removed from the pool of substrate available for metabolic breakdown which returns carbon to the atmosphere. *In situ* studies have shown that soil respiration rates in desert ecosystems may range from very low (e.g. $0.10 \text{ g C m}^{-1} \text{ d}^{-1}$ in the McMurdo Dry Valleys of Antarctica; Burkins et al., 2001) to rates that compare favorably with temperate forests and grasslands ($1.09 - 4.64 \text{ g C m}^{-1} \text{ d}^{-1}$ under *L. tridentata* in the Chihuahuan Desert; Parker et al., 1983). While our R_s values are slight underestimates due to the exclusion/unavailability of data from during and immediately after rain, the annual values for ambient ($56.6 \text{ g C m}^{-2} \text{ yr}^{-1}$) and elevated ($80.3 \text{ g C m}^{-2} \text{ yr}^{-1}$) environments is substantially lower than the $224 \text{ g C m}^{-2} \text{ yr}^{-1}$ estimated by Raich and Schlessinger, 1992 and Raich and Schlessinger, 2001.

Most studies of desert soil respiration have used a portable system where diurnal measurements if taken are done at relatively distant time intervals. While these measurements have yielded valuable information on the R_s of a various soil environments they often do not present a comprehensive picture of the patterns and responses to a variable environment. An *in situ* soil respiration system with static chambers such as those used in this study together with the potential temporal fluctuations of atmospheric CO_2 levels in the FACE plots presents a unique set of problems that needed to be addressed in order to generate valid data. Our experiment with the isolated chamber demonstrated clearly that the system, including the chambers, did not generate an artifactual R_s measurement (Fig. 15). In the absence of a source or sink for CO_2 our system generated a stable zero baseline thereby confirming that the system works.

Figure 14. Diurnal soil respiration response to precipitation for the three soil locations (under *L. tridentata*, under *A. dumosa*, and under no canopy) are shown for 15 July 2004, prior to precipitation (black lines) and 17 July 2004 after precipitation (red lines).

Figure 15. Soil respiration rate as measured with the soil respiration chamber isolated from the soil.

Although the FACE system at the NDFF maintained elevated CO₂ for over 98% of the time, it did shut down under specific conditions – namely when wind speeds exceeded a certain threshold and when air temperatures dropped below a certain threshold. Our measurements showed that temporary suspension of CO₂ fumigation increased R_s rates of elevated plots for short periods of times (~3 hours) (Fig. 15). But when CO₂ fumigation resumed, R_s of elevated plots was decreased for a similar period of time, essentially evening out the initial transitory increase. The initial increase was probably due to the temporarily lowered partial pressure of CO₂ in the air causing CO₂ to be extruded from soil pores; and the subsequent decrease following resumption of FACE fumigation was due to the increase in the partial pressure of CO₂, causing a reversal of the earlier extrusion. Comparison of R_s data from days with partial CO₂ control (when the system shutdown for part of the day) and days with full CO₂ control showed no difference between these days, and therefore days with partial control were not culled from the data used in this report.

While some questions, such as the sources of R_s remain to be answered, our study shows that in the northern Mojave Desert, R_s is both spatially and temporally variable, and that at the ecosystem level, R_s was enhanced under conditions of elevated CO₂. At the ecosystem level, the 42% increase in mean annual R_s found in our study of a desert ecosystem suggests that a significant return of assimilated C occurs back to the atmosphere, potentially reducing, if not negating, increases in C sequestration due to elevated atmospheric CO₂.

Figure 15. Effects of fluctuations in CO₂ elevation. (A) Air temperature dropped below 3 C on 24 March (dotted line) but not on 25 March (solid line). (B) CO₂ elevation was shut down during period when air temperature dropped below 3 C (dotted line). (C) There were no systematic changes to CO₂ concentrations in non-elevated plots during period of system shut-down. (D) Soil CO₂ efflux from the elevated CO₂ plots at first increased greatly during the first 3 hours of system shutdown then reduced substantially when CO₂ elevation initially resumed on 24 March (dotted line) compared to the same times on 25 March (solid line). (E) System shut-down did not elicit an increase in CO₂ efflux in the non-elevated plots during system shut-down.

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