RESPONSE OF MEDITERRANEAN-TYPE ECOSYSTEMS TO ELEVATED ATMOSPHERIC CO_2 AND ASSOCIATED CLIMATE CHANGE

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ABSTRACT

This research incorporated an integrated hierarchical approach in space, time, and levels of biological/ecological organization to help understand and predict ecosystem response to elevated CO_2 and concomitant environmental change. The research utilized a number of different approaches, and collaboration of both PER and non-PER investigators to arrive at a comprehensive, integrative understanding.

Central to the work were the CO_2-controlled, ambient Lit, Temperature controlled (CO_2LT) null-balance chambers originally developed in the arctic tundra, which were re-engineered for the chaparral with treatment CO_2 concentrations of from 250 to 750 ppm CO_2 in 100 ppm increments, replicated twice to allow for a regression analysis. Each chamber was 2 meters on a side and 2 meters tall, which were installed over an individual shrub reprouting after a fire. This manipulation allowed study of the response of native chaparral to varying levels of CO_2, while regenerating from an experimental burn. Results from these highly-controlled manipulations were compared against Free Air CO_2 Enrichment (FACE) manipulations, in an area adjacent to the CO_2LT null balance greenhouses. These relatively short-term results (5-7 years) were compared to long-term results from Mediterranean-type ecosystems (MTEs) surrounding natural CO_2 springs in northern Italy, near Laiatico, Italy. The springs lack the controlled experimental

rigor of our CO_2LT and FACE manipulation, but provide invaluable validation of our long-term predictions.

Arid and semi-arid ecosystems, worldwide cover about 50% of the terrestrial surface, about 25% of the carbon in biomass and soil carbon, and 16% of the NPP. The chaparral is felt to be a good model ecosystem for understanding and predicting the response of arid/semi-arid perennial, woody ecosystems to variations in CO_2 and moisture availability. Because of the interest in the range of interactions and feedbacks likely to act in nature, a field approach was felt to be critical. The California chaparral, a Mediterranean-type ecosystem, is a warm, semi-arid, nutrient-adequate ecosystem which we predicted to be particularly responsive to changes in CO_2 and moisture availability. The presence of lignotubers was hypothesized to provide a strong sink for photosynthate. A range of responses and interactions to elevated CO_2 are possible including those which would limit nutrient availability, as well as those which would increase it. While water use efficiency was predicted to increase under elevated CO_2, compensatory responses via nutrient limitation, or an increase in leaf area per unit ground area, could potentially constrain and or prevent any ecosystem level increases in water yield. While not specifically addressed, the integrated responses studied will help in predicting potential responses of chaparral under a warmer, CO_2 enriched environment and any feedbacks on changes in the fire frequency. A change in the fire frequency, due to

an increase in biomass, could in and of itself, dramatically alter the water yield of the system, as could any large scale change in the community structure.

In the CO_2 LT chambers the dominant plant species, *Adenostoma fasciculatum*, in the 750 ppm CO_2 chamber showed increased leaf-level photosynthesis, and decreased transpiration and stomatal conductance relative to controls at ambient CO_2. Measurements of above ground growth and leaf area index (LAI) showed no increase over the first three years of the study, though GPP increased 56% over this time, suggesting most of the response was below ground. Net ecosystem exchange (smaller source or larger sink) increased under increasing CO_2 treatment through time with effects being less during wet years. In the last 2 years of treatment, a 200% increase in sink strength was observed in the plants treated with 750 ppm CO_2 compared to those at ambient. The plants grown at this high, CO_2 concentration showed a 150% increase in aboveground biomass.

A. fasciculatum in the FACE ring (550 ppm), exhibited typical long-term acclimation responses to increased CO_2 during the spring relative to plants growing in the control ring (ambient). Photosynthetic capacity and Rubisco activity were reduced on a leaf area basis, but leaf area increased. In the summer, plants showed a response more typical of short-term exposure to increased CO_2, with 97% higher photosynthesis rates than controls. There were no differences in photosynthesis between treatments during the winter. These leaf level measurements were similar to those made on the plants in the null balance treatment.

There is evidence that both litter quality and quantity has changed in the FACE treatment. In the spring, total non-structural carbohydrates (TNC) were 32% higher in leaves of FACE plants. Biogenic hydrocarbon emission, measured in the null balance chambers has also increased, suggesting increased C allocation to plant secondary compounds. Root production and rhizodeposition of C has increased substantially, based on ^{13}C isotope data from the chamber experiment.

In the null balance chambers, soil respiration rates measured *in situ* with a Li-Cor 6200 soil chamber were significantly lower in the 750 ppm CO_2 treatments. This contrasts with the increased soil respiration measured in the FACE treatment using a Clapp cuvette (122.5cm×122.5cm×82.0cm). While this discrepancy could reflect many differences in methodology and experimental conditions between the FACE site and the null balance chambers, the negative effect of CO_2 on respiration found in the chambers could be related to the smaller spatial scale of the respiration measurements, indicating that when root respiration is excluded, microbial respiration is actually lower in the high CO_2 treatments.

Two oaks (*Quercus ilex* and *Q. pubescens*) growing under elevated CO_2 from natural springs in Italy were investigated for evidence of any down regulation of photosynthesis. Current year leaves showed no evidence of photosynthetic downregulation early in the growing season. In the case of *Q. ilex*, where older leaves were measured, down regulation was observed. The long term effect of elevated CO_2 on

both plants was an increase in LAI and while WUE was higher in the control areas, improved water relations of the elevated CO_2 growing trees was not observed.

Brief Summary and list of publications, highlights and presentations resulting from this award

In a Mediterranean system exposed to natural sources of CO_2, *Quercus pubescens* response to elevated CO_2 was that of increased photosynthesis based on measurements made in the spring when sink strength was the greatest (Stylinski *et al*., 2000). While this may result in increased biomass, other studies do not conclusively show that these higher photosynthetic rates in the spring will result in larger steady-state carbon pools. The possibility of down regulation during other times of the years, differential response of older leaves, and the response of belowground processes preclude a definitive statement on the response of the ecosystem to elevated CO_2.

Growth chamber data from chaparral species did not show marked increases in carbon uptake under elevated CO_2, in part due to down regulation (Oechel *et al*., 1995).

Elevated CO_2 experiments in the field on chaparral plants suggest greater carbon sequestration, in part due to changes in soil structure. The greater carbon sequestration increased with years of treatment. Possibly of greater importance is that the change in soil structure may result in the stabilization of soil and help to mitigate soil degradation and erosion that might be accelerated by change in climate and land use and result in loss of carbon from the system (Rillig *et al*., 1999).

Baldocchi D., Valentini R., Running S., Oechel W., and Dahlman R. 1996. Strategies for measuring and modeling carbon dioxide and water vapor fluxes over terrestrial ecosystems. Global Change Biology 2: 101-110.

This paper highlights the need, and a plan for measuring and modeling carbon and water flux of terrestrial ecosystems. While the effects of elevated CO_2 on carbon flux are not specifically addressed, under a changing climate scenario, a change in the photosynthesis of plant under elevated CO_2 could potentially change any conclusions drawn from "present day estimates" of flux.

Harney, S.L., Edwards F., and Allen M.F. 1997. Identification of arbuscular mycorrhizal fungi from *Artemisia californica* using the polymerase chain reaction. Mycologia 89: 547-550.

Ecological field studies focusing on arbuscular mycorrhizal fungi are hampered by the in ability to make accurate identification of species and hence changes in community composition. A method was developed using polymerase chain reaction to identify AM fungi.

Klironomos J.N., Rillig M.C., and Allen M.F. 1996. Below-ground microbial and microfaunal responses to *Artemisia tridentate* grown under elevated atmospheric CO_2. Functional Ecology 10: 527-534.

Elevated CO_2 has a qualitatively different effect on below-ground microbial and microfaunal communities depending upon whether or not additional nutrients were added. When nutrients were not added, carbon flow was shifted to a more mutualistic-closed mycorrhizal-dominated system while CO_2 and nutrients resulted in a more opportunistic-open, saprobe/pathogen dominated one.

Klironomos J.N., Rillig M.C., and Allen M.F. 1999. Designing belowground field experiments with the help of semi-variance and power analyses. Applied Soil Ecology 12: 227-238.

Low sample size and the cryptic nature of the soil often results in low statistical power and high type II error rates. Using an elevated CO_2 treatment, a geostatistical analyses was used to describe the spatial distribution of organisms and processes coupled with a power analysis to determine the required sample size. These a priori efforts helped determine which organisms are suitable for student under the scales of interest.

Moreno JM, and Oechel WC (eds.). 1995. Global Change and Mediterranean-Type Ecosystems. 117. Springer Verlag, New York, 527 pp.

Regions with Mediterranean-type climates include parts of California, South America, Australia, and of course, Europe. The effect of global climate change on these heavily populated areas will have major social and political ramifications. This volume addresses issues in these areas, from processes at the leaf level to the individual, ecosystem, and landscape levels.

Moreno J.M., Cruz A., and Oechel W.C. 1999. Allometric relationships in two lignotuberous species from Mediterranean-type climate areas of Spain and California. Journal of Mediterranean Ecology 1: 49-60.

This study contrasts the dominant lignotuber producing plants in the Mediterranean regions of central Spain and southern California. Plants originating from southern California developed greater lignotuber biomass and penetrated deeper into the soil than those from central Spain. It is believed that the less favorable growth conditions (less precipitation) as well as the clear role of fire in southern California to a large degree explain the results. These data provide a foundation upon which to assess potential belowground carbon storage differences between the two regions.

Oechel W.C., Hastings S.J., Vourlitis G.L., Jenkins M.A., and Hinkson C.L. 1995. Direct Effects of CO_2 in Chaparral and Mediterranean-Type Ecosystems. *In*: Moreno J.M., and Oechel W.C. (eds.). Global Change and Mediterranean-Type Ecosystems. Ecological Studies 117. Springer, New York, NY, pp. 58-75.

For the three, major, southern California chaparral species studied, photosynthetic acclimation to elevated CO_2 was observed, possibly due to low nutrient availability and the lack of available sinks for the excess carbohydrate fixed. Asymbiotic nitrogen-fixing bacteria may have alleviated nitrogen stress in coast live oak, prolonging the initial stimulatory effects of elevated CO_2 on photosynthesis early on. While an increase in WUE was observed under elevated CO_2, transpiration per unit of leaf area either remained unchanged or increased. In two of the species studied, increases in leaf area under elevated CO_2 resulted in greater whole plant water use and put into question any benefits in water yield from chaparral water sheds under elevated CO_2.

Reece C.F., Krupa S.V., Jager H.-J., Roberts S.W., Hastings S.J., and Oechel W.C. 1995. Evaluating the effects of elevated levels of atmospheric trace gases on herbs and shrubs: a prototype dual array field exposures system. Environmental Pollution 90:25-31.

A Free air CO_2 enrichment (FACE) system was developed as an economical alternative to the available technology to researchers. Highlighting the design was a dual array system with the outer array intended to reduced CO_2 use, a major cost in

all FACE experiments. Good control and spatial homogeneity of the desired trace gas (CO_2) was achieved with the anticipation that the out array which vented ambient air as an air curtain would reduce CO_2 usage.

Rillig M.C., and Allen M.F. 1999. What is the role of arbuscular mycorrhizal fungi in plants to ecosystem responses to elevated atmospheric CO_2? Mycorrhiza 9: 1-8.

This paper highlights the importance of taking a holistic, process orientated research approach when looking at belowground responses to elevated CO_2. A specific plant species-fungus interaction may or may not be modified by CO_2 treatment. The point is made that a hierarchical approach with emphasis on changes in processes should be the bottom line when evaluating the potential role of mycorrhizal fungi in evaluating ecosystems responses to elevated CO_2 and not simply species specific observations.

Rillig M.C., Wright S.F., Allen M.F., and Field C.B. 1999. Rise in carbon dioxide changes soil structure. Nature 400: 628.

A number of field studies of the effect of elevated CO_2 have resulted in an increase in soil aggregation, specifically, an increase in glomalin concentrations. This observation is important in it represents an increase in carbon sequestration by the ecosystem. However, the importance of this observation goes beyond absolute amounts of carbon sequestered as an additional effect may be a decline in soil degradation and erosion under elevated CO_2 such that current soil carbon stocks are stabilized.

Rillig M. C., Treseder K.K, and Allen M.F. 2001. Global change and mycorrhizal fungi. *In*: Mycorrhizal Ecology, van der Heijden M., and Sanders I. (eds), Ecological Studies Series, Springer Verlag.

Roberts S., Oechel W.C., Hastings S.J, and Bryant P. 1998. A field fumigation system for elevated carbon dioxide exposure in chaparral vegetation. Functional Ecology 12: 708-719.

Further development of a Free Air CO_2 enrichment system was accomplished focusing on a reliable, inexpensive CO_2 delivery system and straightforward algorithms that require little tuning or adjustment by an operator. The performance of the system was on par with other systems and spatially homogenous within a 11 meter diameter portion of a 16-meter ring. Differences in photosynthesis and water stress of *Adenostoma fasciculatum* was found within 6 weeks of treatment.

Stylinski, C.D., Oechel W.C., Gamon J.A.,Tissue D.T., Miglietta F., and Raschi A. 2000. Effects of lifelong CO_2 enrichment on carboxylation and light utilization of

Quercus pubescens Willd. examined with gas exchange, biochemistry and optical techniques. Plant Cell Environment 23:1353-1362.

After lifelong exposure to elevated atmospheric CO_2 from a natural spring, *Quercus pubescens* was not found to down regulate its rate of net photosynthesis. Maximum assimilation at saturating CO_2, electron transport capacity, and Rubisco content, activity and carboxylation capacity were identical in CO_2 exposed and control plants. As these measurements were made early in the growing season when sink strength was likely the greatest, extrapolation to greater biomass at the per meter ground area basis is not necessarily valid.

Treseder K. K. and Allen M.F. 2000. Black boxes and missing sinks: Fungi in global change research. Mycological Research 104:1281-1283.

The point is made that to often, focus on ecosystem response to elevated CO_2 is made on the plant, either ignoring below ground processes, or treating them as a black box. It is pointed out that recent advances in genetic analyses and stable isotope techniques can be combined with traditional approaches to identify the fungal groups and their role in ecosystem function. With mounting evidence that soils may forma sink for C under elevated CO_2 to help mitigate global warming undermines the importance of integrating research of mycologists and ecosystem ecologists.

Treseder K. K., and Allen M.F. 2000. Mycorrhizal fungi have a potential role in soil carbon storage under elevated CO_2 and nitrogen deposition. New Phytologist 147: 189-200.

A significant fraction of soil organic matter and below-ground biomass is comprised of mycorrhizal tissue. Current literature indicates that ecosystems exposed to elevated CO_2 result in increased carbon sequestration by mycorrhizal fungi, potentially serving as a negative feeback on the rise in atmospheric CO_2 levels. Preliminary studies indicated that N deposition might increase turnover rates of fungal tissue and diminish or eliminate the sequestration potential of mycrorrhizal hypae. Studies on potential changes in mycorrhizal community composition under elevated CO_2 and increased N are critical if we are to have a predicative capability with respect to ecosystems response to increases in CO_2 and nitrogen.

In manuscript:

Baraldi R., Oechel W.C., Hastings S.J., Bryant B., and Miglietta F. Recent growth in atmospheric CO_2 is sufficient to increase biogenic volatile hydrocarbon (BVOCs)

emissions in Mediterranean-type ecosystems and provide a pathway for negative feedback on regional warming. (*in manuscript*).

Cheng Y., Oechel W.C., Hastings S.J., Bryant P., and Major J. The effect of Elevated CO_2 on carbon flux of Southern California chaparral by using Free Air CO_2 Enrichment. (*in manuscript*).

Cheng Y, Oechel W.C., Hastings S.J., Major J., and Bryant P. Ecosystem carbon flux under elevated CO_2 by CO_2 LT Chambers in southern California chaparral. (*in manuscript*).

Harazono Y., Vourlitis G.L., Roberts S.W., Hastings S.J, and Oechel W. C. Eddy covariance measurements of net CO_2 flux and energy balance of chaparral ecosystems across a fire-induced age gradient. (*in manuscript*).

Harazono, Y., Hastings S.J., Vourlitis G.L., and Oechel W.C. Differences of physical and physiological characteristics of chaparral vegetation of a canopy level within different ages. (*in manuscript).*

Hinkson C., Oechel W.C., Roberts S., Miglietta F., and Raschi A. Photosynthesis and water-use of a Mediterranean oak after long-term CO_2 enrichment by a natural CO_2 spring. (*in manuscript*).

Ibanez I., Oechel W.C., Hastings S.J., and Tissue D. Patterns of and controls on photosynthetic acclimation to long term exposure to elevated CO_2 in a semi-arid Mediterranean-type shrubland ecosystem. (*in manuscript*).

Langsford D.H. and Oechel W.C. Diurnal and seasonal water potential patterns among and within *Ceanothus greggii* shrubs in southern California chaparral. (*in manuscript*).

Oechel W.C., and Jenkins M.A. 1995. Interactions of pre-industrial and predicted future atmospheric CO_2 concentrations and drought on growth and photosynthesis of chaparral shrubs. (*in manuscript).*

Treseder K.K., Cheng Y., Allen M.F., and Oechel, W.C. ^{13}C tracers of carbon allocation to roots under a CO_2 gradient in intact southern Californian chaparral. (*in manuscript*).

Presentations:

Allen M.F., Karen O., Klironomos J., Rillig M., and Harney S. Fungal responses to elevated CO_2 in Mediterranean-type ecosystems. Ecological Society of America 84th Annual Meeting, August 8-12, 1999.

Anderson A.E., and William, K.S. Effects of elevated atmospheric CO_2 on a southern California chaparral arthropod community. ESA 1997 annual meeting, 10-14 August, Albuquerque, New Mexico. Suppl. Bull. Ecol. Soc. of America. 78(3): 127.

Anderson A.E., and K.S. Williams. Effects of elevated CO_2 on southern California chaparral arthropod populations: Initial results. MEDECOS VIII, October 18-26, 1997, San Diego, California. p 14.

Anderson A.E., and Williams K.S. The effect of atmosphric CO_2 concentrations on chaparral arthropod populations. Ecological Society of America 84th Annual Meeting, August 8-12, 1999.

Baraldi R., Rapparini F., Miglietta F., Oechel W.C., Hastings S.J. and Cheng Y. Effects of elevated CO_2 concentrations on terpenoid emission from chaparral ecosystems. An International Workshop, Montpellier, France, 22-23 March 2001.

Cheng Y., Oechel W.C., Hastings S.J., Major J., Bryant P., and Qian H. Ecosystem carbon flux under elevated CO_2 by FACE technique in southern California chaparral. Ecological Society of America 86th Annual Meeting, August 6-10, 2001.

Cheng Y, Oechel W.C., Hastings S.J., Major J., and Bryant P. Ecosystem carbon flux under elevated CO_2 treatment by FACE and Null Balance chamber in Southern California Chaparral. Ecological Society of America 85th Annual Meeting, August 6-10, 2000.

Cheng Y., Oechel W.C., Hastings S.J., Major J., and Bryant P. Plot-scaled Carbon and Water Vapor Flux measuring by CO_2 LT Chambers in Southern California Chaparral. Southern California Academy of Sciences Annual Meeting May 4 - 5, 2001.

Gamon J.A., Qiu H-L., Roberts D.A., Ustin S.L., Fuentes D.A., Rahman A., Sims D., and Stylinski C.D. 1999. Water expressions from hyperspectral reflectance: implications for ecosystem flux modeling. Proceedings of 1999 Airborne Geoscience Workshop. Jet Propulsion Laboratory, Pasadena, CA.

Hinkson C.L., Oechel W.C., Miglietta F., and Raschi A. Plant- and soil-nitrogen dynamics after long-term CO_2 enrichment by a natural CO_2 spring. ESA 1997 annual meeting, 10-14 August, albuquerque, New Mexico. Suppl. Bull. Ecol. Soc. of America. 78(3): 101.

Ibanez I., Tissue D., Ogle K., Hastings S.J., and Oechel W.C. Long-term photosynthetic response of southern California chaparral to CO_2. Ecological Society of America 84th Annual Meeting, August 8-12, 1999.

Moreno, J.M., Cruz A., and Oechel W.C. 1997. Biometric relationships in two lignotuberous species from Spain and California. MEDECOS VIII, October 18-26, 1997, San Diego, California. p 48.

Oechel W.C. 1994. Effects of global change on unmanaged ecosystems. Review. Dutch National Program. Maastricht December 1994.

Oechel W.C., and Moreno J.M. Symposium Organizers. MEDECOS/GCTE Symposium on Effects of Global Change on Mediterranean-type Ecosystems. Vina Del Mar, Chile. October 23-29, 1994.

Oechel W.C., Hinkson C., and Miglietta F. CO_2 and climate effects on photosynthesis and productivity of Mediterranean-type shrublands and oak woodlands. MEDECOS/GCTE Symposium on Effects of Global Change on Mediterranean-type Ecosystems. Vina Del Mar, Chile. October 23-29, 1994.

Oechel W.C, Hastings S.J, Vourlitis G., Bryant P., and Zulueta R. Predicting the effects of global change from long-term chaparral research at the SDSU Sky Oaks

Biological Field Station. Ecological Society of America 84th Annual Meeting, August 8-12, 1999.

Ogle K., Oechel W.C., Bryant P., and Hastings S.J. Leaf area, shoot elongation and inflorescence response of *Adenostoma fasiculatum* dominated chaparral to elevated CO_2 using FACE and CO_2LT approaches. MEDECOS VIII, October 18-26, 1997, San Diego, California. p 52.

Ogle K., Tissue D., Ibanez I., Hastings S. and Oechel W. 1999. Leaf level response of chamise chaparral grown under pre-industrial, ambient and elevated CO_2 concentrations. Ecological Society of America 84th Annual Meeting, Spokane, WA, August 8-12, 1999.

Stylinski C.D., Oechel, W.C., Miglietta, F., Raschi, A., and Ustin, S. Effects of long-term CO_2 enrichment on chlorophyll concentration and leaf reflectance of three woody species. . ESA 1997 annual meeting, 10-14 August, albuquerque, New Mexico. Suppl. Bull. Ecol. Soc. of America. 78(3): 101.

Stylinski,C.D., Oechel W.C., Raschi A., and Gamon J.A. Effects of long-term CO_2 enrichment on narrow-band leaf reflectance, PSII light-use efficiency and photosynthesis of querus pubescens trees. MEDECOS VIII, October 18-26, 1997, San Diego, California. p 65.

Stylinski C.D., Gamon J.A., and Oechel W.C. Seasonal variations in narrow-wave ban reflectance, photoprotective pigments, and photosynthesis of several evergreen chaparral species. Ecological Society of America 84th Annual Meeting, August 8-12, 1999.

Thesis/Dissertation:

Heffernan L.C. 1996. Measuring the effect of increased levels of CO_2 on soil bacteria in a Mediterranean type ecosystem. M.Sc. Thesis, San Diego State University.

Williams-Anderson A.E. Effects of Whole-Ecosystem Atmospheric Carbon Dioxide Concentration Manipulation on Abundance and Species Diversity of Arthropods in a Post-fire Chaparral Community, Ph. D. Dissertation, UC-Davis and San Diego State University.

Initial progress
For the period

September 1, 1993 to August 31, 1996

RESPONSE OF MEDITERRANEAN-TYPE ECOSYSTEMS TO ELEVATED ATMOSPHERIC CO_2 AND ASSOCIATED CLIMATE CHANGE

Walter C. Oechel

INTRODUCTION AND BACKGROUND

There has been uncertainty and discussion of the potential response of unmanaged ecosystems to elevated CO_2 (Bazzaz, 1990; Graham *et al.*, 1990; Idso *et al.*, 1991; Körner *et al.*, 1992; Melillo *et al.*, 1990; Mooney *et al.*, 1991; Norby *et al.*, 1986; Shaver *et al.*, 1992). This stems in part from the range of responses found in laboratory and field situations. In the salt marsh environment, long-term stimulation of photosynthesis and productivity by elevated CO_2 have been found (Drake, 1992) although recently homeostatic adjustment ("down regulation") has been reported (Jacob *et al.*, 1995). In the arctic, photosynthesis and net ecosystem carbon flux quickly adjust to elevated CO_2 so that at the leaf level, photosynthesis exhibits complete homeostatic adjustment within three weeks, and ecosystem-level homeostatic adjustment of net CO_2 flux begins within the first season of exposure (Grulke *et al.*, 1990) and is complete within the third year (Oechel *et al.*, 1994).

Loblolly pine cultivated in pots in the field show little response to elevated CO_2 after the first year when grown under nutrient levels which simulate field nutrient supply rates (Strain and Thomas, 1992).

Under sufficiently warm temperatures, ecosystems may respond positively to elevated CO_2 over prolonged periods (Drake, 1992) and this may be the case for some ecosystems. However, in other cases nutrients appear to limit the potential long-term response to elevated CO_2 (Strain, 1991; Owensby *et al.*, 1990; Bazzaz, 1990; Norby *et al., 1986;* Körner *et al.*, 1992; Larigauderie *et al.*, 1988). While increased nitrogen fixation has been demonstrated at elevated CO_2 (Bentley, 1992), the interacting effects of nutrient immobilization and nutrient fixation under field conditions have not been fully examined.

Ecosystems may be simultaneously limited by a large number of factors (Billings, 1952) that vary over time. It is possible for water, temperature, CO_2, and nutrients to simultaneously limit ecosystem productivity and carbon storage in certain ecosystems (Chapin, 1991; Field *et al.,* 1992; Mooney *et al.,* 1991; Graham, *et al.,* 1990). In other ecosystems, these factors may limit productivity at various time scales (Oechel and Billings, 1992). Time scales can be critical in consideration of response to elevated CO_2. For example, it is possible for CO_2 to be limiting in the short term (minutes to hours), but not over longer time scales (Tissue and Oechel, 1987; Grulke *et al.*, 1990, and Oechel *et al.* 1994) when nutrients may

become more limiting (Shaver *et al.*, 1992). However, at still longer time scales, the nutrient mass or species composition may change, again allowing response to elevated CO_2 (Oechel and Billings, 1992).

Both resources and processes can limit ecosystem response to elevated CO_2, and the importance of these factors can change over time. In short time scales, leaf biochemistry and physiology may control ecosystem photosynthetic rates (Drake, 1992; Strain and Thomas, 1992). At longer time scales, morphology (Woodward, 1987), growth, species composition, vegetation, and evolution can be important (Graham, *et al.,* 1990; Oechel and Billings 1992).

The response of ecosystems to elevated CO_2 can be viewed at a number of different time scales. In this research, we were primarily interested in the response of ecosystems to elevated CO_2 which occurs over minutes to decades or even centuries. The short-term response helps in understanding mechanisms and feedbacks significant in controlling responses at seasonal to decadal time scales (Sage *et al*., 1989; Stitt, 1991a,b).

For terrestrial plants, atmospheric CO_2 concentration appears to universally limit short-term photosynthesis rates (Drake, 1992). As a result, an increase in CO_2 concentration of the air results in a rapid short-term increase in photosynthesis. However, long-term photosynthetic response to elevated atmospheric CO_2 concentration is much more variable. This is due to the fact that there are additional

controls and feedbacks on long-term photosynthetic response to elevated CO_2 compared to short-term responses. Short-term response (minutes) depends almost entirely on fast-acting processes (e.g. leaf biochemistry and physiology). Longer-term responses depend on these levels of organization, and on other longer-term processes including anatomical, growth, demographic, community, and ecosystem levels of organization. Over even longer time scales, ecotypic, dispersal, and genetic factors can begin to influence the plant and ecosystem response to elevated CO_2 (Oechel and Billings, 1992). These adjustments can be homeostatic; that is, they can tend to maintain ecosystem photosynthetic rates at similar levels, or they can potentially further exaggerate the effect of elevated CO_2 on photosynthesis by increasing the plant or ecosystem response to elevated CO_2 (as would occur if extant species were replaced by those better able to utilize additional atmospheric CO_2).

Chaparral

Chaparral and other Mediterranean-type ecosystems were selected for study since they are good model ecosystems in which to investigate the controls on homeostatic adjustment of photosynthesis to elevated CO_2. These systems are predicted to respond to elevated CO_2 due to their warm, water-limited, and nutrient-moderate status (Oechel *et al.*, 1995, Strain and Thomas, 1995). Preliminary data indicate a range of potential homeostatic adjustments to elevated CO_2 that allow tests of CO_2 and moisture interactions. Chaparral forms a valuable comparison to

other ecosystems which have been, or are being investigated including arctic (Oechel *et al.*, 1992; Oechel and Billings, 1992), salt marsh (Drake, 1992), prairie (Owensby, 1990), eastern deciduous forest (Norby, 1986), conifer forests (Strain and Thomas 1992; Ball *et al., 1992*) and deserts (planned, Seeman *et al.* pers. comm.).

In this research, we concentrated primarily on the homeostatic processes and controls which affect the response of CO_2 and H_2O flux to altered atmospheric CO_2 and moisture availabilities. To adequately address and understand limitations on responses to elevated CO_2, it is necessary to investigate the range of processes and mechanisms which can feed back on, and affect photosynthesis. Biochemical, physiological, growth, demographic, community, and ecosystem processes were studied. Other factors will affect ecosystem response to CO_2 in the chaparral. Moisture availability, a significant limiting factor in Mediterranean-type ecosystems (MTEs), which is affected by CO_2 level, was investigated. Effects on and feedbacks with other environmental factors including nutrient mass and supply rates were also studied.

Tested were the effects of CO_2 concentration from nominally pre-industrial CO_2 concentrations to more than double current CO_2 (250 to 750 ppm) in 100 ppm CO_2 increments. The interacting effects on the growth, water yield, and trace gas flux of chaparral shrub vegetation was measured.

Higher atmospheric CO_2 has the potential to increase plant growth in a variety of ways including greater photosynthesis (McGuire *et al.*, 1995), relief of nutrient stress (through various mechanisms including increased nutrient-use efficiency, symbiotic and assymbiotic nitrogen fixation, and higher nutrient uptake by the roots) (Norby *et al*., 1986), relief of drought stress Tolley *et al*., (1991) and increased water yields (due to greater water-use efficiency) (Wigley and Jones, 1985), depression of respiration (Poorter *et al*., 1992), or delay of leaf senescence (Tissue and Oechel, 1987; Curtis *et al.*, 1989). The mechanisms that increase water-use efficiency and decrease transpiration should promote growth in arid and semi-arid ecosystems. However, there is great uncertainty as to whether these mechanisms operate for prolonged periods in natural ecosystems. If elevated CO_2 does stimulate the growth of woody vegetation, this could lead to long-term increases in carbon storage in terrestrial ecosystems.

RESULTS 1993-1996

CO_2LT Chambers

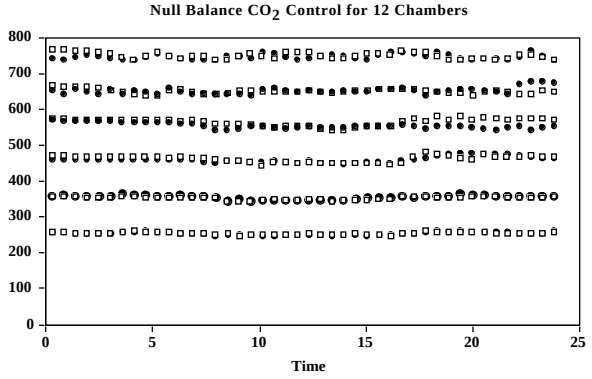

Figure 1. CO_2 control in the twelve CO_2LT chambers operating at 250 to 750 ppm CO_2 with two chambers at each concentration (February 16, 1996).

The null-balance CO_2LT chambers designed for CO_2 control and measurement under ambient Light and controlled Temperature (Tissue and Oechel, 1987; Grulke *et al*., 1990; Oechel *et al*., 1992) were adapted for use in the chaparral. The chambers were placed on chaparral regenerating after fire, and initial measurements of the effects of CO_2 concentration on net ecosystem flux were made. As the plants regrew, the original CO_2LT chambers were redesigned and expanded to 2 x 2 x 2 meters high, the cooling capacity was increased, and the air handling hardware was improved. In addition, "blow out panels" were installed to allow the chamber sides to be opened in the event of a system failure, thereby preventing over or under temperature conditions. The original control and data logging software was completely re-written in "Lab View" software. A color image of the current chamber configuration can be viewed at the San Diego Union Tribune web site at: "http://www.uniontrib.com" under "Science" or at: "http://www.uniontrib.com:80/science_city/environment/environment960228/climate2.html."

The current experimental design is for 12 chambers, running two each at 250 to 750 ppm CO_2 in 100 ppm increments. Regression analysis of response to CO_2 concentration is being used. A water stress (75% of ambient precipitation) is

planned for one-half of the chambers. Precipitation is collected in equal area collectors and routed to the chambers in the amount of actual precipitation.

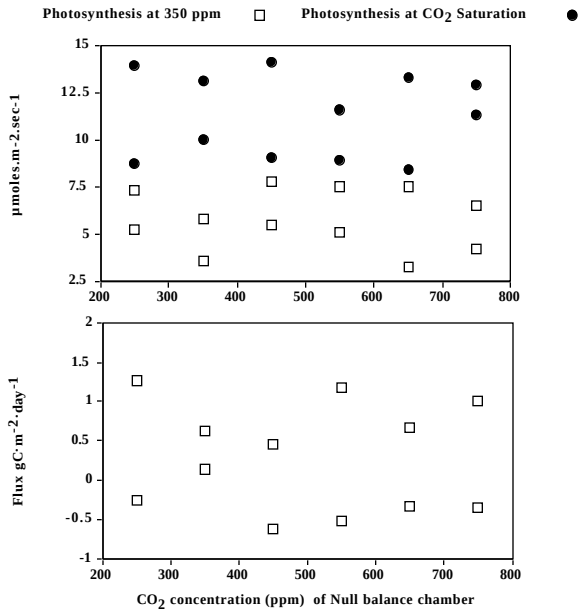

Figure. 2. Patterns of photosynthesis at ambient and saturating photosynthesis (top) and net ecosystem CO_2 flux for chamber pairs assigned to differing subsequent CO_2 treatment levels.

Performance

Chamber performance of CO_2 (Figure 1) and temperature control following modifications is very good (Oechel et al. 1993), however, refinement of control algorithms and hardware continues.

Prior to initiation of long-term elevated CO_2 treatments in the CO_2LT chambers, variability in photosynthetic rates (at ambient and saturating CO_2 levels) were determined (Figure 2). Also determined were the net ecosystem CO_2 fluxes. Results show no difference in any of these factors among the chambers allocated to different treatment CO_2 levels. This gave confidence that future differences in carbon flux would be due to treatment effects, and not to chamber to chamber variability.

Net Ecosystem CO_2 Flux

Short-term net ecosystem CO_2 flux is significantly stimulated by elevated CO_2 (Figures 3 and 4). Exposure to 750 ppm CO_2 increases net ecosystem CO_2 flux by almost 80%. Net midday sequestration is much more affected than is nighttime respiration. Laboratory studies indicate that long-term response to elevated CO_2 will depend in part on water availability (Oechel *et al.*, 1995; Jenkins 1993).

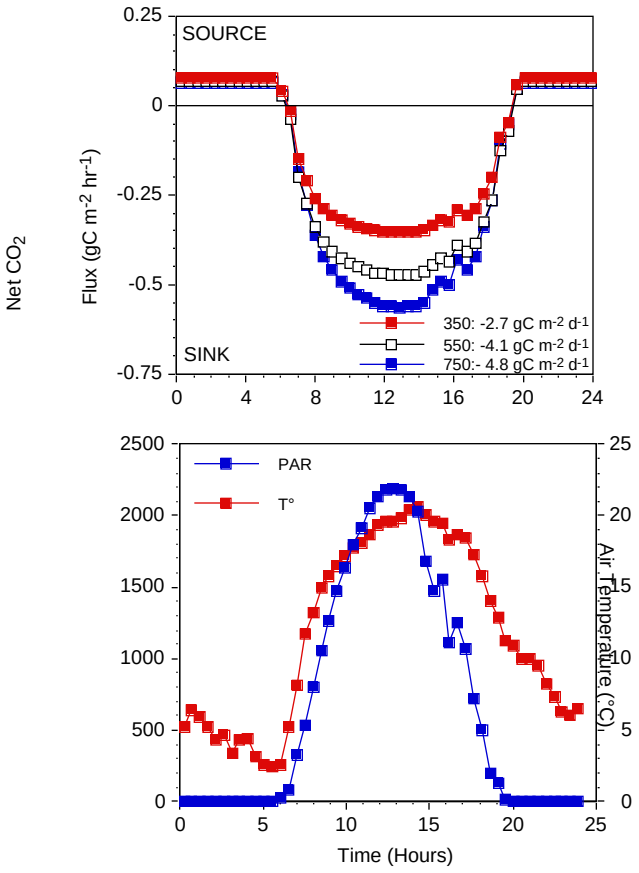

Figure 3. Diurnal net ecosystem CO_2 flux at 350, 550, and 750 ppm CO_2 and accompanying light and temperature in May 1995. Net diurnal ecosystem fluxes at 750 ppm CO_2 were almost 80% higher than those at 350 ppm CO_2.

Studies on the response of chaparral recovering from fire to ambient and experimentally manipulated levels of atmospheric CO_2 (250 to 750 ppm CO_2) are being undertaken in an experimental area comprised of a mix of *Adenostoma fasciculatum* resprouts and *Adenostoma fasciculatum* and *Ceanothus greggii* seedlings. Water availability is typically increased immediately after fire (Hastings, Oechel, and Sionit, 1989). Due to the reduced drought following fire, reduced water

treatments will not commence until later in the experiment as significant drought develops. This approach will allow a period of time with duplicate chamber treatments to allow replication and baseline measurements before the reduced water manipulations begin. Water treatments will include normal ambient and 75% ambient precipitation.

The use of six CO_2 levels allows regression analysis of the resultant information, production of response surfaces to CO_2 concentration, and avoids pseudo-replication (Hurlbert, 1984). Temperature and dew point are being maintained at contemporary ambient levels.

Photosynthesis measurements made after only 6 weeks of exposure to elevated CO_2 show a strong down regulation of photosynthetic capacity (maximum photosynthetic rate) (Figure 5**,** top). This shows rapid photosynthetic adjustment under elevated CO_2, at least under conditions of adequate winter moisture and cool winter temperatures. Despite the down regulation of photosynthesis, elevated CO_2 was sufficient to convert ecosystem flux from a source to the atmosphere, to a sink during this winter period (Figure 5 bottom).

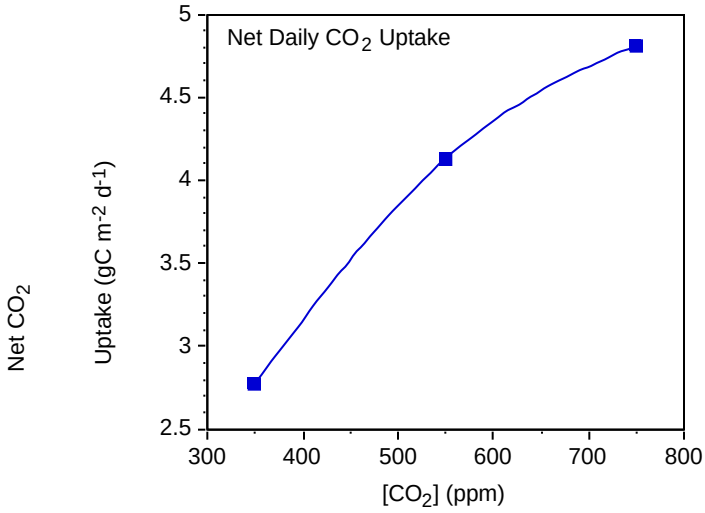

Figure 4. Short-term response of diurnal net ecosystem CO_2 flux to CO_2 concentration (May 1995).

Free Air CO_2 Enrichment (FACE)

Obviously the size and complexity of ecosystems very nearly makes ecosystem-level studies intractable, and yet this very complexity also makes it difficult to generalize the results of laboratory or greenhouse studies to natural ecosystems. We are using FACE technology to validate the results from our CO_2LT chambers, and to treat sufficient area to allow adequate sampling for belowground processes. FACE systems use a chamberless array of stand pipes which release CO_2 into a study plot based on wind direction and speed. Although the CO_2 consumption is high compared to other technologies, FACE systems allow CO_2 control over relatively large areas, in this case, 177 m^2, and eliminates chamber effects.

The FACE approach is one of the least invasive methods for treating natural systems with elevated CO_2, as well as being the most cost effective when figured on a cost/area basis (Kimball, 1992). It also treats a sufficiently large area to capture many ecosystem processes of interest (e.g. plant germination, establishment, competition; most nutrient dynamics of interest; plant-microbe interactions; certain plant-insect interactions). We developed a FACE system as a validation for our CO_2LT chamber study, and to provide a large enough area to allow adequate sampling for most processes of current interest. A 16 m FACE ring has been built and tested in conjunction with Sagar Krupa and Clive Reece of University of Minnesota (Reece *et al.*, 1995). Subsequent refinements included further software and hardware development, movement to a single ring from the original double ring configuration to reduce possible environmental effects on wind speed or shading, and addition of a 34 ton receiver which allows us to run for 25 days without refilling.

Upon completion of FACE construction, concentrations were within $\pm$ 20% of the set point for one-minute averages for at least 80% of the measurement period (see e.g. Hendrey *et al*. 1993, Table l). Initially, control at a desired set point was accomplished by using a particular mix of algorithms and fan speeds. One algorithm predicted the mass flow of CO_2 required to achieve a set point CO_2 concentration based on the cross-sectional area of the ring and the wind speed. The other two algorithms used PID loops, one based on the error in meeting the CO_2 set point while the other on the error of the mass flow of CO_2 delivered. Only one algorithm could be used at a time. The program has been modified so that a mix of algorithms

is used, weighted as a function of wind speed. The algorithm used is now dependent on wind speed, where each individual coefficient can be independently specified.

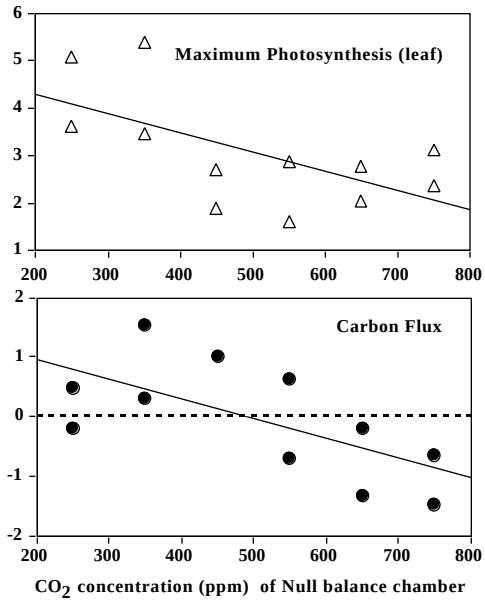

Figure 5. Net photosynthesis (maximum observed, February 17, 1996) (top) and net ecosystem flux after 6 weeks of treatment at the CO_2 concentrations indicated (Feb 16, 1996).

The CRT screen output program has been rewritten to show real time value of the weight of each algorithm and coefficient as well as percent time between $\pm$ 10 and 20% of the set point for the cumulative one minute average, and over minute averages over 10-min. 30-min., 1-hr, 6-hr, 12-hr, and 24-hr periods. The real time data analysis allows the user to receive immediate statistical analysis and evaluate how well a set of parameters is controlling.

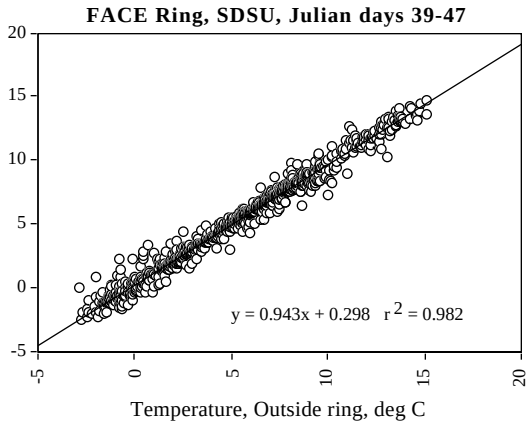

Figure 6. Average of 16 thermocouple readings every 10 min., (1,164 total readings) made at 0.7 m from ground in and outside the FACE ring. Control readings outside of ring were taken from a location 30 m from the edge of the ring.

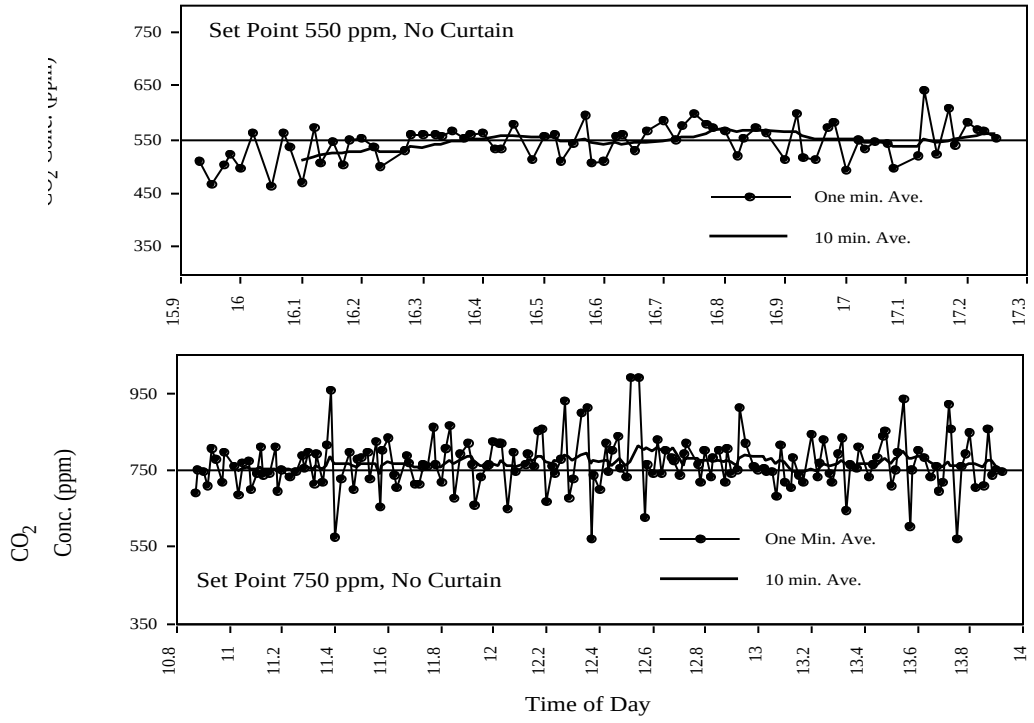

Figure 7. One minute average (circles) and ten minute running mean (solid line) CO_2 concentrations within the MedFACE ring with a set point of 550 ppm (top) and 750 ppm CO_2 (bottom). At 550 ppm CO_2, 92% of the time the CO_2 concentration in the ring was ± 10%, while 100% of the time it was ± 20% of the set point while at 750 ppm percentages were 80 and 95 percent respectively. Mean control CO_2 concentration was 544 and 766 ppm. Data collected at SDSU's MedCO_2RE FACE facility (unpublished data).

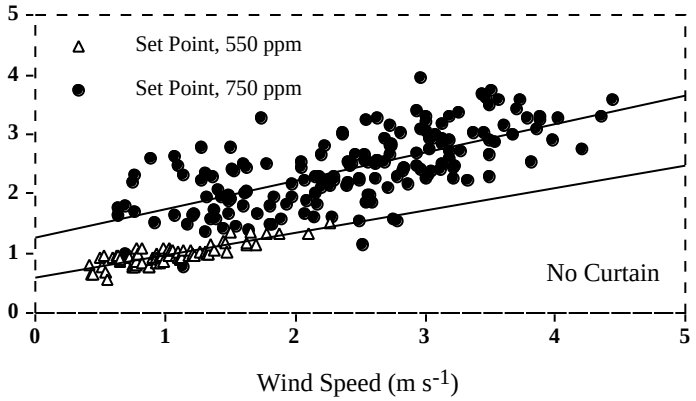

Figure 8. MedFACE CO_2 consumption as a function of wind speed and CO_2 set point determined in the field, winter 1995.

There is minimal affect on temperature of the FACE apparatus, even with the outer barrier plenum in place. Figure 6 shows the near 1:1 correspondence between inside and outside temperatures. There was a slight decrease inside the ring at higher temperatures (daytime) while at lower temperatures (nighttime) a slight increase. This is due to the impacts of increased wind movement during the day at

high temperatures, and a disruption of the still, radiatively cooled air at night during still wind conditions.

This system performed very well with respect to CO_2 control (Figure 7) and CO_2 consumption (Figure 8). The performance was very good "at turn on" (Reece *et al*., 1995), and subsequently improved through hardware and software improvements described above CO_2 consumption, increases with wind speed (and CO_2 set point concentration) in the manner predicted (Figure 8).

In developing our MedFACE system, it was decided that the control code and construction and operation materials will be provided upon request to interested researchers, and all aspects of the system will be published in the open literature to allow maximal availability to the research community. The FACE system will complement our existing ongoing investigation of chaparral ecosystem response to elevated CO_2 by validating in an open environment the results obtained using chamber technology at the same site.

Table 1. Preliminary CO_2 control and consumption data for MedFACE ring. These are initial values and reflect initial control algorithms and variable conditions during testing.

CO_2 Set Point (ppm)	Ave. CO_2 Conc. (ppm)	SE	Wind Speed m s^{-1}	% of 1 min. average within 10% of set point	% of 1 min. average within 20% of set point	CO_2 use kg CO_2 h^{-1}
550	544	4	1.0	92	100	64
550	589	3	2.4	72	92	78
550	560	5	2	72	95	136
550	515	3	1.7	81	100	73
550	575	5	0.4	85	100	36
750	717	7	1.9	71	94	167
750	760	5	1.9	78	96	136
750	766	5	2.8	80	94	149

Baseline measurements

Extensive measurements were made within the FACE ring and on soil beneath similar age vegetation (part of the same burn as the FACE study) in the surrounding areas with respect to soil respiration. Rates were in general similar other than one control area east of the FACE ring and situated on a south-facing slope (Figure 9).

Soil respiration was determined using an opaque cuvette (150 cm^2) during midday on six replicate areas including the FACE ring treatment area, and areas east and west of the FACE ring site; within the null-balance chambers; and within control plots laid out for comparison with the chambers. The respiration rates were surprisingly uniform at this time. We anticipate that the soil respiration rate will increase within the FACE ring due to increased allocation of carbon belowground. An increase in water-use efficiency, and a decrease in transpiration rate due to elevated CO_2 could result in higher soil moisture which would tend to increase decomposition rates of soil organic matter and thereby further increase soil respiration rates.

Diurnal water potentials and net photosynthesis measurements were determined for *A. fasciculatum* in the FACE ring and in the control plot. The pattern of water stress before initiation of treatment was nearly identical in the two areas (Figure 10). Minimum values were observed at midday of -5 MPa, and only partially recovering to -4 MPa in the evening. Full recovery took a number of hours when pre-dawns values of -3 to -2.5 MPa were recorded.

After 5 weeks of treatment at elevated CO_2, there is an indication of improved moisture status under the elevated CO_2 (Figure 10). This is consistent with predictions of initially reduced leaf and canopy transpiration rates at elevated CO_2. (Jenkins, 1993; Oechel et al., 1995).

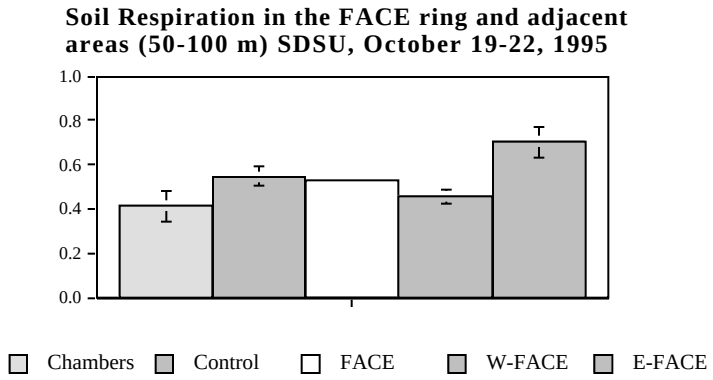

Figure 9. Soil respiration in and around the FACE ring site.

Before initiation of the CO_2 treatment, photosynthesis was slightly higher in the plants found within the FACE ring (1.5 to 1.8 µmoles/m^2/sec) compared to those found in the control area (ca. 1 µmole/m^2/sec). Photosynthesis and water potential data indicate a low level of metabolic activity in both regions at this time.

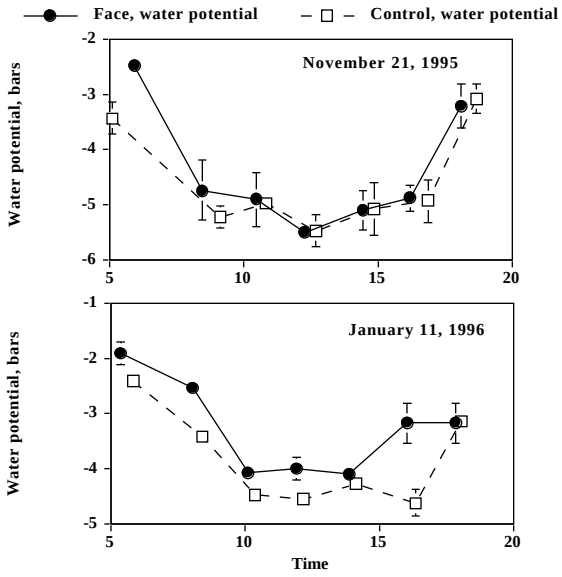

Figure 10. Average water potential for six plants within the FACE ring and six in a designated control area [from November 21, 1995 (before the start of the continuous treatment) and on January 11 (after about 5 weeks of elevated CO_2 treatment)]. Standard errors are shown on the figure.

Measurement of photosynthetic rates after initiation of continuous CO_2 treatment on December 1, 1995, indicates rapid and significant down regulation of photosynthesis after only 5 1/2 weeks of elevated CO_2 treatment (Figure 11), even with the improvement of water status within the treatment area.

Long-term Responses to Elevated CO_2: Research at the Laiatico CO_2 Springs

An opportunity to study long-term effects of elevated CO_2 on ecosystems exists at a natural CO_2 spring near Laiatico, Tuscany in northern Italy. This site has been visited and found to exhibit a large area of CO_2 enrichment of over 0.6 ha as a

result of a CO_2 release estimated at over 8 tons per day. While some sulfur is present in the CO_2, sulfur release is low and is estimated at less than 60 grams of sulfur per day in 8 tons of CO_2. Previous studies indicate that the sulfur should pose little or no biological effect at a moderate distance from the CO_2 vent and over most of the area enriched. In addition, since most of the CO_2 is emitted from one major vent, it is possible to remove most of the sulfur from the CO_2 emissions should this be deemed prudent. No symptoms of sulfur (SO_2, H_2S) toxicity were noted at the site. Additional analyses are needed, however, including soil pH and leaf sulfur content.

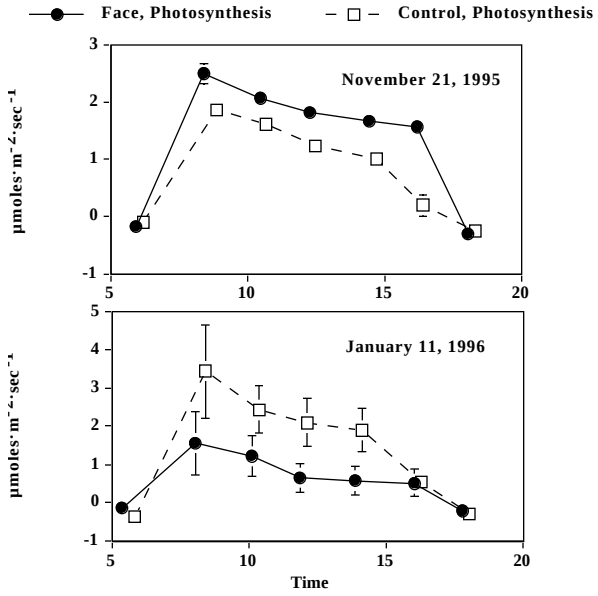

Figure 11. Average net photosynthesis for six plants within the FACE ring and six in a designated control area. From November 21, 1995 (before the start of the continuous

treatment) (top) and on January 11 (after about 5 weeks of elevated CO_2 treatment) (bottom). Standard errors are shown on the figure.

CO_2 concentrations were measured to be on average more than 2,500 ppm at 5 m from the origin of the transect (about 10 m from the spring) to less than 600 ppm at 80 m to the east of the spring. A north-south transect, 20 m to the east of the spring ranged from > 2,000 ppm CO_2 to < 550 ppm CO_2 at 40 m (Figure 12). At the top of the canopy, leaf samples were analyzed for ^{14}C content to yield average CO_2 concentration (Figure 13). The concentrations were lower than those reported near the ground (Figures 12 and 13), but were still significantly elevated.

The vegetation of the area is a coppiced oak forest approximately 25 years of age containing typical Mediterranean mattoral and forest species including *Quercus ilex, Q. pubescens, Pistacia lentiscus, Arbutus linedo,* and several nitrogen-fixing species. There appear to be good control areas and control watersheds in the region. The area is apparently one of strong carbon sequestration, and one of increased sequestration potential with a longer coppicing interval.

The elevated CO_2 springs in northern Italy allow the study of the effects of very long-term CO_2 exposure (probably over millennia). The period of past enrichment can be estimated by ^{14}C dating of wood and soil organic material. Since the CO_2 originates from subvention of ancient carbonate rocks, its emission is ^{14}C depleted. This estimate will allow easy determination of the average concentration

of CO_2 experienced by recent plant material, as well as indicating the minimum period of enrichment.

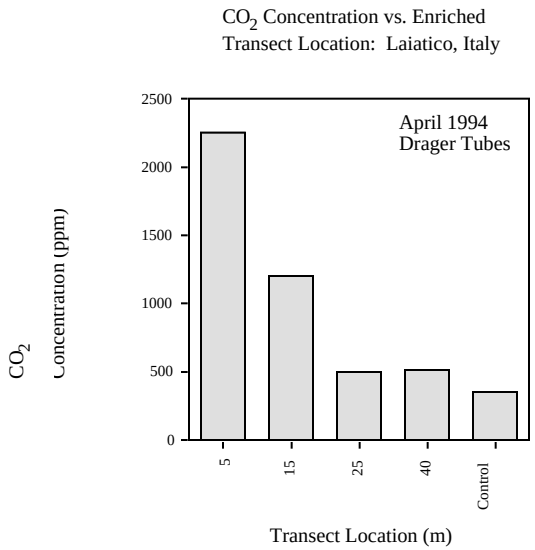

Figure 12. CO_2 concentration along a north-south transect at Laiatico CO_2 springs in Laiatico, Italy. CO_2 concentrations were generally averaged over 4-6 hours on one-two days in April 1994. CO_2 concentrations were determined by Draeger diffusion tubes and by infrared gas analysis. Good agreement was found between Draeger tubes and integration of IRGA measurements when averaged over the same period in the field (Miglietta *et al.*, unpubl).

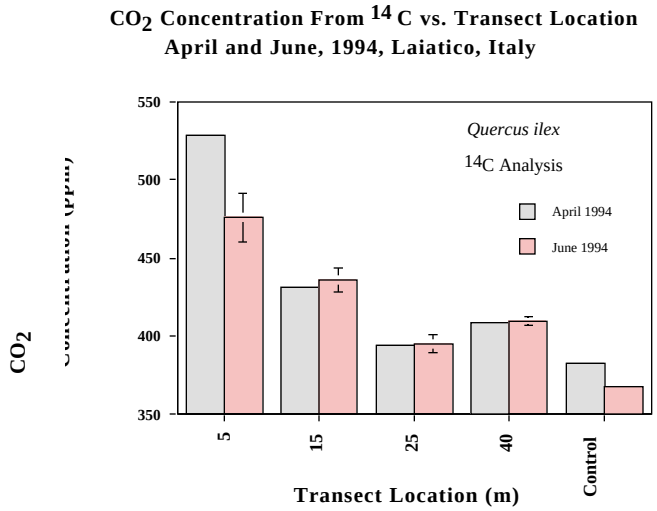

Figure 13. CO_2 concentration from the top of the plant canopy along a north-south transect at Laiatico CO_2 springs in Laiatico, Italy determined from ^{14}C analysis.

Initial measurements were made, including $A:C_i$ curves of photosynthesis, and leaf samples were taken for nutrient analysis. Initial nutrient analysis of the leaves indicates that leaf total nitrogen concentration significantly decreased with proximity to the CO_2 spring (increasing CO_2 concentration; Figure 14).

Preliminary investigation indicates significant "down regulation" and homeostatic adjustment of *Quercus ilex* to elevated CO_2 (Figure 15). "Down regulation" of $A:C_i$ curves is of the order 15 m > 25 m > 40 m, and is in the direction to homeostatic adjustment. However, in June 1994, the homeostatic adjustment was totally relaxed (Figure 16). Reasons are uncertain, but formation of new leaf tissue

and depletion of carbohydrate reserves during the spring growth flush may have accounted for the loss of "down regulation." This is fruitful area for insight into the controls on homeostatic adjustment to elevated CO_2, and the interactions with other factors.

It is premature to attempt to estimate the combined effects of "down regulation" of A:C_i curves, elevated CO_2, and changes in leaf area on plant and ecosystem carbon flux and carbon sequestration. It is clear, however, that feedbacks and adjustments are occurring at the physiological level. The use of these springs should give a real-world look at possible physio-logical, nutrient flux, genetic, and landscape responses to long-term CO_2 enrichment in native forest tree and shrub species.

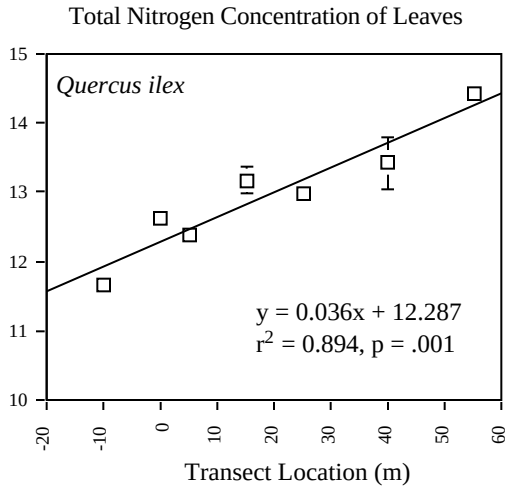

Figure 14. Nutrient concentration in *Quercus ilex* with distance from CO_2 source at Laiatico CO_2 springs at Laiatico, Italy. Distances given are relative to an arbitrary starting point, approximately 20 m from the CO_2 spring. Distance from the CO_2 vent is approximately 20 m plus the value indicated. Total nitrogen concentration was determined using TKN extraction and a Technicon auto-analyzer (Bran & Luebbe Analyzing Technologies, Elmsford, N.Y.). Symbols represent the mean of 12 samples (three leaf ages, two branches per tree, two trees per transect location). Means and standard errors are shown (n=2 trees per transect location). Data were analyzed using a one-way ANOVA. Since there were non-significant differences in nitrogen concentration between years for each tree, the data from each of the three years of leaf age classes were pooled for each tree (Hinkson, Oechel, and Miglietta, unpubl. data).

Evaluation of predawn and midday tissue water potentials indicate no substantial improvement of water status from elevated CO_2 in either Summer or Fall. Again, while the data are preliminary, it appears as though decreases in transpiration rate on a leaf area basis are at least offset by increases in leaf area index (Figure 17) in areas of elevated CO_2 concentrations.

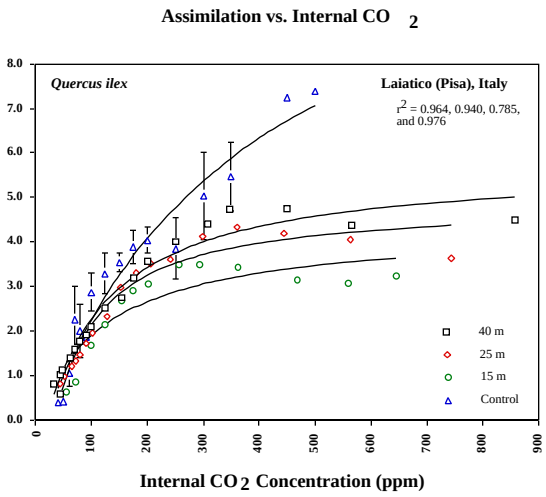

Figure 15. Assimilation versus internal CO_2 concentration of *Quercus ilex* at the Laiatico CO_2 spring in Laiatico, Italy in April 1994. Standard error bars reflect variability among three individuals under ambient CO_2 conditions at 60, 80, and 100 m locations south along the north-south transect running 20 m east of the spring. Individuals measured at 40, 25, and 15 m locations north along the north-south transect were under enriched CO_2 concentrations, the 15 m location being closest to the CO_2 source and the most enriched.

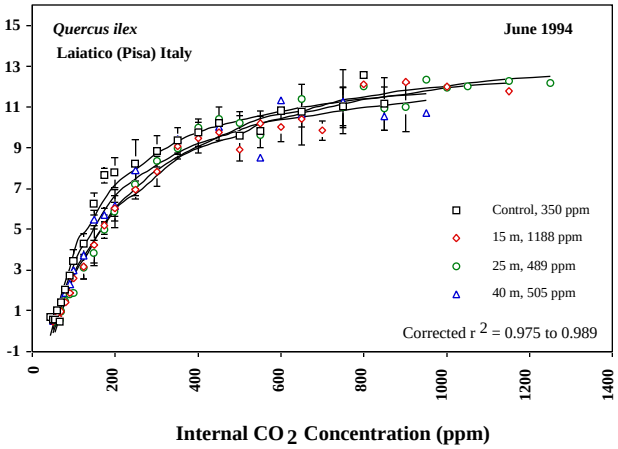

Figure 16: Assimilation versus internal CO_2 concentration of *Quercus ilex* at the Laiatico CO_2 spring in Laiatico, Italy in June, 1994 (bottom). Standard error bars reflect variability among three individuals under ambient CO_2 conditions at 60, 80, and 100 m locations south along the north-south transect running 20 m east of the spring. Individuals measured at 40, 25, and 15 m locations north along the north-south transect were under enriched CO_2 concentrations, the 15 m location being closest to the CO_2 source and the most enriched.

The effect of intermittent stimulation in photosynthesis, and spreading of the C:N ratio appears to increase the growth rate as indicated by internode elongation and ring widths (Hinkson, Oechel, and Miglietta, unpublished data).

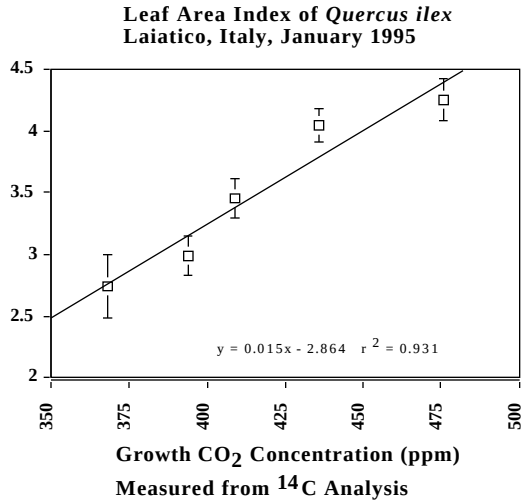

Figure 17. Relationship between leaf area index and CO_2 concentration as measured by ^{14}C dilution.

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Ecosystem carbon flux under elevated CO_2 by CO_2 LT Chambers in southern California chaparral

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Abstract

Ecosystem carbon flux was studied using six different CO_2 treatments with CO_2 LT ($\underline{CO_2}$ controlled, Natural Lit, Temperature controlled) null balance chambers in Southern California chaparral dominated by *Adenostoma faciculatum* H. & A. between 1997 and 2001. Using the chamber technique, net ecosystem flux was measured directly and continuously. Missing data were modeled using non-linear curve fitting techniques with meteorological data. The annual net ecosystem carbon flux under different CO_2 treatments was accumulated from daily carbon flux values. There is a significant CO_2 effect ($p=0.049$) and year effect ($p=0.000$) for annul NEE from 1997 to 2000 with a strong seasonal pattern of elevated CO_2. Elevated CO_2 delayed water stress. Although elevated CO_2 enhanced leaf-level photosynthesis and decreased transpiration and conductance regardless of water availability, whole-ecosystem rates of net CO_2 flux and plant water status were not significantly altered by elevated CO_2 when water availability was high. Aboveground biomass (e.g. Plant Growth and leaf area index) did not show a significant effect of different CO_2 treatment, while there is a significant CO_2 effect on belowground bulk root biomass ($p<0.015$), which indicates the chaparral ecosystem

above ground response to the environment is very slow, which might mask, if just looking at above ground, the CO_2 effect.

Introduction

World CO_2 concentration has increased about 30% over pre-industrial levels (Houghton *et al.*, 1990, Alcamo *et al.* 1996). Unfortunately we do not even know with certainty the sign for net ecosystem exchange for most ecosystems (Oechel *et al.*, 1994) now or their potential response to future CO_2 concentration (Ryan 1991, Amthor 1995, Drake *et al.* 1999, Hymus *et al.* 2002). Studies at different scale across varieties of ecosystems have been reported (Strain & Thomas 1992), including studies of the arctic tundra (e. g. Billings *et al.* 1984, Oechel *et al.* 1994, Joes *et al.* 1998, Press *et al.* 1998), Californian grasslands (Field *et al.* 1995, Luo *et al.* 1997, Cardon *et al.* 2001), alpine grassland (Schäppi & Körner 1996, Diemer and Christian 1998), a prairie (Owensby *et al.* 1993, Hunt *et al.* 1998, Williams *et al.* 2000, Lee *et al.* 2001), a wetland (Drake 1992, Nungesser *et al.* 1999, Dakora and Drake, 2000), a crop (Yeo, 1999, Okada *et al.* 2001) and a desert (Huxman *et al.* 1998, Hamerlynck *et al.* 2000, Nowak *et al.* 2001). All these studies have given us valuable information to understand systematic dynamic carbon exchange and interaction with other biogeochemical cycles. However, a continuous and long term carbon flux monitoring across different ecosystems and regions is needed to get sufficient information for a better estimation of global carbon budget both in the present and in the future.

Plant photosynthesis is more responsive to elevated CO_2 (i.e. less homeostatic adjustment to elevated CO_2) at higher temperatures (Luo and Reynolds 1999, Hamerlynck *et al.*, 2000), under water stress (Gifford, 1979, Tolley and Strain, 1985,

Sgherri *et al.,* 2000), and under adequate nutrients (Larigauderie, *et al.*, 1988, Strain, 1992, 1995). This indicates ecosystems which are warm, drought stressed, and moderately fertile (e.g. chaparral), should be more responsive to elevated CO_2 than other ecosystems. NEE and plant growth in chaparral (and related Mediterranean-type ecosystems), should therefore be fairly responsive to elevated CO_2.

The responses of arid and semi-arid woody ecosystems to elevated CO_2 are very important to understand because arid and semi-arid woody shrublands comprise approximately 51% of the global terrestrial surface area, 24% of the global soil organic carbon, and 16% of the global above-ground biomass (Atjay *et. al.*, 1979). This ecosystem is likely to be especially sensitive to changes in climate, atmospheric CO_2 due to the arid environment, while the data on CO_2 flux from this important ecosystem is poorly known. To date, there are relative few field experiments on the effects of elevated CO_2 on carbon sequestration in native woody ecosystems (Mooney *et al.*, 1991). Understanding how these ecosystems will respond in the future to higher CO_2 concentrations is very critical.

CO_2 effects on carbon flux and carbon storage of unmanaged and lightly and managed ecosystem depends on complex aboveground and belowground interactions of biological and physical processes (Field *et al.*, 1992, Jach *et al.* 2000, Heijmans *et al.* 2001). Elevated CO_2 can directly affect the growth and water use of vegetation through effects on transpiration, photosynthesis, and water use efficiency (Grodzinski 1992, Palicz *et al.* 2000). To understand the response of the ecosystem on elevated CO_2 concentration, we need to study the ecosystem under different CO_2 concentration and

monitor the environmental factors of different ecosystem concerned. This paper discusses the effect of different CO_2 concentration on the net ecosystem exchange, plant water status and morphological response. By using *in situ* measurement, we are able to test the following hypotheses that elevated CO_2 would (1) increase ecosystem carbon sequestration, (2) improve plant water status and (3) increase biomass accumulation (both aboveground and belowground).

Materials and methods

The study took place at the Sky Oaks Biological Field Station (33º23'N, 116º37'W), located in Southern California, at about 1,420m elevation and 75 km from the coast. Major shrubs present include *Adenostoma sparsifolium Torr., Adenostoma faciculatum H. & A.,* and *Ceanothus greggii Gray*. Treatments were carried out using CO_2-controlled, ambient Lit, Temperature controlled null-balance chambers (Tissue and Oechel, 1987, Grulke *et al.*, 1987, Oechel *et al.*, 1992). The site consisted of a chaparral stand burnt in July of 1992, having previously burned back in 1898 (Marion and Black, 1988, Keeley, 1992, Zedler, 1995). *Adenostoma fasciculatum* is the dominant species (70%) in California chaparral (Hanes 1977). This species regenerates after fire by seeds and by resprouting from a lignotube (Stohlgren *et al.* 1989, Keeley, 1992, Zedler, 1995).

Twelve closed 2×2×2-m chambers are each centered around individual *Adenostoma faciculatum* and surrounding herbaceous plants. Atmospheric CO_2 concentrations within the chambers are maintained at levels ranging from 250- to 750-ppm in 100-ppm increments (n = 2). The treatment started in December 1995. The

chambers are naturally lit (about 93% of ambient), and temperature tracked the ambient environment (Oechel *et al.* 1992). Precipitation is collected from the chamber roof and induced into a bucket. A rain distribution system was set up to spray all the rainwater from above down to canopy to minimize the chamber effect.

Measuring technique

More detailed descriptions of CO_2LT null balance are provided by Oechel *et al* (1992). The carbon flux was measured automatically by the chamber technique, which provided replicated *in situ* ecosystem level measurements at the 6 different CO_2 concentrations. Temperatures were monitored by copper-constantan (type T) thermocouples. Carbon dioxide concentrations of each chamber was measured with an infrared gas analyzer (IRGA) (Bio 4B.2, Leybold-Heraeus, Hanau, Germany).

There are three major parts of the CO_2 control and carbon flux measurements: CO_2 injection, CO_2 scrubs and chamber leakage. The CO_2 injection was controlled by computer program, which sent command to solenoid valve and mass flow controller to determine the amount of the pure CO_2 to be injected to each chamber. The mass flow controller sent the output of actual injection to computer data acquisition system accordingly. CO_2 scrub is also controlled by computer program. When the chamber CO_2 concentration was above the set point, the computer sent command to a solenoid valve to direct gas through a PVC tube filled with soda lime. CO_2 scrub tubes of different sizes were used according to the CO_2 set point. The amount of CO_2 scrubbed was determined by the flow rate passing through each soda lime tube and scrubbing time. Test showed that regular soda lime check (every 3 days) is enough and critical to make sure the

sufficiency of the soda lime. Chamber leak rate was calculated by the amount of N_2O (trace gas) injection and N_2O concentration measured for each chamber by the N_2O IRGA. With the difference between chamber and ambient CO_2 concentrations, the leakage of CO_2 for each individual chamber was calculated. Net ecosystem exchange was calculated by CO_2 injection, CO_2 scrub, CO_2 leakage and the variation of the CO_2 concentration within each chamber for every three minutes.

Data analysis

The data acquisition system recorded all the raw data automatically at 3-minute basis for all the chambers, and then we reduced all the raw data to hourly basis, missing data are modeled by curve fitting method with meteorological data. "CO_2 Exchange"(Viktor, 1995, Vourlitis *et al*., 2000) program was used to estimate the missing data and to model the GPP, Ecosystem Respiration. The "CO_2 Exchange" program was designed to provide a full set of daily and seasonal estimations for the CO_2 exchange between plant's ecosystem and the atmosphere. The program uses statistical analysis based on the *in situ* flux measurement to estimate the carbon flux (gross primary productivity, net ecosystem exchange and ecosystem respiration). For each day's measurement, the program use the hourly summed measured flux data and the weather data either from chambers data acquisition system or the weather station to make the daily estimation. The weather data will mainly include the solar radiation (in PAR) and the chamber temperature (equal to air temperature in this study).

Ecosystem respiration (ER) was modeled as an exponential function of temperature (Billings et al. 1977, Bunnell et al., 1977 Oechel et al., 1995, Vourlitis et al. 2000)

$$ER = a\exp(bT)$$

Where a is the base estimation of the ER at the temperature 0C, b is the ER sensitivity to the temperature and T is the chamber (ambient in the study) temperature.

Hourly gross primary production (GPP) was described as a hyperbolic function of photosynthetic active radiation (PAR):

$$GPP = \frac{aQb}{aQ + b}$$

Where a is the estimation estimated quantum yield; Q is PAR, and b is the photosynthesis capacity of the PAR. All of the parameters were estimated by nonlinear regression. Net ecosystem exchange (NEE) is the difference of the GPP and ER.

Plant morphology measurement

Plant stem growth was measured in each chambers and chamber controls during the study period. In general there was very little plant growth between August to March, so that growth data reported here are generally accumulated from March to July. Six stems of *Adenostoma faciculatum* were randomly selected before plant growth in each year. The length of the selected stems was measured from the basal connection to the other stems. Shoot growth was intensively measured (every other weeks) during the growing season. Leaf area index (LAI) was calculated by the volume of plant canopy (length*width*height) and an empirical equation (Ref.).

Plant stem water potential measurement

Predawn stem water potentials were measured each month or every other month for each chamber and in the FACE and their controls by using a pressure bomb

(Scholander *et al.* 1965). Two samples were collected from each chamber and outside chambers as control. Six samples were collected in the FACE (treated at 550 ppmv CO_2) and FACE control for the comparison of the chamber plants and chamberless plants for the same CO_2 concentration treatment to test the chamber effect regarding water stress. Samples are general takes between 3 and 4 in the morning for the stable water potential measurement. Samples from each treatment were measured immediately after the cutting.

Statistics

Relationships between annual NEE and CO_2 concentration and accumulative effect (year in this case) were tested though linear regression analyses using Systat9.0 (SPSS 1999) and SigmaPlot 2000 (SPSS 2000). Analysis of variance (ANOVA) was performed to test the effect of different CO_2 treatments on plant shoot and leaf area index. Pair wise comparisons were conducted using a Kolmogorov-Smirnov test. Plant water potentials were analyzed using T-test for a difference between the elevated CO_2 treatment (550 ppm) and ambient treatment (365ppm) as well as between seasons. In all cases, results were considered significant when $P < 0.05$.

Results

Carbon flux (net ecosystem exchange)

Mean average daily net ecosystem exchange in wet and dry season showed different pattern (fig. 1). The mean of the night time ecosystem respiration (as seen from night time NEE) was ranged from 0.051 to 0.134 $gC/m^2/h$ in January and from 0.113 to 0.247 $gC/m^2/h$ in June for all four years, which was 184% to 222% percent enhancement

of the nighttime ecosystem respiration. which shows higher temperature enhanced ecosystem respiration. Daytime NEE in June was a stronger sink or smaller source in June than those in January especially for very high CO_2 growth concentrations (e.g. 650 ppm and 750 ppm). The mean maximum of NEE (highest sink) in the day for January ranged from –0.176 $gC/m^2/h$ to -0.379 $gC/m^2/h$ for January and from –0.216 $gC/m^2/h$ to –0.628 $gC/m^2/h$ for June, which shows 122% to 165% increment of daily NEE. For both January and June, higher CO_2 treatment enhanced ecosystem carbon sink strength (fig. 1), the daily maximum NEE difference between lowest treatment and highest treatment for the January was 0.127 $gC/m^2/h$, while for the June was 0.267 $gC/m^2/h$, which was 210% percent increment. This indicates that the effects of elevated CO2 was stronger during growing season (e.g. June) verse now-growing season (e.g. January).

Annual net ecosystem exchange CO_2 fertilizer effect in the 1997 to 2000 (fig. 2) indicated that there were significant CO_2 treat effect (p=0.049) and precipitation/year effect (p=0.000) (table 1) , while there is no significant effect of the interaction of CO_2 and year effect on the annul NEE. The strongest CO2 fertilizer effect is in 1999, for the highest CO2 treatment (750 ppm), the CO2 fertilizer effect was 1104 $gC/m^2/h$, which is more than 3 times more than that in 1998. Annual gross primary productivity (GPP) in 1997 to 2000 (fig. 3) also showed that there is a significant CO_2 effect (p=0.000) and year effect (P=0.003), while no CO_2 and year interaction effect (p=0.928). The CO_2 effect (as deduced from the slope of the regression line) on annul carbon flux were significant different on the four year basis, which shows during the driest year (1999, with the

precipitation 247mm fig. 4), the CO_2 effect is the highest (as the slope is steepest), and it is the lowest for the wettest year. (1998, with the precipitation 725mm, fig. 4) (fig. 3).

The gross primary productivity (GPP) shows there was a tend for the increment for the both with CO_2 concentration increment and year increment (fig. 3) although there were some exception like GPP in 1999 for most treatment showed less than 1998 (wettest year) and GPP under 550 ppm CO_2 in 1999 (driest year) was significant lower than 450 ppm treatment. These were also due to the reason stated above.

Seasonal pattern of the elevated CO_2 (550 ppm) effect on plant water relation can also be seen in the predawn water potentials (fig. 4). The seasons were divided into two for statistically analysis: dry (monthly precipitation less than median) and wet (monthly precipitation greater than median). There is a significant season effect (comparing dry season and wet season) on the water potential (p=0000), no significant CO_2 treat effect in both dry season (p=0.074) and wet season (p=0.055). However, if just comparing the two different treatments by chambers, there was significant difference in the dry seasons (p=0.027) although there was no significant difference in wet season (p=0.308). There was significant difference between plant water potential in FACE (treated by 550 ppm CO_2) FACE control (ambient 365ppm) in the dry season (p=0.017) and no significant difference in wet season (p=0.423). There is significant difference for the chamber treatment and chamberless treatment at both 550 ppm (p=0.037) and 350 ppm treatment (p=0.041) in the dry season only. This indicated that both elevated CO_2 and chamber can delay water stress.

Plant morphological response

There is no significant CO_2 effect on plant growth and LAI although there is a trend they are higher in higher CO_2 growth concentration (Table 2). The low number of replicates (n=2) reduced the statistical power, and the chaparral ecosystem above ground response to the environment is very slow (Black, 1987), which might mask the CO_2 effect temporally. Belowground studies at the same site showed there is significant CO_2 enhancement for the root biomass especially for upper layers (Treseder *et al.* personal communication).

Discussion

In global change researches, it is still an elusive goal to generalize the responses of different plant species to elevated atmospheric CO_2 concentrations. However, different ecosystem or species might respond differently because of the unique character and environmental factors (Pearcy & Ehleringer, 1984; Curtis *et al.*, 1989). From this study, we knew that the average annual NEE of all treatments showed 136.5 $gC/m^2/h$, 58.4 $gC/m^2/h$, 338.8 $gC/m^2/h$, -45.4 $gC/m^2/h$ for 1997, 1998, 1999 and 2000 respectively, which was the tend to increase sink strength to the atmospheric CO_2 when the plants were getting older and bigger, except for 1999, which is the driest year for the record. The average of the NEE of the four years for different CO_2 444.8 $gC/m^2/h$, 357.3 $gC/m^2/h$, 234.3 $gC/m^2/h$, 302.6 $gC/m^2/h$, -65.4 $gC/m^2/h$, -68.8 $gC/m^2/h$ for the six different CO_2 concentration treatments (from 250 ppm to 750 ppm). There is a tend for the CO_2 enhancement as the CO_2 concentration increases except for 550 ppm treatment, which

was highly due to the air conditioning system failure in 1999 almost killed the plants inside one of 550 ppm CO_2 treated chambers which made the average NEE in 1999 was a strong source to the atmosphere.

The results reported here indicate that chaparral ecosystems are likely to increase carbon sequestration under potential future elevated $CO_{2,}$ which increased water use efficiency that might avoid the water stress of the plant and the entire ecosystem (Strain & Thomas 1995). Both daily NEE and annul NEE response to elevated CO_2 shows strong seasonal pattern, which is, during the wet seasons, there is no significant difference for the NEE response to elevated CO_2, while during dry seasons there is a significant difference for the elevated CO_2 and ambient CO_2 treat. This is consistent to leaf level scaled carbon flux measurement. Net photosynthetic measurements at the growth CO_2 concentration (A_{growth}), show significant effects of the CO_2 treatments on net photosynthetic rates (Ibanez *et al.* 2001). Elevated CO_2 enhancing effects varied by season and year, with the greatest CO_2 effect during the dry and growing seasons.

Photosynthesis rate was consistently enhanced on average by 66% by reviewing of experiments with trees treated by plus 300 μmol mol^{-1} elevated CO_2 (Norby et al., 1999). A primary response of C_3 plants to elevated atmospheric CO_2 concentrations is believed to increase in the net assimilation rate and an associated decrease in the transpiration rate at leaf scale (Morrison, 1987; Kimball et al., 1995). Intrinsic differences in photosynthetic biochemistry structure should lead to markedly different responsiveness to elevated CO_2 which is supported by theory and early field studies (Pearcy & Ehleringer, 1984; Curtis *et al.*, 1989).

The results reported here also show that the elevated CO_2 enhancing NEE (fig. 1 and fig. 2) cannot be detected by aboveground morphological responses (table 2), while there is a significant CO_2 effect on root biomass Several studies have suggested that more carbon is fixed due to a larger increase in leaf-level or canopy-level NEE in systems exposed to elevated CO_2 than could be subsequently found in plant biomass and soils (Norby, *et al.* 1992, Körner *et al.* 1996, Treseder *et al.* 2001). Interestingly, there is an significant carbon sink for one of 650 ppm treatment, but no new growth was detected in that year. The results agree with findings for the Eurasian *Avena barbata* in Californian grassland (Jackson *et al*. 1995) and for *A. barbata*, *Aegilops cylindrica and Aegilops neglecta* in southern France (Navas *et al*. 1995, 1997) .There is no significant difference regarding biomass production of these species treated by 700ppm CO_2 compared to current ambient CO_2 concentration. To detect all the carbon flux and carbon allocation through the whole ecosystem, we need to make the simultaneous, accurate measurement of the systematic carbon dynamics (input and output). However, considering the chaparral situation, rocky soil and deep roots, it will await more effort in studying belowground carbon dynamics.

In water-limited ecosystems, the effects of elevated CO_2 on Carbon balance often involve interactions with water (Owensby *et al*. 1999). Combined with the FACE water potential results, it is clearly that there is significant difference for the ambient CO2 treatment and elevated CO_2 treatment in the dry season by analyzing the chamber treatment or chamberless treatment separately. There is no significant difference in the wet season. The results also showed that there is a strong chamber effects on plant stem

predawn water potential, especially in dry season. Plants in a close chambers environments may have less water loss since the relative humidity in the chambers was substantially higher than outside environment.

In conclusion, for *A. faciculatum* growing in the nature field, the effect of elevated CO_2 on net ecosystem exchange is generally increasing carbon uptake with the increase of the CO_2 although there were some exception. There is a strong seasonal pattern of this effect that is during relative dry season or dry year, the CO_2 effect is bigger than the relative wet scenario. There is no significant difference for the plant morphological response detected. There was a big variation for the different CO_2 effect, to get more confident on what the effects of CO2 on the complicated ecosytem, a longer *in situ* experiments are strongly suggested.

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Figure 1. Mean (±SE) average daily net ecosystem exchange (NEE) ($gC/m^2/h$) for chaparral ecosystem (dominated by *Adenostoma fasciculatum* with the age 5-8 years) in Sky Oaks of Southern California in January (wet and non growing season) and June (dry and growing season) between 1997 and 2000.

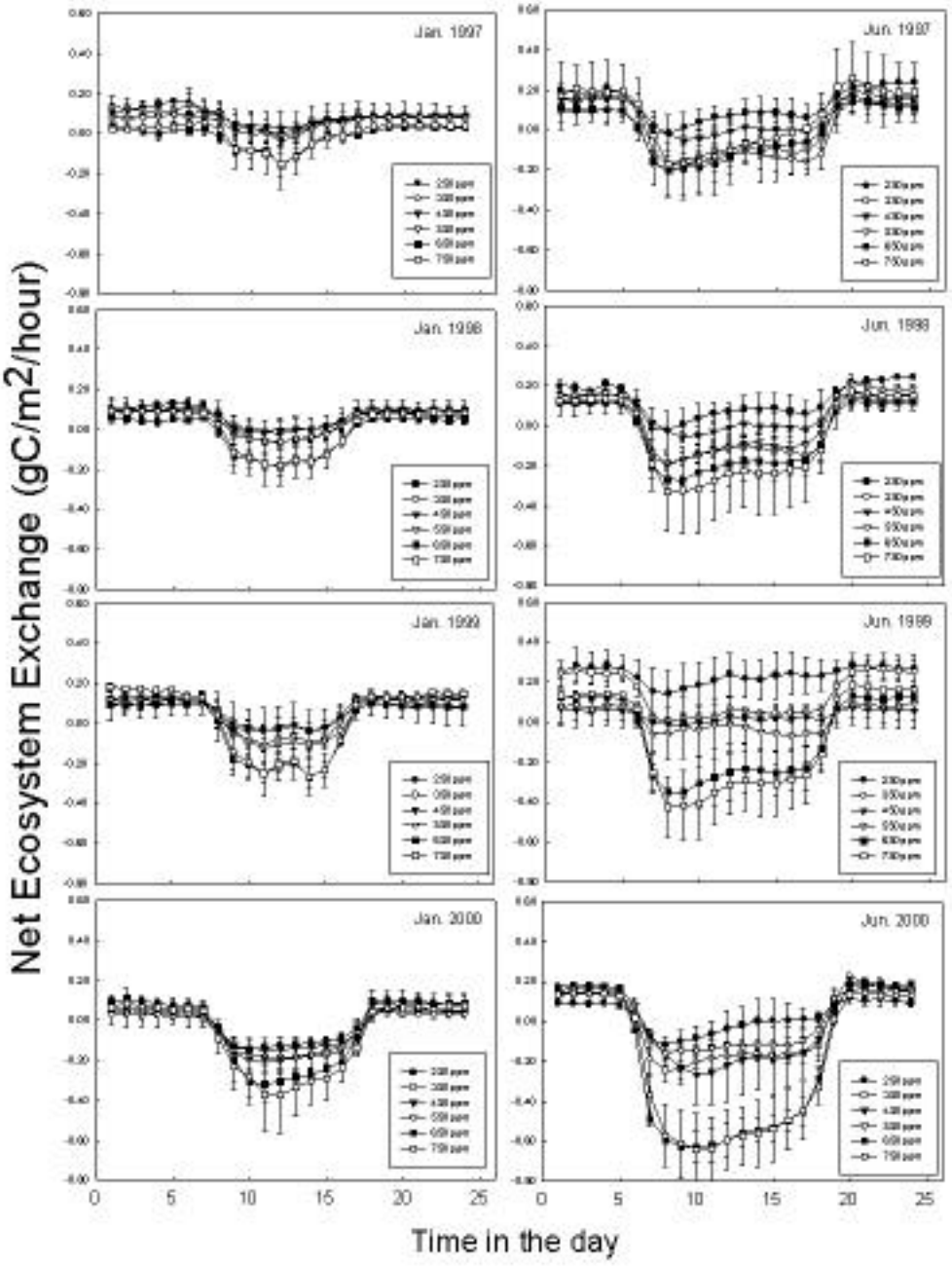
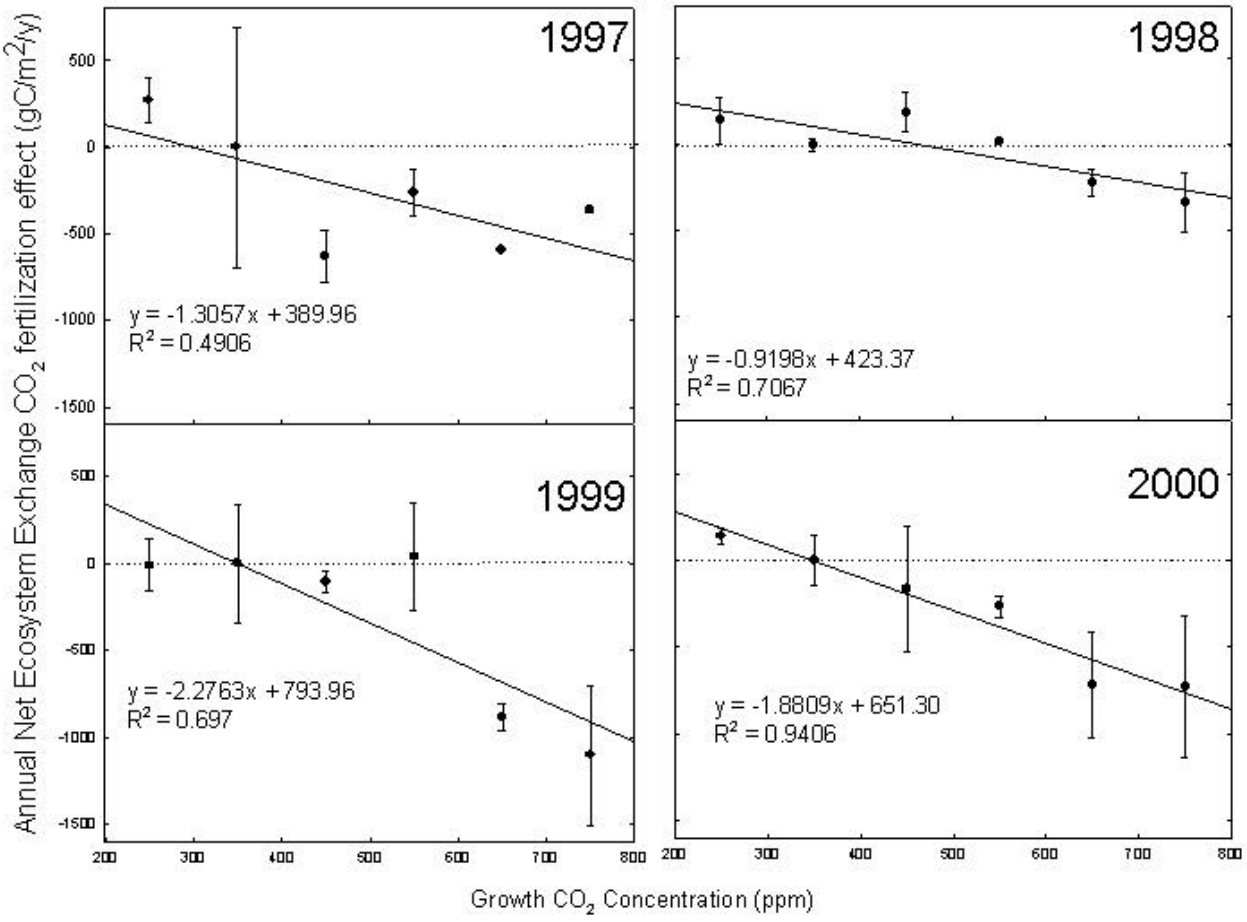


Figure 2. Mean (±SE) annual net ecosystem exchange (NEE) fertilization effect ($gC/m^2/y$) verse growth CO_2 treatment for chaparral ecosystem (dominated by *Adenostoma fasciculatum* with the age 5-8 years) in Sky Oaks of Southern California between 1997 to 2000. The CO_2 fertilization effect is the difference between the annual NEE at different CO_2 treatment and ambient treatment (350 ppm). Regression line and its r^2 are showed in each graph for different year.



N=2.

Figure 3. Annual Gross Primary Productivity (GPP) ($gC/m^2/y$) verse growth CO_2 treatment for chaparral ecosystem (dominated by *Adenostoma fasciculatum* with the age 5-8 years) in Sky Oaks of Southern California between 1997 to 2000.

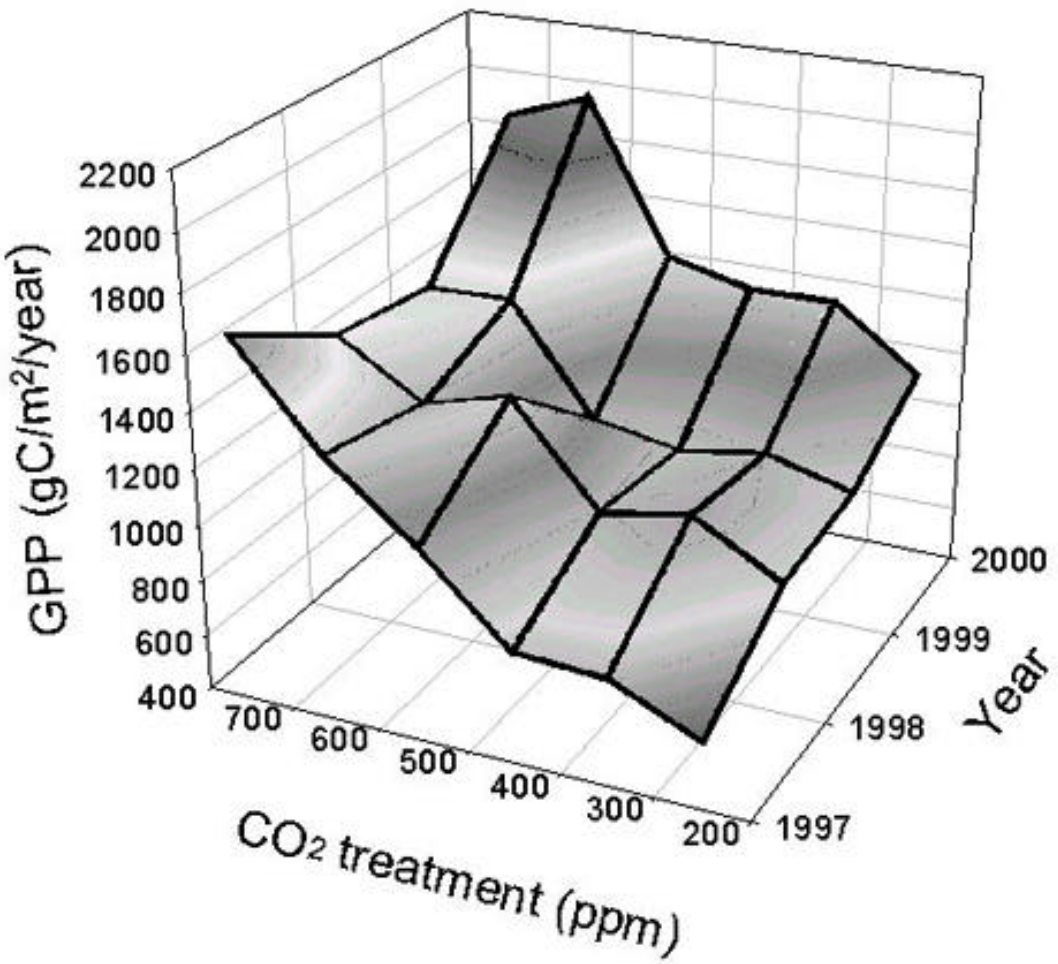


Figure 4. Monthly accumulative precipitation (mm) in Sky Oaks Biological Field Station from the weather station near null balance chambers from January 1997 to December 2000. The annual precipitations are 415mm, 725mm, 247mm and 234mm for year 1997, 1998, 1999 and 2000 respectively.

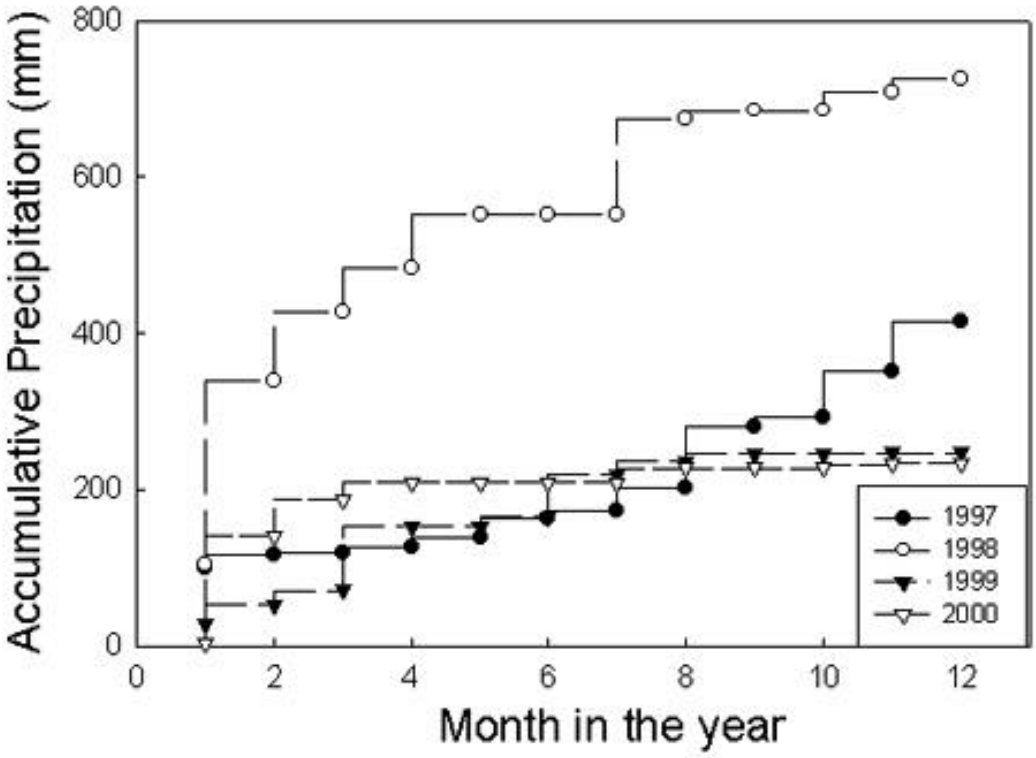
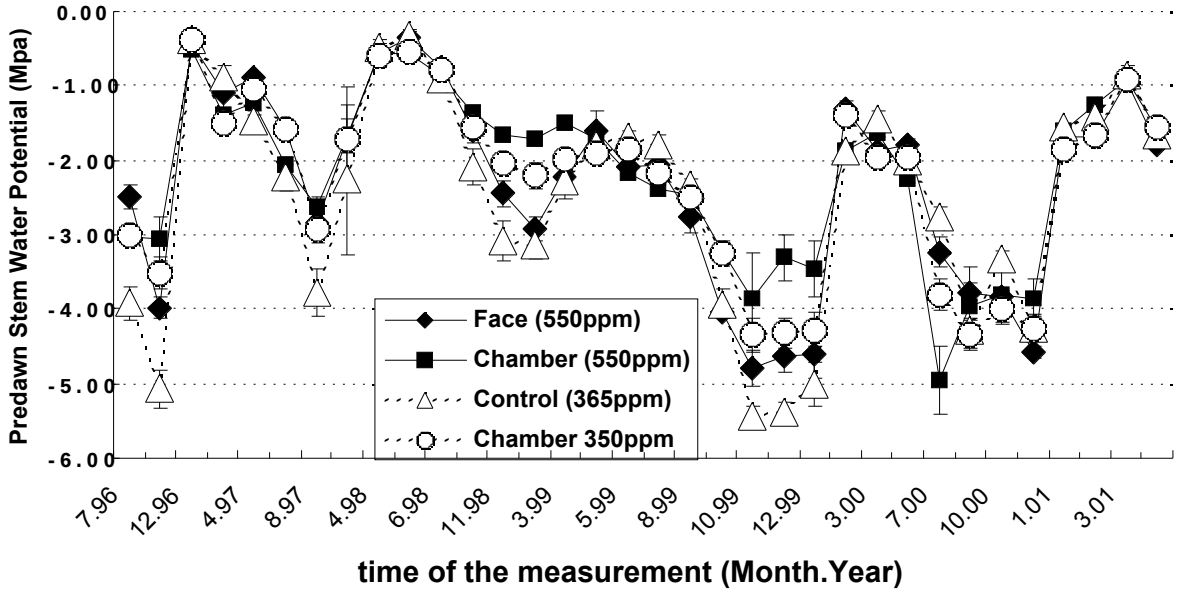


Figure 5. Mean ± SE of predawn stem water potential measured by Pressure bomb under elevated CO_2 (550ppm treated by free air CO_2 enrichment (FACE) and chambers) and ambient (by FACE control (C) and chambers maintained at 350ppm between 7-96 to 4-01. There is a significant season effect on the water potential (p=0000), no significant CO2 treat effect in both dry season (monthly precipitation less than medium) (p=0.074) and wet season (monthly precipitation greater than medium)



(p=0.055).

Table 1. General linear model analysis (two-way ANOVA) of the annual NEE with CO_2 treatment and annual precipitation effect and the CO_2 cross year effect.

Source	Sum-of-squares	df	Mean-Square	F-ratio	P
Annual precipitation	1295776.345	3	431925.448	3.639	0.027
Treat (CO_2)	4017924.567	5	803584.913	6.771	0.000
Treat _ Annual precipitation	1535612.010	15	102374.134	0.863	0.609

Table 2. Plant stem growth and leaf area index of *Adenostoma fasciculatum* individuals grown at different CO_2 concentrations by CO_2 null balance chambers.

parameter	year	Growth CO2 concentration						P-Value	R-square
		250ppm	350ppm	450ppm	550ppm	650ppm	750ppm		
Stem growth	1997	3.35	8.8	8.5	4.2	36.8	18.4	0.151	0.427
	1998	7.25	5.27	9.3	5.61	9.39	4.51	0.752	0.016
	1999	0.75	1.6	2.1	0.52	2.8	0.18	0.508	0.002
	2000	1.25	2.17	4.34	0.62	4.9	2.37	0.479	0.010
leaf area index	1997	1.06	1.54	0.65	0.94	1.45	1.80	0.357	0.215
	1998	1.25	2.33	1.05	1.37	2.08	1.64	0.203	0.027
	1999	1.19	2.56	1.19	1.29	2.35	1.59	0.319	0.016
	2000	1.09	2.29	1.23	1.30	2.44	1.62	0.403	0.087

Negative feedbacks on global change already exist from a Mediterranean-type ecosystem

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The increase in atmospheric CO_2 of approximately 30% since the beginning of the industrial revolution (IPCC, 1995) and the potential links to recent and potential greenhouse warming (Mann, *et al*., 1998; Osborne *et al*., 2000) are well known. Much less certain are the feedbacks that could affect the rate of growth in atmospheric CO_2 (Woodward, 2002), and its effectiveness in surface warming (IPCC, 1995; Gielen and Ceulemans, 2001). In this study we show that increases in atmospheric CO_2 that have occurred in recent decades are sufficient to enhance net ecosystem carbon sequestration in a Mediterranean-type ecosystem as well as the rate of biogenic hydrocarbon emissions. This is the first report that CO_2 increases since the industrial revolution are sufficient to have already increased biogenic volatile organic compound (BVOC) emissions which are relevant not only because of their impact on air quality, but because they are precursors to

airborne particulates (aerosols). It is well recognized that aerosols can increase reflectance of incoming shortwave radiation and thereby act to cool the surface in opposition to the direct effects of greenhouse active trace gasses (Charlson *et al*., 1992; Russell, Hobbs and Stowe, 1999; Andreae and Crutzen, 1997). The enhancement of net ecosystem carbon sequestration also tends to provide a negative feedback on greenhouse warming. Critical to accurate prediction of future patterns of growth of CO_2 in the atmosphere, and concomitant global warming, is a robust knowledge of feedbacks that may act to ameliorate this increase of atmospheric $CO_{2,}$ or its effectiveness in surface warming.

Mediterranean-type ecosystems are predicted to be especially sensitive to elevated CO_2 and global climate change (Strain and Bazzaz, 1983; Moreno and Oechel, 1992) but their response to increasing atmospheric CO_2 remains largely unexplored. In particular, the role of rising CO_2 in the emission of biogenic volatile organic compounds is a subject of active debate since the production of these trace gasses by the vegetation is of importance for local and regional tropospheric ozone pollution and the occurrence of complex feedback effects on climate. This study analyzed the CO_2 response of a regenerating chaparral ecosystem containing the resprouting shrub species *Adenostoma fasciculatum*, the most common species of the southern California chaparral, and the obligate seeder, *Ceanothus greggii*, a nitrogen fixer. This natural plant community regenerating after fire has been exposed for 4 consecutive years to CO_2 concentrations from preindustrial (250 μmol mol^{-1}) to double ambient (750 μmol mol^{-1}) CO_2 levels in 100 μmol mol^{-1} increments in naturally lit null-balance chambers (Oechel *et al*., 1992)

configured to precisely track ambient conditions (except for the variable of study, CO_2). During the measurement periods, Net Ecosystem Exchange (NEE) responded to higher treatment CO_2 levels (Fig.1) with a positive linear increase. The ecosystem was a C-sink at the highest CO_2 concentration both in December and in June. However, when exposed to sub-ambient and ambient CO_2 concentrations these regenerating ecosystems were a small sink in June and a source in December. CO_2 concentration was positively correlated with NEE both in autumn and in spring. In agreement with previous work, monoterpenes were the main biogenic hydrocarbons emitted by the plants in this study with only traces of isoprene (Winer *et al*., 1992). Eighteen different compounds were identified and are detailed in Table 1 with α-pinene, camphene, β-myrcene, p-cymene and limonene, accounting for more than 70% of the total emission. The composition of the compounds emitted, as a fraction of the total emissions, was not affected by the CO_2 concentration. Total trace gas emissions exhibited a diurnal pattern with increasing values in the morning and a maximum at midday (Fig.1). In winter, with maximum photosynthetically active radiation (PAR) of 1200 μmol m^{-2} s^{-1} and temperatures ranging from 0 to 14° C, emission levels expressed on a ground area basis, were low (mean = 1.04 x 10^{-5} g h^{-1} m^{-2}) and did not show any correlation with CO_2 concentrations. However, in June, with higher light levels (PAR maximum of 2000 μmol m^{-2} s^{-1}) and warmer temperatures (ranging from 15 to 30° C) BVOC emissions were an order of magnitude greater (mean = 2.13 x 10^{-4} g h^{-1} m^{-2}) and responded positively to rising CO_2 concentrations (Fig.1). Emission rates varied during the daytime but the cumulative daily emission increased with CO_2 from 1.56 x 10^{-3} g m^{-2} at 250 μmol mol^{-1} to 2.67 x 10^{-3} g m^{-2}

at 750 μmol mol^{-1} CO_2. Emission rates and NEE were well correlated throughout the day (Fig.1) while the overall carbon loss due to monoterpene emission was negligible ranging from 0.002 to 0.3% of the net carbon gain at the ecosystem level, independent of the season of measurement. Based on the experimental results, early summer production of reactive volatile organic compounds may have already increased by 15% since the beginning of the industrial revolution and NEE in the more productive early summer period should have almost doubled. Furthermore, the data indicate that BVOC emissions will be further enhanced by an additional 26% concomitantly with a four-fold increase in C-uptake in response to the predicted increment of atmospheric CO_2, by another 50% in this century (Oechel *et al*., 1995).

The total annual estimated global emission of biogenic hydrocarbons of 1,150 $TgCyr^{-1}$, dominates over anthropogenic non-methane organic emissions (Guenther *et al*., 1995) and the degradation of these reactive biogenic compounds affects directly and indirectly the atmospheric concentration of air pollutants and greenhouse gases such as ozone, carbon monoxide and methane (Fehsenfeld *et al*., 1992). Recent modeling simulations of the impact of VOC on tropospheric chemistry indicate that biogenic hydrocarbon oxidation increases the longevity of methane and carbon monoxide at the surface and, in the presence of air pollutants, almost doubles the net photochemical production of ozone in the troposphere (Poisson, Kanakidou and Cruzen, 2000). Biogenic emissions are of particular relevance in an environment characterized by high temperature and high radiation, typical of regions with Mediterranean-type climates (Seufert *et al*., 1995; Thunis and Cuvelier, 2000). These areas are often well populated or

over populated because of the favorable climatic conditions and, accordingly, are already known to be major source of anthropogenic pollutants capable of promoting the chemical transformation of VOCs leading to photochemical pollution. It is therefore obvious that if the stimulation of anthropogenic and biogenic VOC emissions that have occurred in the recent past continue in the future, there will likely be serious consequences for mean tropospheric ozone concentrations. On the other hand, a significant atmospheric aerosol-forming potential of the most prevalent biogenic hydrocarbons has been established and characterized under simulated laboratory conditions (Griffin *et al*., 1999; Hoffman *et al*., 1997; Virkkula *et al*., 1999). Moreover, *in situ* experiments of gaseous and particulate atmospheric species demonstrated that under natural atmospheric conditions, photo-oxidation of terpenes yields relatively non-volatile secondary products (i.e. organic acids) which condense to form organic aerosols (Yu *et al*., 1999; Pio, Alves, and Duarte, 2001; Kavouras, Mihalopoulos and Stephanou, 1998; 1999; Kanakidou *et al*., 2000). These airborne particles affect climate by scattering and absorbing solar shortwave radiation and thus generating feedback mechanisms for the planetary radiation balance (Dowd, 2001). Model calculations indicate that aerosols can cause a net negative climate forcing (cooling of the atmosphere) which could partially offset the current anthropogenic greenhouse gas warming of the atmosphere (Climate Change, 1994).

In conclusion, a predicted increase of BVOC flux to the atmosphere on the order of 5% per decade will potentially trigger photochemical reactions of the unsaturated hydrocarbons. These changes will affect the oxidative balance of the troposphere, the photochemical ozone formation and the secondary organic aerosol production. While

relative growth rates are unknown, these increases in BVOC are in addition to any increases in anthropogenic VOC emissions over the coming decades, and both sources of increases in VOC are likely to be significant.

Methods

Measurements of the volatile organic compounds were made by collecting 2-3l of the air surrounding the vegetation grown inside the chambers into two-stage glass traps. Sampling was carried out in the morning, at midday and in the afternoon to follow the diurnal course of the emissions and repeated for three days in June and December. Separation, identification and quantification of the collected hydrocarbons were done by means of thermal desorption gas chromatography and mass spectrometry (Baraldi *et al*., 1999). Environmental control and CO_2 manipulation were conducted in null balance, environmentally controlled, field chambers similar to those previously described (Oechel *et al*., 1992; Oechel *et al*., 2001). Response of BVOC emissions are a combination of overall plant response to elevated CO_2, and include effects of growth CO_2 on emission rates and leaf area development. A similar combination of responses, and a similar net response is expected in nature. However, changes in temperature would be expected to exacerbate the effect reported here. Changes in species composition with climate change which changes the mix of BVOC emitting species to relatively non-emitting species would also effect the emissions under expected future conditions.

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Table 1. Percent composition of volatile organic compounds identified in chaparral emissions

Compounds	December 1999 (%)	June 2000 (%)
Isoprene	0.9 ± 0.1	3.8 ± 1.0
α-thujene	0.6 ± 1.1	3.6 ± 1.8
α-Pinene	22.0 ± 1.6	22.3 ± 1.1
Camphene	16.0 ± 1.5	12.7 ± 2.0
Sabinene	5.7 ± 1.0	4.2 ± 1.9
β-Pinene	5.2 ± 0.1	4.3 ± 0.9
β-Myrcene	2.4 ± 0.1	11.2 ± 1.1
α-Phellandrene	0.1 ± 0.1	1.0 ± 0.1
Δ 3-Carene	0.3 ± 0.1	0.2 ± 0.1
α-Terpinene	0.4 ± 0.1	0.7 ± 0.3
ρ-Cymene	2.5 ± 0.1	16.3 ± 3.2
β-Phellandrene	0.5 ± 0.1	1.6 ± 0.2
Limonene	37.0 ± 2.5	12.9 ± 1.2
Cis-o-cymene	0.2 ± 0.1	0.5 ± 0.1
Trans-o-cymene	0.3 ± 0.2	1.0 ± 0.3
γ-Terpinene	1.0 ± 0.3	2.1 ± 0.6
α-Terpinolene	0.3 ±0.1	0.8 ± 0.2
Linalool	0.4 ± 0.2	0.4 ± 0.1
Camphor	0.6 ± 0.1	0.3 ± 0.1

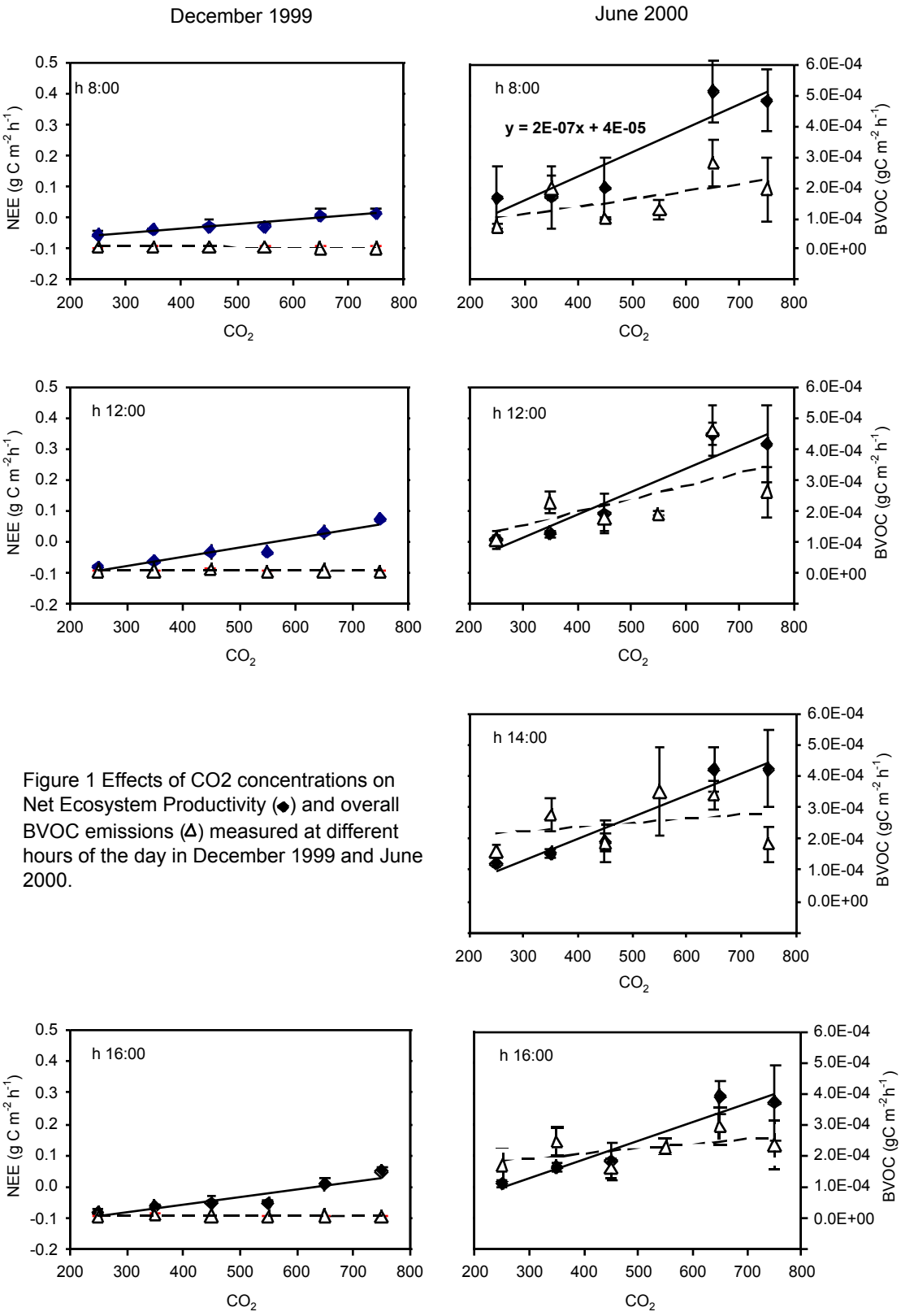

Figure 1 Effects of CO2 concentrations on Net Ecosystem Productivity (◆) and overall BVOC emissions (Δ) measured at different hours of the day in December 1999 and June 2000.

^{13}C tracers of carbon allocation to roots under a CO_2 gradient in intact southern Californian chaparral

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Abstract

We examined root dynamics in intact chaparral exposed for 3.5 years to atmospheric CO_2 levels ranging from subambient (250-ppm) to more than twice ambient (750-ppm) in 100-ppm intervals. Because the fossil fuel-derived CO_2 used for CO_2 enrichment was depleted in ^{13}C relative to the atmosphere, stable isotope tracers allowed us to tease apart the fates of carbon fixed before and after the inception of CO_2 enrichment. Bulk root biomass (including both live and dead roots) increased 7-fold with elevated CO_2, and this increase was significantly more pronounced in the upper soil layers. The factors underlying this increase appeared to vary with CO_2 level. Based on root signatures of ^{13}C, accumulation of recently-fixed root C in the highest (750-pm) treatment seemed to contribute to relatively high bulk root carbon, potentially due to increased production of roots constructed after initiation of the experiment or reduced turnover of new roots. In contrast, a relatively high accumulation of older C in roots appeared to raise bulk root carbon in intermediate (450- and 550-ppm) CO_2 treatments. Root diameters declined significantly with increasing CO_2, from 0.53- to 0.34-mm in the

250- and 750-ppm treatments, respectively. The relatively large CO_2 effect in chaparral may be attributable to the arid nature of this environment and may have a marked effect on contribution of roots to soil C dynamics in chaparral.

Keywords: ^{13}C stable isotopes, chaparral, elevated CO_2, root biomass, soil carbon

Introduction

Numerous studies have examined effects of elevated atmospheric CO_2 on root dynamics in crop systems, forests, and grasslands. The majority report increases in root biomass or production (reviewed in Bazzaz 1990; Berntson and Bazzaz 1996; Pritchard and Rogers 2000; Rogers et al. 1992; Rogers et al. 1994; Tingey et al. 2000) with some exceptions (Arnone et al. 2000). Responses of other systems to CO_2 manipulation are less well understood, especially for long-term field-based experiments (Norby 1994). Arid and semi-arid lands, in particular, constitute about 16% of net primary production and can be important components of global carbon cycling (Roberts et al. 1998). Natural ecosystems in the southwestern United States, such as southern Californian chaparral, can be particularly water-limited; net primary productivity (below- and above-ground) here may respond strongly to increases in water use efficiency typically conferred by CO_2 enrichment (Bazzaz 1990). It is also possible that any augmentation of plant growth may enhance demand for water (Berntson and Woodward 1992), with a consequent downward shift in the vertical distribution of roots in chaparral to access deeper water sources. Mean root diameter may also decrease to improve foraging efficiency for water (Norby 1994; Tingey et al. 1995). These potential changes in the allocation of biomass belowground could alter soil carbon dynamics, and, potentially, carbon storage (Norby 1994; Rogers et al. 1994; Van Kessel et al. 2000a; Van Kessel et al. 2000b) and are considered in global change models (Woodward and Osborne 2000).

Documented changes in standing root biomass could be a result of several factors, including increased root production, increased longevity, or decreased decomposition

rates (Pregitzer et al. 1995; Tingey et al. 2000). Studies that have explicitly measured root growth generally have reported increases in woodlands (Pregitzer et al. 1995; Tingey et al. 2000) and crops (Pritchard and Rogers 2000; Rogers et al. 1992), but not necessarily in native grasslands (Arnone et al. 2000). Moreover, increases in biomass or production can occur disproportionately in the top layers of soil in crop plants (Chaudhuri et al. 1990; Prior et al. 1994; Pritchard and Rogers 2000) natural grasslands (Arnone et al. 2000), forests (Larigauderie et al. 1988), and other systems, though not always (Berntson and Woodward 1992; Chaudhuri et al. 1986; Rogers et al. 1992; Thomas et al. 1999). Root longevity and decomposition rate tend to vary in response to elevated CO_2 (Allen et al. 2000; Johnson et al. 2000; Pregitzer et al. 2000; Thomas et al. 1999; Verburg et al. 1998), and generalizations at this point are difficult (Arnone et al. 2000; Pritchard and Rogers 2000). As such, static measures of root biomass provide only a limited understanding of mechanisms underlying CO_2 effects on belowground biomass and carbon storage (Pritchard and Rogers 2000).

Because the fossil fuel-derived CO_2 used in studies of CO_2 enrichment is depleted in ^{13}C relative to the atmosphere, stable isotope tracers allow us to tease apart the fates of carbon fixed before and after the inception of CO_2 enrichment (Hungate et al. 1996; Nitschelm et al. 1997; Van Kessel et al. 2000a; Van Kessel et al. 2000b). With this approach, we may focus on the integrated allocation to roots of recently acquired C and assess changes in root processes in addition to changes in root biomass. We examined root dynamics in intact chaparral exposed for 3.5 years to atmospheric CO_2 levels ranging from subambient (250-ppm) to more than twice ambient (750-ppm) in 100-ppm intervals.

This experimental design conferred novel aspects to our study by allowing us to consider plant responses over CO_2 concentrations ranging from pre-Industrial Revolution levels to those anticipated to occur within the next century. In addition, since all treatments received ^{13}C-depleted CO_2 to stabilize CO_2 levels, we were able use stable isotopes of carbon as a tracer at even the lower CO_2 concentrations; stable isotope analyses in most other CO_2 studies have been restricted to the CO_2-enriched treatments only. We hypothesized that elevated CO_2 would (1) increase root biomass, (2) shift root allocation to deeper soil layers, (3) reduce root diameter, and (4) increase allocation of recently-fixed carbon to roots.

Materials and methods

Site

The Sky Oaks CO_2 enrichment study (operated by San Diego State University) is located near Temecula, California in chaparral vegetation dominated by *Adenostoma fasciculatum* (chemise) shrubs. Twelve closed 2- x 2- x 2-m chambers are each centered around an individual of *Adenostoma* and its surrounding herbaceous plants. Atmospheric CO_2 concentrations within the chambers are maintained at levels ranging from 250- to 750-ppm in 100-ppm increments, with two replicates for each treatment. Ambient CO_2 levels are about 360-ppm. Concentrations of CO_2 are measured every three minutes, and CO_2 is scrubbed or augmented to maintain appropriate levels. As a result, CO_2 is occasionally added to even the lower CO_2 levels. The chambers are naturally lit (about 93% of ambient), and temperature is maintained near ambient (Oechel et al. 1992).

Precipitation is collected from the chamber roof and channeled inside to the soil surface through several small tubes. The site was burned in 1992, and CO_2 enrichment started in December 1995.

Sample collection

In October 1999, we obtained soil from the chambers with 4-cm diameter corers for measurements of root biomass. One core was collected from underneath the canopy of each *Adenostoma* at 5-, 10-, 20-, 30-, 40-, 50-, 60-, 70-, and 80-cm depths. Samples were stored on ice for transport back to the laboratory.

Root sampling

Root collections were made to measure the bulk (live + dead) biomass of roots in the each layer of soil. In addition, bulk and live roots from the 5-cm depth samples were subjected to stable isotope analyses, measurements of carbon concentrations, and assessments of root diameter. To obtain live *Adenostoma* roots (as an estimate of the ^{13}C signature of recently-fixed C), we used forceps to sift through whole soil and pull out any roots encountered. These roots were washed three times in deionized water. At least five live *Adenostoma* roots < 1-mm diameter were selected from each sample based on color, morphology, and texture, and these were oven-dried at 60 °C for 72 hrs, and isotopic signatures were measured as described below. We could not collect enough root material from one of the 550-ppm chambers to measure ^{13}C accurately, so we report only one replicate for that treatment. To determine pre-treatment ^{13}C signatures of root material, we also selected live *Adenostoma* roots from five soil cores obtained from an undisturbed area about 50-m from the chambers.

To obtain roots for biomass measures, soil samples were sieved through 1-mm mesh, and material remaining on the sieve was examined. Root fragments greater than 4-mm in length were selected regardless of condition or species identity. These roots were washed three times in deionized water. A precision caliper was used to measure the diameter of each root from the 5-cm depth samples. All roots were then oven-dried at 60 °C for 72 hrs and weighed. Root biomass at each depth was calculated as dry weight per unit area of soil. Roots from the 5-cm depth samples were then analyzed for ^{13}C content and %C as detailed below.

Stable isotope analyses and calculations

To determine $d^{13}C$ and %C of roots, oven-dried samples were ground to a fine mesh, enclosed in tin capsules, and run through a continuous flow mass spectrometer coupled with a gas chromatograph and elemental analyzer (Europa Integra, Stable Isotope Facility, University of California – Davis). $d^{13}C$ is calculated as $[(^{13}C/^{12}C_{sample})/(^{13}C/^{12}C_{standard})–1] \times 1000‰$, where $^{13}C/^{12}C_{sample}$ and $^{13}C/^{12}C_{standard}$ are the isotope ratios of the sample and standard (Pee Dee Belemnite), respectively (Ehleringer and Osmond 1991).

The tank CO_2 added to the chambers was derived from fossil fuels, which are depleted in ^{13}C relative to the atmosphere ($d^{13}C$ of added CO_2: –20‰; atmospheric CO_2: –8‰). This difference in isotopic signature enabled us to trace the C that had been added to the system during the CO_2 experiment (referred to as “new C”). We used a mass balance equation to calculate the amount of new C in bulk roots. The $d^{13}C$ signature of belowground C in each treatment can be represented as a linear equation in which the

mean $d^{13}C$ of live *Adenostoma* roots from each chamber is used as the signature of the "new" C (fixed since onset of experiment) and the mean $d^{13}C$ of live roots from a nearby undisturbed area represents the "old" C (fixed before CO_2 enrichment):

$$d^{13}C_{bulk\ roots} = [(d^{13}C_{old} * \%old\ C) + (d^{13}C_{new} * \%new\ C)] / 100,$$

where $d^{13}C_{bulk\ roots}$ is the signature of live + dead roots; $d^{13}C_{old}$, the isotopic composition of old C; $d^{13}C_{new}$, the signature of new C; %old C, the proportion of C that is old; and %new C, the proportion of C that is new. Total amounts of old versus new C in bulk roots were estimated using percent new C and measurements of root biomass and %C:

$$New\ C = (\%newC/100) * (\%C/100) * (biomass) * (depth),$$

where %newC represents the percentage of root C that is new; %C, the concentration of C in roots; biomass, live + dead root weight (g/cm^2); and depth, the depth to which soil was collected (5-cm). Old C is calculated as total root C minus new root C. This tracer method has some limitations—the live roots we selected to represent the signature of new C may include some roots present before the onset of CO_2 enrichment 3.5 years earlier. Alternately, stored old C may have been transferred to live roots grown during the experiment. In either case, we would overestimate to amount of new C in bulk roots. In addition, the stable isotope approach would not detect C that had both entered and exited the system during the experiment.

Statistics

Relationships between each dependent variable (e.g. root biomass, root $d^{13}C$, etc.) and CO_2 concentration were tested though linear or non-linear regression analyses using Systat10 (SPSS 2000b) and SigmaPlot 2000 (SPSS 2000a). For root diameter, the mean

from each chamber was used for the regression. To test for significant depth by CO_2 interactions on bulk root biomass, data were ranked and subjected to a fully factorial analysis of variance (ANOVA) with CO_2 and depth as independent variables and biomass as the dependent variable. We also performed linear regressions separately on samples from each depth. To display results, we grouped samples by 20-cm depths (0- to 20-cm, 20- to 40-cm, etc.). We employed a Kruskal-Wallis test to assess significant differences in ^{13}C signatures among bulk roots, live roots, and roots from the undisturbed area. Pairwise comparisons were conducted using a Kolmogorov-Smirnov test. In all cases, results were considered significant when $P < 0.05$.

Results

Biomass, carbon content, and morphology of bulk roots

Bulk root biomass (including both live and dead roots) increased with elevated CO_2, and this increase was significantly more pronounced in the upper soil layers. Total biomass (0- to 80-cm) rose significantly and 7-fold with increasing CO_2 (Fig. 1a; $P < 0.015$; $r^2 = 0.510$). Specifically, root biomass increased significantly at 0- to 5-cm (Fig. 1b; $P < 0.046$; $r^2 = 0.341$) and at 5- to 10-cm (data not shown; $P < 0.005$; $r^2 = 0.625$) depths. In contrast, at lower depths, we found no significant increases with CO_2 (Fig. 1a). As a result, CO_2 concentration and soil depth interacted significantly on root biomass ($F_{15, 24} = 5.450$; $P < 0.0001$), indicating an overall shift in rooting depth upward. Total carbon sequestered in roots from the uppermost soil layer (0- to 5-cm) increased 6-fold with elevated CO_2 (Fig. 1b; $P < 0.035$; $r^2 = 0.372$), even though %C in root tissue did not vary

significantly (data not shown). Root diameters declined significantly with increasing CO_2, from 0.53- to 0.34-mm in the 250- and 750-ppm treatments, respectively (Fig. 2; $P < 0.027$; $r^2 = 0.401$), although intra- and inter-chamber variation was high in the lower treatments.

Carbon dynamics in roots

The increase in standing root biomass with CO_2 could have been a result of increased accumulation of new roots or decreased disappearance of old roots. Because recently produced "new" roots had a lower ^{13}C-content than "old" roots present before CO_2 manipulation, we were able to tease apart these effects in the top 5-cm of soil. The original ^{13}C signature of roots (before onset of the experiment) was indicated by the $d^{13}C$ of live roots collected outside the chambers (Fig. 3; –26.9‰ ± 1.2‰ SE); roots in the chambers that had been constructed before CO_2 manipulation began should have had that approximate signature. Live roots collected from the chambers were significantly more depleted in ^{13}C (Fig. 3; –31.7‰ ± 0.8‰; $P < 0.001$). Bulk (live + dead) roots from the chambers had intermediate signatures (Fig. 3; –30.6‰ ± 0.8‰) that were significantly different from roots representing original signatures ($P < 0.001$) but not significantly different from live roots in the chambers. In addition, ^{13}C contents of live roots from the chambers decreased significantly with CO_2 concentration (Fig. 3; $P < 0.043$; $r^2 = 0.380$) while bulk roots did not.

Based on the ^{13}C signatures of roots from the top 5-cm of soil, we calculate that across treatments, 80.2% ± 11.7% SE of carbon in bulk roots had been fixed since the onset of the experiment. The percentage of "new" carbon in bulk roots did not change

significantly with treatment (Fig. 4). Four chambers displayed proportions of new C that were greater than 100%; this result occurred wherever live roots where more ^{13}C-enriched than bulk roots from the same chamber due to the relatively high degree of variability in signatures. Because the pool of C in bulk roots increased with elevated CO_2 (Fig. 1b), total amounts of "new" root C (on a per meter basis) increased significantly as well (Fig. 5a; $P < 0.012$; $r^2 = 0.482$). In contrast, total amounts of "old" C in roots were significantly higher in the intermediate-level CO_2 treatments, with a best-fit non-linear regression of

$OldC = 31\ e^{-0.5\left(\frac{CO_2 - 509}{58}\right)^2}$ (Fig. 5b; $P < 0.022$; $r^2 = 0.574$).

Discussion

In this chaparral ecosystem, elevated CO_2 increased bulk root biomass in upper soil layers (Fig. 1a,b), and the factors underlying this increase appeared to vary with CO_2 level. Based on root signatures of ^{13}C, a greater accumulation of "new" root C in the highest (750-pm) treatment (Fig. 5a) seemed to contribute to relatively high bulk root carbon there, potentially due to increased production of roots constructed after initiation of the experiment ("new" roots) or reduced turnover of new roots. In contrast, a relatively high accumulation of "old" C in roots appeared to raise bulk root carbon in the intermediate (450- and 550-ppm) CO_2 treatments (Fig. 5b). Decomposition of roots constructed prior to onset of CO_2 manipulation ("old" roots) may be slowed in those treatments. Alternately, old roots may have longer lifespans under these conditions. While stable isotopes are helpful in determining relative patterns of these processes across treatments, the absolute amounts of new or old root C derived from this approach

should be interpreted with caution for several reasons. First, $d^{13}C$ signatures of bulk and live roots, as well as roots from outside the experimental chambers, were highly variable (Fig. 3) and even differed in live roots by as much as 6.4‰ between the two 750-ppm chambers. This variation reduces the precision of our estimates accordingly. Second, live roots from the chambers may have included roots produced before the onset of CO_2 manipulation. If so, we overestimate allocation of new C to bulk roots. The degree of this error would depend on the longevity of fine *Adenostoma* roots, which is unknown. If fine roots turn over within months to a few years, error is likely to be small. However, if they tend to live longer 3.5 years (the length of CO_2 enrichment before sampling), we may have significantly overestimated the absolute amount of new C in standing roots. Likewise, if plants allocated stored old C to newly-produced roots, we would also have overestimated amounts of new C. Gaudinski et al. (2000) found that roots in a mixed deciduous forest in central Massachusetts received only recently-fixed C, but C dynamics in chaparral may not be comparable. For these reasons, we use ^{13}C signatures only to indicate the relative importance of production and turnover across CO_2 treatments, and not necessarily as an estimate of flux rates or pool sizes (Ehleringer et al. 2000).

Why would root production or longevity increase under elevated CO_2 in chaparral, as indicated by ^{13}C signatures of roots? As is thought to occur in other systems, an increase in water use efficiency conferred by CO_2 enrichment may have ameliorated water limitation and extended the growing season in this water-stressed environment (Arnone et al. 2000; Bazzaz 1990; Berntson and Woodward 1992); augmentation of below- and above-ground productivity—or increased investment in root maintenance—is a possible

result. In addition, a higher demand for nutrients following increased growth may have contributed to this effect. The observed enhancement of root biomass is consistent with the findings of the majority of CO_2 studies, although the magnitude of the increase (7-fold) in our study is much greater than usual. For example, reviews of CO_2 studies report increases as high as 58% in native grasslands (Arnone et al. 2000) and 200–300% in agricultural systems (Rogers et al. 1992; Rogers et al. 1994). The relatively large CO_2 effect in chaparral may be attributable to the arid nature of this environment.

Potential reasons for the increase in amounts of old C at intermediate CO_2 concentrations are not straightforward (Fig. 5b). In this system, we have found that mycorrhizal abundance is highest at intermediate levels, and then declines at 750-ppm (K. Treseder, unpublished data). The presence of these fungi may have influenced root longevity, although it is not clear why old roots and not new roots would be affected. Alternately, the composition of the general community of soil microbes may have shifted under the CO_2 gradient such that decomposers may have become less effective at 450- and 550-ppm. We know of no other study that has examined the fate of old C at intermediate CO_2 levels.

We observed a shift in distribution of roots upward (Fig. 1a); this result has also been found in crops (Chaudhuri et al. 1990; Prior et al. 1994; Pritchard and Rogers 2000), natural grasslands (Arnone et al. 2000), and forests (Larigauderie et al. 1988). However, we expected that rooting depth would increase in the chaparral so that plants could tap water in deeper horizons; soil in this site is dry and powdery at the surface for most of the year. Instead, plants may have increased foraging capacity for nutrients in the upper

layers in response to increased nutrient demand. There was also a shift in root morphology toward finer roots (Fig. 2) that tend to be more efficient at procuring nutrients from the soil. A decrease in root diameter has been observed in other systems (Norby 1994; Tingey et al. 2000).

Since turnover rates of C can vary with soil depth due to changes in temperature and moisture, and decomposition rates of roots may increase as diameter decreases, these shifts in root architecture could have implications for carbon dynamics in the soil. Namely, the flux of C through roots may increase. Coupled with the increase in pool size of C in standing root biomass via greater accumulation of new C and decreased removal of old C, elevated CO_2 may have a marked effect on contribution of roots to soil C dynamics in chaparral. We suggest that other natural, water-stressed systems may have strong responses to CO_2 enrichment and merit closer examination, especially to improve our understanding of large-scale C cycling under global change.

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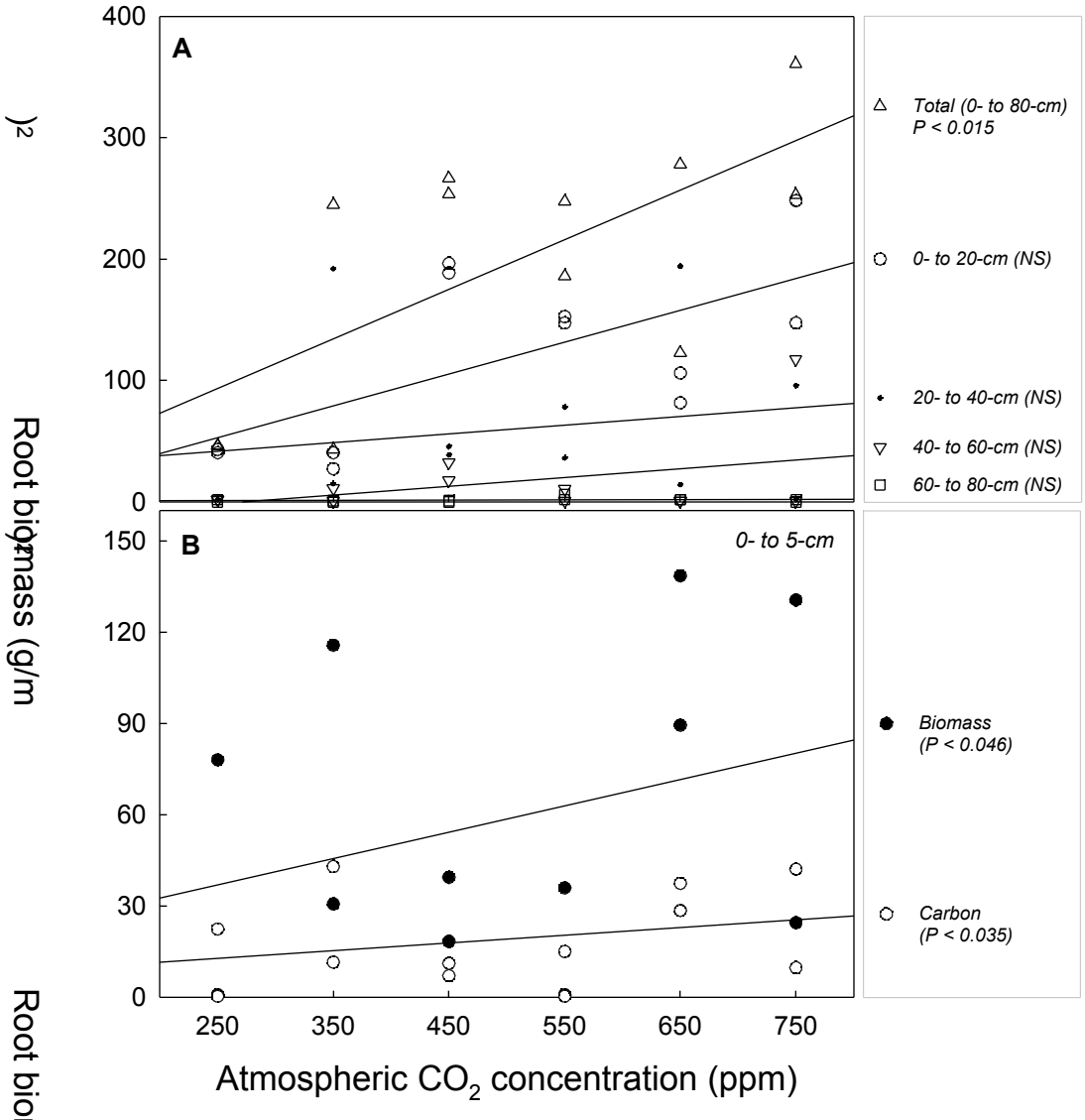

Figure 1. Biomass and carbon of bulk (live + dead) roots from (a) the top 80-cm of soil and (b) the top 5-cm of soil. Each symbol represents one chamber; lines indicate best fit linear regressions.

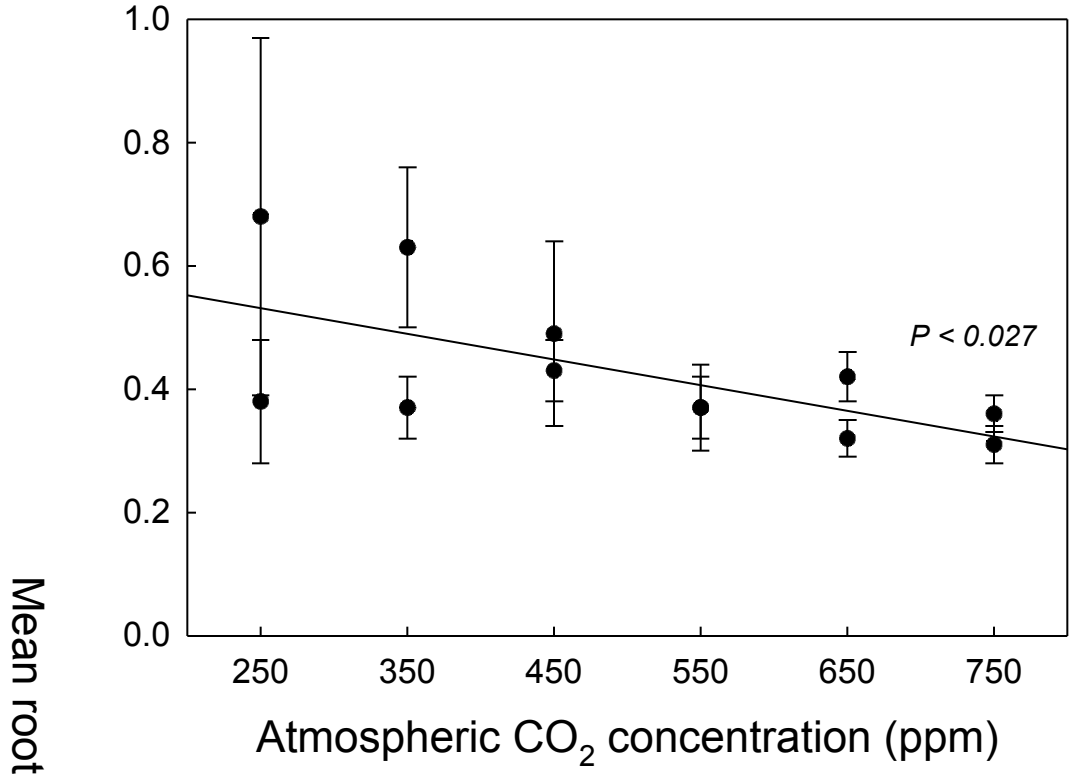

Figure 2. Variation in diameter of bulk roots with CO_2 level. Symbol represent means (± 1 SE) of all roots collected within each chamber; the line indicates a best fit linear regression through the means.

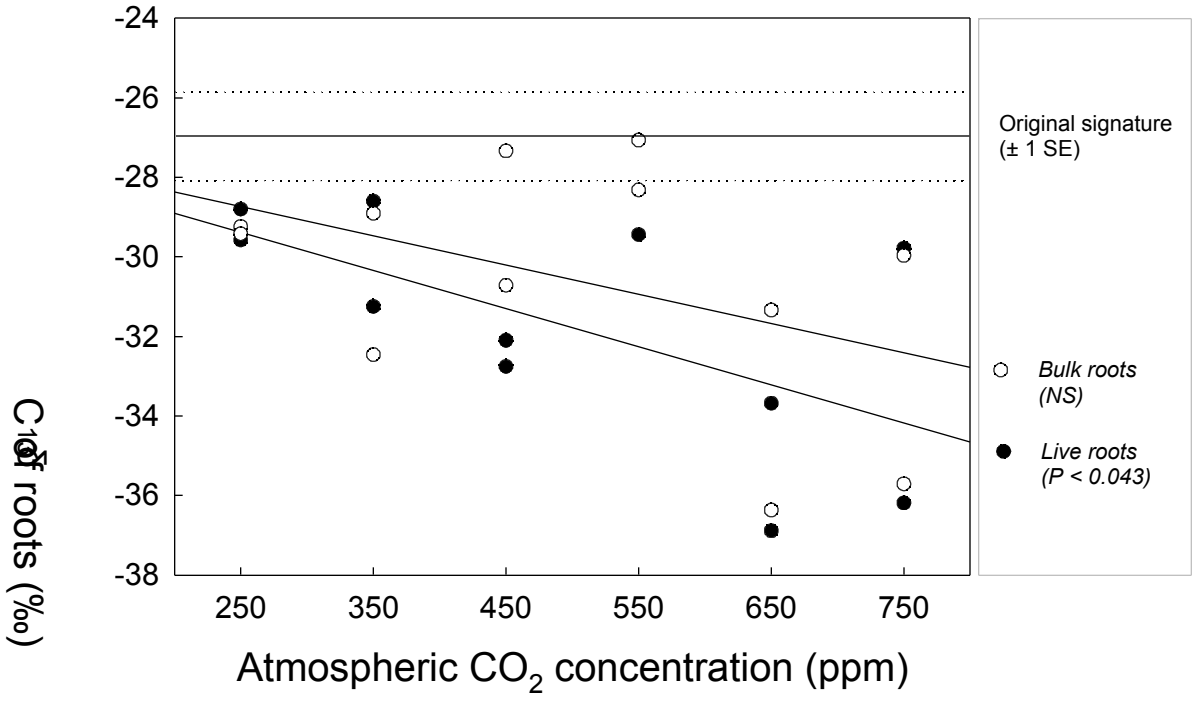

Figure 3. d^{13}C signatures of roots from the top 5-cm of soil. Bulk roots (❍) represent all roots (live and dead) in chambers. Live roots (●) indicate the signature of recently-fixed C in the chambers. Each symbol represents one chamber. Best fit linear regressions through bulk and live roots are represented by lines, as is the ^{13}C content (±1 SE) of roots collected outside the chambers (representing the "original signature" of roots before CO_2 fumigation commenced).

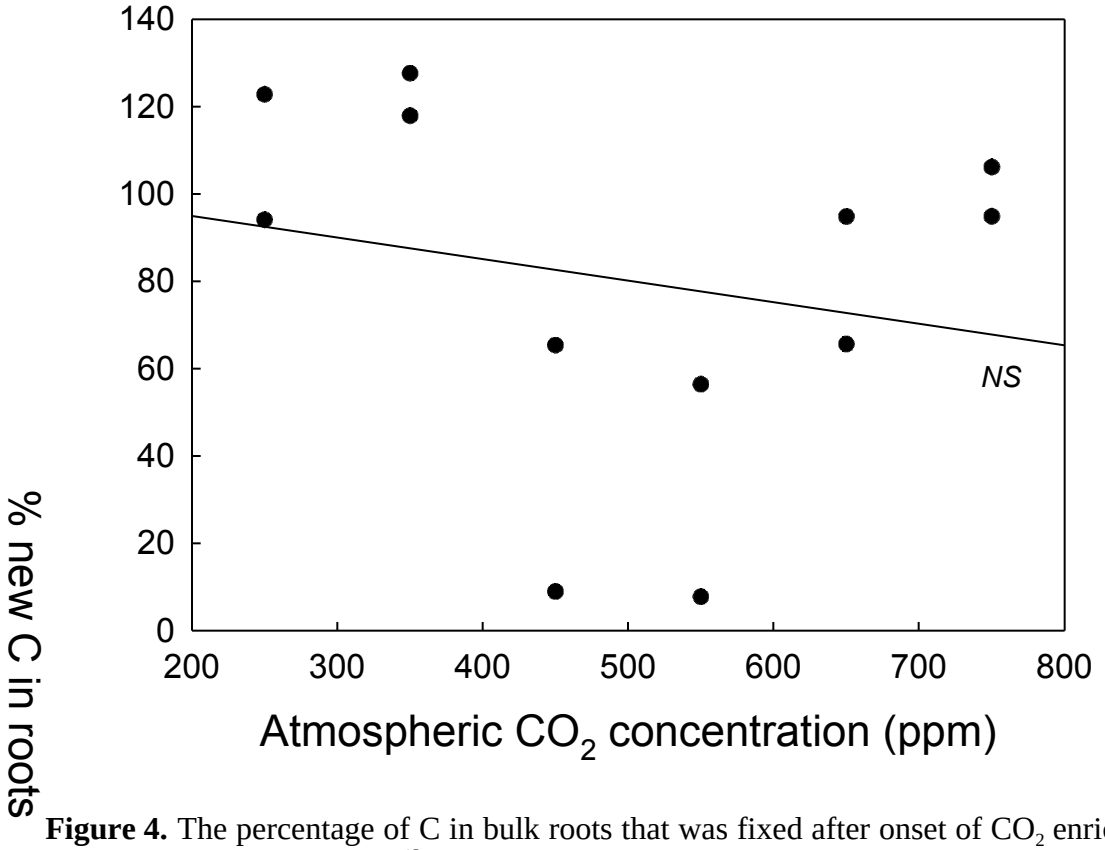

Figure 4. The percentage of C in bulk roots that was fixed after onset of CO_2 enrichment ("new" C), calculated from ^{13}C signatures. Each symbol represents one chamber. The line indicates a best fit linear regression through all points. NS = no significant CO_2 effect.

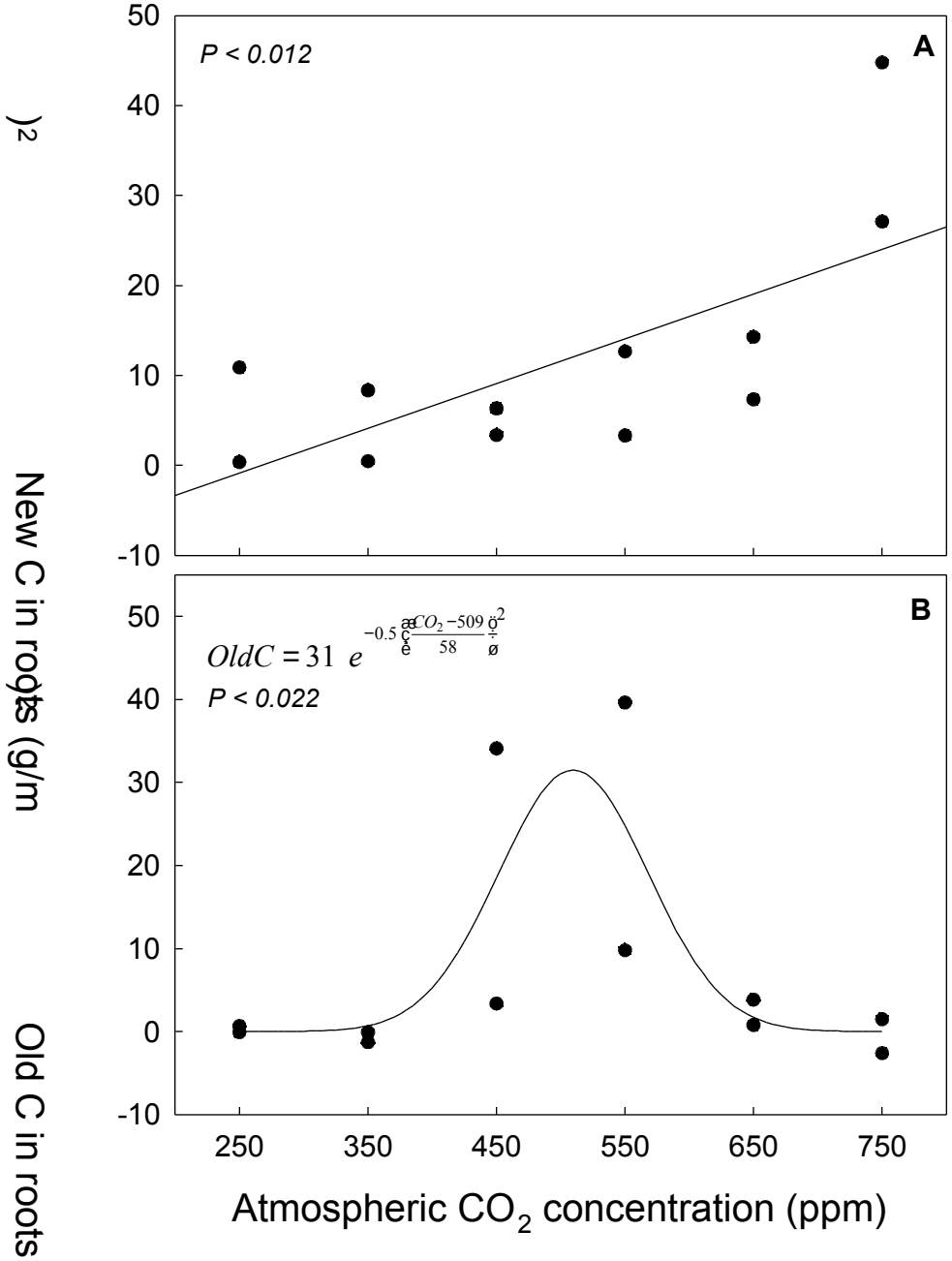

Figure 5. Pools of C in bulk roots that were fixed (a) after onset of CO_2 enrichment ("new" C), and (b) before onset ("old" C). Each symbol represents one chamber. Lines indicate a best fit linear regression in (a) and non-linear regression in (b).

Alteration of soil carbon by communities of mycorrhizal fungi in a chaparral ecosystem exposed to elevated CO_2

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Abstract

We examined the potential of arbuscular mycorrhizal (AM) fungi to increase soil carbon storage under elevated atmospheric CO_2. With antisera labeling, we assessed the community composition and total lengths of live AM hyphae associated with soil macroaggregates under varying CO_2 levels. We then used stable isotopes to calculate C input to these macroaggregates. Carbon concentrations and AM hyphal lengths were correlated and rose 30-fold and 10-fold, respectively, with increasing CO_2. The AM genera *Scutellospora* and *Acaulospora* responded strongly and positively to CO_2 concentration, while *Glomus* and *Gigaspora* did not. AM fungi appear to increase macroaggregate-C under CO_2 enrichment, possibly due to changes in community structure as well as stimulation of hyphal production. The C content of bulk soil may rise as macroaggregates disintegrate and release C into other soil fractions.

Introduction

Carbon allocation, sequestration, and turnover in terrestrial ecosystems remain critical issues in global carbon modeling and ecosystem science. In particular, changes in soil carbon dynamics under atmospheric CO_2 enrichment are not well understood. The possibility that soils may partially sequester carbon emitted through fossil fuel burning and deforestation has been the subject of much recent attention and debate (e.g. Schlesinger 1990; Hungate *et al.* 1996; Trumbore 1997; Batjes 1998; Schlesinger 1999). By focusing on the responses of various soil organisms to elevated CO_2, we may gain a greater understanding of the mechanisms that may (or may not) produce this potential scenario.

In this paper, we address the influence of mycorrhizal fungi (microbes that symbiotically colonize plant roots) on soil C dynamics under CO_2 enrichment. Numerous greenhouse and field chamber experiments have indicated that elevated atmospheric CO_2 typically stimulates growth of mycorrhizal fungi (reviewed in Diaz 1996; Hodge 1996; Staddon and Fitter 1998; Cairney and Meharg 1999) with varying responses among genera (Klironomos *et al.* 1998). These fungi acquire all or most of their C directly from the host plant. Estimates of C allocation to mycorrhizal fungi range from 5 to 85% of net primary production, with 10 to 20% considered typical (Allen 1991). Arbuscular mycorrhizal (AM) fungi, in particular, are widespread. This group associates with over 80% of the world's plant species ranging from the tropics to the arctic and from deserts to rainforests (Smith and Read 1997). In addition, AM fungi are the sole producers of the

glycoprotein glomalin (Wright and Upadhyaya 1996; Wright *et al.* 1996; Wright *et al.* 1998; Wright and Upadhyaya 1999), and their cell walls contain large portions of chitin and chitosan (Muzzarelli 1977). These compounds are relatively recalcitrant to decomposition and could be long-lived in the soil (Gooday 1994; Wright and Upadhyaya 1996). Thus, much of the C allocated to AM fungi may not be rapidly mineralized to CO_2. To date, the capacity of mycorrhizal fungi to alter soil C content under elevated CO_2 has been frequently suggested (e.g. Allen *et al.* 1995b; Rygiewicz *et al.* 1997; Staddon and Fitter 1998; Rillig *et al.* 1999b; Rillig and Allen 1999) but not directly examined.

Mycorrhizal fungi also play a critical role in the formation of soil structure. Their hyphae bind together soil particles to form macroaggregates (1−2 mm diameter) which remain stable when wet (e.g. Tisdall and Oades 1979; Oades 1984; Miller and Jastrow 1990; Oades and Waters 1991; Jastrow *et al.* 1998). After hyphae deteriorate, residual glomalin may maintain these connections (Wright *et al.* 1998; Rillig *et al.* 1999b). Macroaggregate-rich soils tend to be resistant to wind and soil erosion (Emerson 1977; Lyles *et al.* 1983). In addition, because the organic material within these clusters is physically protected from microorganisms and external chemical processes, macroaggregates often differ from bulk soil in C turnover times and concentrations of organic C (Tisdall and Oades 1982; Jastrow 1996; Jastrow *et al.* 1996). A recent study has reported that macroaggregates are more prevalent under elevated CO_2 in two Californian ecosystems, most likely due to an increase in the mass of mycorrhizal glomalin (Rillig *et al.* 1999b).

In this study, we consider the role of AM community composition and biomass in soil carbon storage under elevated CO_2. We evaluated the abundance and composition of AM hyphae occupying the macroaggregates and bulk soil from a chaparral ecosystem exposed to CO_2 levels ranging from 250- to 750-ppm. Stable isotope techniques were then used to link AM hyphal biomass to rates of C input with increasing CO_2. These data were used to test the hypotheses that CO_2 enrichment (1) alters the amount of incorporated mycorrhizal biomass (and hence C allocation) within macroaggregates, and (2) modifies the AM community profile, and (3) in turn, such changes contribute to increased soil C storage with elevated CO_2.

Materials and methods

Site

The Sky Oaks CO_2 enrichment study (operated by San Diego State University) is located near Temecula, California in chaparral vegetation dominated by *Adenostoma fasciculatum* (chemise) shrubs. Twelve closed 2- x 2-x 2-m chambers are each centered around individual *Adenostoma* and surrounding herbaceous plants. Atmospheric CO_2 concentrations within the chambers are maintained at levels ranging from 250- to 750-ppm in 100-ppm increments (n = 2). The chambers are naturally lit (about 93% of ambient), and temperature is maintained at ambient levels (Oechel *et al.* 1992). Precipitation is collected from the chamber roof and channeled inside. In addition, a separate 10-m diameter free-air CO_2 enrichment (FACE) ring maintains CO_2 levels within the ring at 550-ppm. An undisturbed area slightly uphill from the FACE ring serves as a control (about 350-ppm). The site was burned in 1992, and CO_2 enrichment started in December 1995.

Sample collection and macroaggregate extraction

In May 1999, we gathered about 2-g of full sun-exposed, green *Adenostoma* leaves from the four cardinal directions of each plant, and we compiled soil from four 5-cm deep pits located at each cardinal direction within the canopy of those *Adenostoma* individuals. In June 1999, we collected leaf and soil samples from *Adenostoma* in the FACE ring and control area (n = 5). Soil was stored at 4°C for transport back to the laboratory, where particles greater than 2-mm were sieved out and discarded. The

remaining “bulk” soil was divided into three subsamples. For measurement of stable isotopes and C concentrations in bulk soil, one set of 5-g subsamples was oven-dried at 60°C for 72 hours. Leaves were also oven-dried for isotope analyses.

Within three hours of collection, water stable aggregates 1-2 mm in diameter were extracted from the second set of subsamples by wet-sieving as detailed in (Jastrow 1996). Fifty grams of soil were sieved through 1-mm mesh. Particles > 1-mm diameter were slowly immersed in deionized water at 20°C and sieved for 20-min through 1-mm mesh while agitating at 30 cycles min^{-1}. Aggregates that did not pass through the mesh were oven-dried at 60°C for 72 hrs, weighed to determine macroaggregate mass, and used for stable isotope analysis. Macroaggregate extraction was performed on the third sample (also 50-g), except the macroaggregates were stored at -20°C until used for immunofluorescence analysis of the mycorrhizal community.

Stable isotope analyses and calculations

To determine $d^{13}C$ and %C of plant tissue and soil, oven-dried samples were ground to a fine mesh, enclosed in tin capsules, and run through a continuous flow mass spectrometer (Europa Integra, Stable Isotope Facility, University of California – Davis). $d^{13}C$ is calculated as $[(^{13}C/^{12}C_{sample})/(^{13}C/^{12}C_{standard})-1] \times 1000‰$, where $^{13}C/^{12}C_{sample}$ and $^{13}C/^{12}C_{standard}$ are the isotope ratios of the sample and standard (PeeDee Belemnite), respectively (Ehleringer and Osmond 1991).

We used a mass balance equation to calculate the amount of new C in macroaggregates and bulk soil. The $d^{13}C$ signature of macroaggregate-C in each treatment can be represented as a linear equation in which the $d^{13}C$ of *Adenostoma* is used

as the signature of the "new" C (fixed since onset of experiment) and the least-negative $d^{13}C$ of soil from the control area represents the "old" C (fixed before CO_2 enrichment):

$$d^{13}C_{macro} = [(d^{13}C_{old} * \%old\ C) + (d^{13}C_{new} * \%new\ C)] / 100,$$

where $d^{13}C_{macro}$ is the signature of macroaggregate-C; $d^{13}C_{old}$, the isotopic composition of old C (-24.0‰); $d^{13}C_{new}$, the signature of new C (-30.7 to -38.0‰); %old C, the proportion of C that is old; and %new C, the proportion of C that is new. The amount of new C is the product of %new C and the total C concentration of the sample. Concentrations of new C were not calculated for the FACE ring versus control, as new C had no distinct signature in the latter.

We then calculated the annual net C accumulation into macroaggregates by using estimates of percent new C and measurements of macroaggregate mass and %C:

$$C\ input = (\%macro/100) * (\%newC_{macro}/100) * (\%C_{macro}/100) * (density_{soil})/t,$$

where %macro is the portion of bulk soil comprised of macroaggregates (by weight); $\%newC_{macro}$, the percentage of macroaggregate C that is new; $\%C_{macro}$, the concentration of C in macroaggregates; t, the time since onset of CO_2 enrichment (3.5 years); and $density_{soil}$, soil bulk density (1.35 g/cm^3 for the upper 10-cm of soil). Across treatments, about 5% of soil mass (by weight) was composed of macroaggregates.

Mycorrhizal measurements

The percentage of macroaggregates and root fragments containing AM hyphae was determined by direct immunofluorescence. Antibodies were raised against each of the four major genera of AM fungi. Rabbits were immunized with whole spore fractions of *Glomus deserticola* Trappe, Bloss & Menge, *Acaulospora laevis* (Nicol. & Gerd.), *Gigaspora margarita* (Becker & Hall), and *Scutellospora calospora* (Nicol. & Gerd.) Walker and Sanders as described in (Egerton-Warburton and Allen 2000). Each antiserum was conjugated to fluoroscein isothiocyanate, and its specificity was determined by evaluating immunoreactivity between all combinations of antisera and spores of each species; no cross-reactions were detected (Allen *et al.* 1999). Duplicate samples of root fragments and entire macroaggregates from each CO_2 concentration were incubated in diluted antisera (1:1 antisera: water) in the dark for 24 hr at 20ºC, rinsed briefly in water and examined and scored under epifluorescence for the presence or absence of immunoreactive hyphae. Only live hyphae fluoresce under this stain (Friese and Allen 1991).

Macroaggregate-bound hyphae were dispersed by incubation in sodium hexametaphosphate solution (39.5 g/L) for 1 hr at 20ºC. For each sample, the extracted hyphae were collected, rinsed in deionized water, allocated to four subsamples and incubated in each of the antisera as described above. Incubated hyphae were collected by pulse centrifugation, rinsed briefly in deionized water, mounted on glass slides, and examined using epifluorescence (Egerton-Warburton and Allen 2000). All hyphae on the slides were measured and then designated as either immunoreactive (indicating the

presence of a particular genus), or non-reactive hyphae. Non-reactive hyphae were further subdivided into AM or non-mycorrhizal hyphae on the basis of color and morphology under transmitted light and differential interference contrast optics. All immunolabeling and measurements were undertaken in duplicate.

Statistics

Relationships between CO_2 treatment and either macroaggregate mass, C content, or mycorrhizal hyphal length were tested though linear regression analyses, and correlations between new C and hyphal length were examined using a Pearson test (SPSS 1998). Changes in community composition of AM fungi were assessed with a Chi-square test.

Results

Mycorrhizal abundance and community composition

We detected live AM hyphae on forty percent of macroaggregates. These hyphae could clearly be seen growing throughout the macroaggregates and intertwined with their component particles (Fig. 1c), as is often reported (e.g. Tisdall and Oades 1979; Oades 1984; Oades and Waters 1991). An additional fraction of macroaggregates was likely associated with dead hyphae that did not show a positive reaction to antisera (Fig. 1b). Fine roots can also form macroaggregates (Tisdall and Oades 1979; Oades 1984; Miller and Jastrow 1990; Oades and Waters 1991; Jastrow *et al.* 1998) and were assessed as another potential input of C into these particles. However, roots are sparse in these soils and were associated with only 2% of macroaggregates.

Hyphal biomass responded to CO_2 concentration in both the chambers and the FACE ring. Lengths of AM fungal hyphae in macroaggregates rose more than ten-fold between the 250- and 650-ppm treatment, then sharply declined at 750-ppm (Fig. 2, linear regression with 750-ppm omitted, $r^2 = 0.635$, $P < 0.006$). AM fungal biomass in macroaggregates was also greater in the FACE ring (258-mm/g) than in the control area (90-mm/g).

Shifts in community composition, as well as biomass, of AM fungi under elevated CO_2 may indirectly affect soil C dynamics. We measured the abundance of four major genera of AM hyphae in macroaggregates from the closed chambers. Hyphal length among genera differed significantly in response to increasing CO_2 (Fig. 3, $X^2 = 55.77$, df

= 15, P < 0.001). *Scutellospora* and *Acaulospora* were the rarest genera in the 250-ppm chambers, but their presence increased markedly with elevated CO_2. In particular, hyphal length of *Scutellospora* increased 50-fold in response to CO_2 treatment. In contrast, *Glomus* and *Gigaspora* had more subtle responses: *Glomus* predominated at low CO_2 levels while the abundance of *Gigaspora* varied little across treatments. Comparable shifts in AM community composition were noted in the FACE rings (data not shown).

Carbon dynamics

We assessed soil C inputs in each CO_2 treatment by using stable isotopes of C as tracers. The tank CO_2 used to augment and stabilize CO_2 levels in the Sky Oaks experiment is depleted in ^{13}C relative to the atmosphere ($d^{13}C$ of tank CO_2: approximately –29‰; atmospheric CO_2: –8‰). As a result, the $d^{13}C$ of tissue from *Adenostoma* declined significantly with greater CO_2 concentrations (linear regression, $P < 0.012$, $r^2 = 0.483$; $d^{13}C$ of *Adenostoma* = -0.0093*[ppm CO_2] – 29.9‰). The $d^{13}C$ of mycorrhizal tissue reflects that of the host plant within reasonable limits (~1.5‰;Staddon *et al.* 1999), so hyphae should have signatures similar to those of *Adenostoma*.

We found that the $d^{13}C$ of macroaggregates decreased significantly with elevated CO_2 in closed chambers ($P < 0.010$, $r^2 = 0.497$; $d^{13}C = -0.0051$*[ppm CO_2] – 23.4‰) and FACE plots (FACE ring = -26.5‰, control = -25.3‰), reflecting an incorporation of tank CO_2 (via plants) into these particles. Bulk soil was depleted in ^{13}C relative to the control area, but did not vary among treatments (average: -25.5‰, $P > 0.05$, $r^2 = 0.054$). We used the difference between signatures of ^{13}C-enriched “old” C and ^{13}C-depleted “new” C to determine the proportion of macroaggregate- or bulk soil-C that had been fixed after the

experiment started. We then calculated the amount of new C in macroaggregates or bulk soil.

Elevated CO_2 affected C accumulation in macroaggregates but not in bulk soil. We found that concentrations of new C in macroaggregates varied almost 30-fold among CO_2 treatments (Fig 2). The mass of new C was near zero in the 250-ppm treatment but increased significantly with CO_2 concentration ($P < 0.046$, $r^2 = 0.342$). In fact, the amount of new macroaggregate C at 350- and 650-ppm CO_2 was equivalent to a net accumulation of 3 versus 14 g C m^{-2} yr^{-1}, respectively. The drop in new C at 750-ppm corresponds to low hyphal biomass in that treatment, and the abundance of new C was correlated with hyphal length across CO_2 levels (Pearson correlation, $r = 0.574$, $df = 10$, $P < 0.06$). Total (old + new) C concentrations in macroaggregates increased with CO_2 (linear regression, $P < 0.091$, $r^2 = 0.259$; %C = 0.00219*[ppm CO_2] + 0.632%). Atmospheric CO_2 concentration did not significantly affect the amount of new C, total (old + new) C, or hyphae in bulk soil (linear regression; $P > 0.05$; $r^2 = 0.012, 0.042, 0.060$, respectively). Finally, we found no significant effect of CO_2 on the prevalence of macroaggregates (i.e. macroaggregate mass per unit bulk soil, linear regression, $P > 0.05$, $r^2 = 0.260$).

Discussion

Macroaggregates were commonly associated with mycorrhizal hyphae (Fig 1), but rarely with fine roots. Mycorrhizal fungi therefore appear to be the primary conduits of C into larger soil particles. We expect that the growth and decomposition of AM fungal tissue should influence the C content of macroaggregates. In particular, changes in mycorrhizal dynamics under elevated CO_2 may alter C storage in this soil fraction.

Mycorrhizal growth responded strongly to elevated CO_2. Hyphal length increased dramatically (by almost an order of magnitude) as CO_2 levels approached 650-ppm, then declined sharply (Fig. 2). The drop in hyphal length at the highest CO_2 treatment is puzzling, but very high levels of CO_2 occasionally inhibit AM fungal growth (Becard and Piche 1989). This response may have occurred in the 750-ppm treatment, especially since soil CO_2 concentrations typically exceed atmospheric levels. Many greenhouse- or growth chamber-based experiments have reported increases in mycorrhizal biomass under a doubling of CO_2 concentration (reviewed in Diaz 1996; Hodge 1996; Staddon and Fitter 1998; Cairney and Meharg 1999). However, at this time, only one other published study has examined hyphal length in intact ecosystems exposed to long-term CO_2. Rillig *et al.* (1999a) observed a nearly two-fold increase in hyphal length in a serpentine grassland (but no significant change in a sandstone grassland) after more than five years of CO_2 enrichment. Other studies more than one year in length have also noted increases in the presence of ectomycorrhizal (Tingey *et al.* 1995) and AM (Klironomos *et*

al. 1997) hyphae in open-top chambers with planted seedlings. None have directly examined the consequences of this biomass response for soil C dynamics.

We assessed the community composition, as well as the biomass, of AM fungi, since this factor could indirectly influence soil C inputs as well. Hyphal lengths of the four major AM genera shifted from a relatively even distribution at 250-ppm CO_2 to dominance by *Scutellospora* and *Acaulospora* at 650-ppm (Fig. 3). Spore biomass among genera followed a comparable pattern in this CO_2 experiment (S. Harney and M. F. Allen, unpublished data). Similarly, in a growth chamber study using single-species inoculations on potted plants, Klironomos and others (1998) demonstrated that hyphal lengths of *Scutellospora calospora* and *Acaulospora denticulata* increased significantly with elevated CO_2, but those of two *Glomus* species did not. Our experiment is the first to document changes in the distribution of AM genera under elevated CO_2 in a natural system.

The four genera of AM fungi may vary in life history characteristics and decomposability of hyphal tissue. For example, glomalin concentrations are lower in *Glomus intraradices* than in *Gigaspora rosea* (Wright and Upadhyaya 1999), which may lead to differences in decomposition rate. Similar variation may occur among the other AM genera and species (e.g. *Acaulospora* and *Scutellospora*). In addition, mycorrhizal genera and species differ in their ecological specificity and capacity to influence the growth and fitness of the host plant (e.g. Mosse 1972; Abbott and Robson 1981a; Abbott and Robson 1981b; Allen *et al.* 1995a). Shifts in mycorrhizal composition can therefore impact the structure, nutrient status, and productivity of the plant community (van der

Heijden *et al.* 1998). Through these mechanisms, changes in community composition of AM fungi could feed back to alter C cycling under elevated CO_2. Since AM community composition shifted and hyphal lengths increased in macroaggregates under elevated CO_2, we expected that C inputs to this soil fraction would change as well. Alterations in soil C dynamics due to short-term CO_2 enrichment are often difficult to measure because the pool of pre-existing C is usually large. However, by using stable isotope ratios ($d^{13}C$) to focus only on C that has been fixed under CO_2 enrichment, we could track the fate of that C (e.g. Hungate *et al.* 1996; Leavitt *et al.* 1996; Nitschelm *et al.* 1997; Lin *et al.* 1999). We calculated that C inputs to macroaggregates increased markedly (almost 30-fold) between the 250-and 650-ppm treatments, and were correlated with hyphal length (Fig. 2). These results indicate that an increase in growth of mycorrhizal hyphae under elevated CO_2 may augment macroaggregate C pools.

This increase in gain of new C could be offset by a potential rise in decomposition rates with elevated CO_2. However, total (old + new) C concentrations in macroaggregates rose under CO_2 enrichment, indicating a net augmentation in C storage in these particles. Our results are consistent with measurements of net ecosystem flux (net primary productivity minus heterotrophic respiration) in the chambers. There appears to be a net ecosystem loss of C at lower CO_2 levels, and a net gain where atmospheric CO_2 exceeds 550-ppm (Y. Cheng, S. J. Hastings, W. Oechel, unpublished material).

The amount of new C in macroaggregates (Fig. 2) is probably not solely attributable to C contained in live hyphae. Rather, the rise in C input in macroaggregates under enriched CO_2 might be due in part to an increase in the pool of residual organic C

that remains after hyphae have decomposed. Chitin, a major constituent of fungal cell walls, is resistant to decomposition (Gooday 1994) and may be a long-lived pool of organic C in macroaggregates. In addition, microarthropods graze preferentially on non-mycorrhizal versus AM hyphae (Klironomos and Kendrick 1996), which may slow turnover rates. Rillig and others (1999b) found that concentrations of glomalin, another potentially recalcitrant component of AM tissue, significantly increased with CO_2 level in Sky Oaks and other ecosystems.

This study by Rillig and colleagues (1999b) documented a significant increase in the abundance of smaller (0.25–1.0 mm in diameter), but not larger (1–2 mm) macroaggregates. We also found no response in the prevalence of the latter size fraction. The main influence of increased hyphal growth in larger macroaggregates appears to be an augmentation of C concentrations within these particles. It has been suggested that C within macroaggregates may turn over more slowly than C in bulk soil because aggregates form a physical barrier to decomposer chemicals and organisms (Tisdall and Oades 1982; Jastrow 1996; Jastrow *et al.* 1996). Supporting evidence for this mechanism is not always straightforward (Jastrow *et al.* 1996), but physical protection could act as another feedback on C sequestration in macroaggregates.

This system had been exposed to elevated CO_2 for 3.5 years at the time of sampling, so possibly only the more responsive fractions of soil (e.g. macroaggregates) were affected in this short time. Hungate and others (1996) found little response to elevated CO_2 in soil organic C and C input after 2–3 years of CO_2 enrichment in two

California grasslands, although other studies have reported increases in C input (Ineson *et al.* 1996; Nitschelm *et al.* 1997) and soil C (e.g. Leavitt *et al.* 1994; Wood *et al.* 1994; Nitschelm *et al.* 1997) within that time. Another consideration in our study is that carbon in macroaggregates represents 2 to 5% of total soil C and is not likely to have an immediate influence on the C content of bulk soil. However, macroaggregates eventually break down into smaller particles as hyphae and fine roots decompose (Oades 1984). Through this process, C allocated to macroaggregates could enter other soil fractions and potentially raise C concentrations of bulk soil. Nevertheless, it remains to be seen whether these mycorrhizal responses represent long-term trends, or if hyphal growth and/or C in macroaggregates will reach steady-state levels within a relatively short time.

Overall, our results suggest that arbuscular mycorrhizal fungi influence the response of soil C dynamics to elevated atmospheric CO_2 levels through changes in hyphal biomass. Shifts in fungal community composition may also have unforeseen consequences for carbon cycling. We note that effects on C input were limited to macroaggregates but may spread to other soil components with time. Since mycorrhizal fungi are widespread, and increases in mycorrhizal growth under elevated CO_2 have been reported in many studies, this feedback could influence C cycling at the global level and potentially slow increases in atmospheric CO_2 concentrations. Further study of mycorrhizal effects on soil dynamics (especially estimates of large-scale hyphal production and biomass under elevated CO_2) could greatly facilitate our understanding of the importance of mycorrhizal fungi to the global C cycle.

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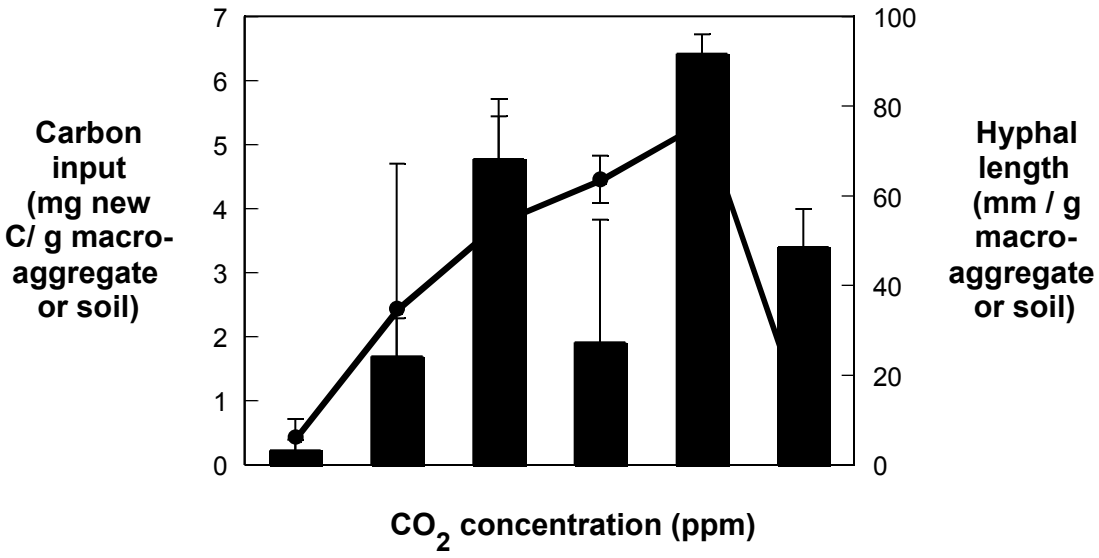
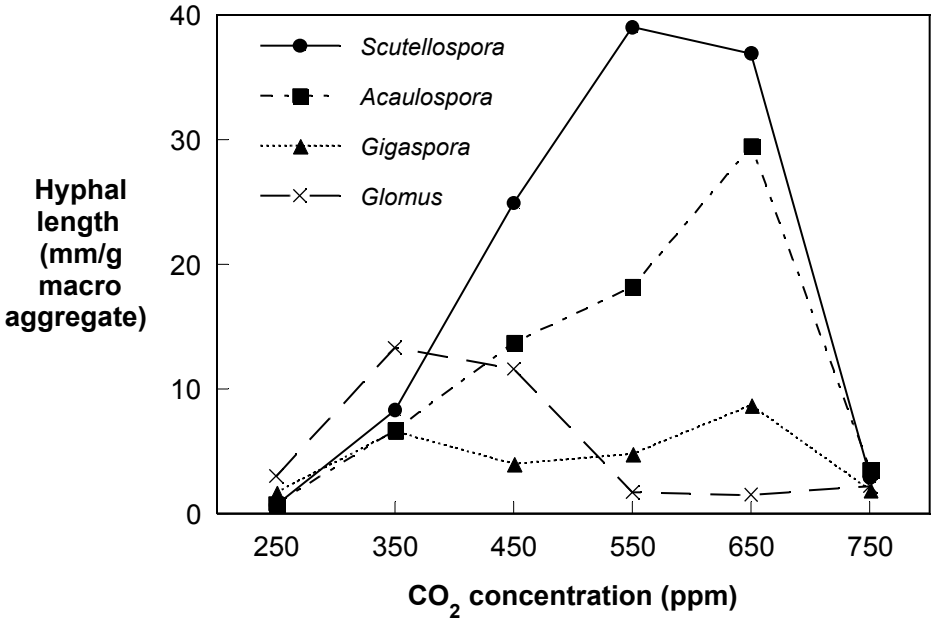
Figure legends

Figure 1. (Note: not shown). Confocal laser scanning microscope images illustrating a control macroaggregate in which arbuscular mycorrhizal (AM) hyphae are absent (a), and macroaggregates with non-immunoreactive AM hyphae bound to the surface (arrows) (b), and immunoreactive (fluorescent) hyphae of *Acaulospora* extending from a macroaggregate (arrows) (c).

Figure 2. Carbon input (lines) and hyphal length of arbuscular mycorrhizal fungi (columns) in 1-2 mm diameter macroaggregates under a range of atmospheric CO_2 levels. Points and columns represent means (±1 SE) of two chambers. Hyphal length and C input varied significantly with treatment ($P < 0.006$ and 0.046, respectively).

Figure 3. Hyphal length of four major genera of arbuscular mycorrhizal fungi in 1-2 mm diameter macroaggregates. Each point represents a mean of two chambers. Abundance of the genera changed significantly with CO_2 concentration ($P < 0.0001$)


Carbon input (mg new C/ g macro-aggregate or soil)
Hyphal length (mm / g macro-aggregate or soil)
0
1
2
3
4
5
6
7
0
20
40
60
80
100
CO2 concentration (ppm)


Hyphal length (mm/g macro aggregate)
40
30
20
10
0
Scutellospora
Acaulospora
Gigaspora
Glomus
250
350
450
550
650
750
CO2 concentration (ppm)

Effects of whole-ecosystem atmospheric carbon dioxide concentration manipulation on arthropod population dynamics in southern California chaparral.

Alison Eileen Williams

Introduction

Research indicates that increased atmospheric CO_2 concentrations will substantially alter the composition and function of Mediterranean-type ecosystems (Oechel *et al.* 1995) and associated arthropod populations (Whittaker 1999). Prior to the turn of the century and the industrial revolution, atmospheric CO_2 concentrations were stable for thousands of years at 270-280 parts per million volume (ppmv). Over the past 100 years the concentration has risen to 364 ppmv, and continues to rise at a rate of 0.4% per year (IPCC 1997). Elevated CO_2 concentrations cause increased growth and photosynthesis rates in most plants (Tissue and Oechel 1987, Fetcher et al. 1988, Sage 1989, Idso and Kimball 1993, Masle et al. 1993, Luo et al. 1994, Sage 1994). This can result in an increase in foliar carbon to nitrogen ratio (C:N), and other chemical changes that affect the performance of insect herbivores and associated arthropod guilds (reviewed by Coviella and Trumble 1999, Whittaker 1999).

CO_2-mediated changes in plant chemistry appear to have altered performance in most insect herbivore species studied (reviewed by Lincoln *et al.* 1993, Watt *et al.* 1995, Lindroth 1996, Bezemer and Jones 1998, Coviella and Trumble 1999, Whittaker 1999). Since CO_2 tends to increase foliar C:N, and nitrogen is limiting in most herbivore diets (Mattson 1980), indirect effects of elevated CO_2 on insect folivore performance have been negative overall (Bezemer and Jones 1998, Coviella and Trumble 1999). In most

cases, folivorous insects appear to have responded to lower N concentrations of elevated CO_2-grown foliage with increased consumption rates (Coviella and Trumble 1999), but were unable to compensate enough to match the performance of those feeding on ambient CO_2-grown foliage (Hattenschwiler and Schafellner 1999). Not only did host plant nitrogen content generally decrease under elevated CO_2, but concentrations of defensive chemicals, such as phenols, that reduce insect performance sometimes increased (Lindroth 1996, Penuelas *et al.* 1996). Foliar concentrations of minerals such as potassium sometimes also decreased (Norby *et al.* 1996). Even when defensive chemical concentrations remained unchanged, higher consumption rates likely increased insect exposure to the chemicals (Lindroth and Kinney 1998). Reduced relative growth rates have been commonly observed (McDonald *et al.* 1999), generally resulting in decreased folivore weight, even when development time was extended (e.g. Hattenschwiler and Schafellner 1999). Sometimes folivore mortality increased under elevated CO_2 (e.g. Fajer *et al.* 1989), and in the few population-level studies that have been done, folivore abundance was lower (Stiling *et al.* 1999, Bezemer and Jones 1998, and Whittaker 1999).

Feeding on plants grown under elevated CO_2 has not always negatively affected folivore performance; sometimes individual growth rate increased and occasionally larval mortality was lower (Bezemer and Jones 1998). Explanations for a lack of effects or positive effects on folivorous insects include: reduced sensitivity to host plant changes of later insect growth stages (Fajer 1989), greater environmental nitrogen availability (Goverde *et al.* 1999), specialized feeding on unaffected plant species or plant parts

(Kerslake *et al.* 1998), and compensation for reduced nutritional value via increased consumption rate (Lindroth 1996).

The majority of elevated CO_2 effects on fluid-feeder performance have been positive, and results have been less consistent than those recorded in folivore studies (Bezemer and Jones 1998, Coviella and Trumble 1999, Whittaker 1999). Positive fluid-feeder responses included increased fecundity and population density (Awmack *et al.* 1996, Salt *et al.* 1996, Smith 1996, Awmack *et al.* 1997, Docherty *et al.* 1997, Bezemer *et al.* 1998, Whittaker 1999). A few fluid feeders exhibited negative responses, including reduced population size (Butler 1985, Newman *et al.* 1999) and reduced fecundity and nymph weight (Docherty *et al.* 1997). Jones *et al.* (1998), recorded increased abundance of one aphid species and decreased abundance of another in the same model ecosystem experiment. Sometimes no effects of elevated CO_2 on fluid-feeders were detected (Butler 1985, Butler *et. al.* 1986, Bezemer *et al.* 1998; Diaz *et al.*, 1998).

Effects of elevated atmospheric CO_2 on soil and litter-dwelling arthropods are only beginning to be investigated, but so far recorded effects on springtails (Collembola, common detritivore-fungivores) have been mixed. Elevated CO_2 apparently resulted in increased total springtail abundance in model ecosystems with “soil that was relatively poor in nutrients,” but some species’ abundance increased while others decreased (Jones *et al.* 1998). However, in open top chambers under elevated CO_2 concentration, springtail abundance decreased when soil nitrogen availability was also limited (Klironomous *et al.* 1997).

Limited studies elevated CO_2 effects at the third trophic level suggest that predators and parasitoids of herbivores will experience reduced population growth. Mortality of gypsy moth parasitoids increased under elevated CO_2 in one study (Roth and Lindroth 1994). In a subsequent study, survival and adult female size decreased in a hymenopteran gypsy moth parasitoid, and maturation was slowed in a dipteran parasitoid, although pupal weights were higher in the dipteran parasitoid (Roth and Lindroth 1995). In agreement with Roth and Lindroth's (1994, 1995) results, the abundance of predatory flies associated with cotton was reduced in open-top elevated CO_2 chambers (Butler 1985). Alternatively, parasitoid attack rate on leaf miners increased in open-top chambers with elevated CO_2 levels (Stiling *et al.* 1999), suggesting that decreased parasitoid performance is not always the case.

In this study we employed a whole-ecosystem field manipulation to investigate effects of elevated CO_2 on an ecological community, a higher and more complex organizational level than that at which most arthropod-CO_2 studies had previously been done (Bezemer and Jones 1998, Coviella and Trumble 1999, Weiner 1996). Our goal was to identify promising candidate effects at the ecosystem level for further study at all hierarchical levels, and to begin to predict community composition under future atmospheric CO_2 levels. As reviewed above, earlier organismal-level research suggested the following population-level responses to elevated CO_2:

- Folivores (such as Lepidoptera) will decrease in abundance.
- Fluid-feeding herbivores (such as Homoptera and herbivorous Heteroptera) will increase in abundance.

- Parasitoids (such as parasitic Hymenoptera) will decrease in abundance.

Methods

Study site

In-situ CO_2 manipulation in this study was accomplished usingpitfall trap arrays in a series of controlled CO_2, ambient Light, controlled Temperature (CO_2 LT) null-balance chambers (Oechel *et al.* 1994) and a Free-Atmosphere Carbon dioxide Enrichment (FACE) ring and control ring plots. The facility was located at the Sky Oaks Biological Research Station run by San Diego State University in a chamise (*Adenostoma fasciculatum*) dominated chaparral system in north-east San Diego County (1400 m elevation; (33° 23'N, 116° 37'W). Co-dominant shrubs and sub-shrubs included cupleaf lilac (*Ceanothus greggii*), flat-top buckwheat (*Eriogonum fasciculatum*), matchweed (*Gutierrezia sarothrae*), and golden yarrow (*Eriophylum confertiflorum*). Redshank (*Adenostoma sparsifolium*) was also dominant in the system, but not present in any of the treatment or control plots. The chambers and ring plots were located within an area that burned in July 1992.

Experimental Design

The CO_2 LT experimental series consisted of 12 walk-in chambers, two each at set points of 250, 350, 450, 550, 650, and 750 ppmv CO_2, enclosing pre-existing habitat. Chambers were roughly cubical with sloping roofs, enclosing an area approximately 1.8 m^3. Treatments began December, 1995. Thus, habitat had been treated for 2 years prior to the first collections, and four years by the time collections were completed.

Arthropods were collected using 50 ml plastic cup pitfall traps filled with approximately 15 ml of propylene glycol. Traps were set for two weeks, with two week intervals between sampling periods. Arthropods were collected in chambers over periods beginning April 14, May 10, August 26, and September 23 in 1997, and over periods beginning April 18, May 17, June 15, July 13, and August 21 in 1998. When not in use, sealed pitfall traps remained in the ground in order to minimize ground disturbance. Three traps were placed in each chamber. Trap locations were selected so that they were approximately a half meter apart in the center of the chamber floor. Collected arthropods were subsequently strained from the propylene glycol and stored in 70% ethyl alcohol.

The FACE design consisted of a 15 m diameter FACE ring, and two proximal ambient control plots of the same dimension, one with a similar ring structure, and one without. The ring structures consisted of large plastic or metal pipes designed to deliver CO_2 into the atmosphere within the plot. An average CO_2 concentration of 550 ppmv was maintained within the FACE ring starting in spring, 1996. The same type of pitfall traps used in the chambers was also used in the FACE and control rings. Thirty locations were selected randomly from within the 177 m^2 ring plots. Arthropods were collected for 24 hours every two weeks in May and June of 1998. Collections were processed as in the CO_2LT chamber study.

Arthropod Identification

Arthropods were identified to class, order, family (Bland 1978, Arnett *et al.* 1980, Stehr 1987, Borrer *et al.* 1992), and numbered morpho-species (putative species based on morphological similarity). Supervised assistants completed much of the identification

using an arthropod morpho-species voucher collection built as the study progressed. Because arthropods are highly diverse, morpho-species are commonly used for large collections in ecological studies. Arthropod identification and classification methods employed in this study were similar to those of Oliver and Beattie (1996), who found that species counts were largely consistent whether supervised non-specialists sorted to morpho-species or specialists sorted to taxonomic species.

Selection of arthropod groups for analysis was based both on functional similarity and abundance of taxonimc groups in collections. Groups analyzed from chamber collections were: **total arthropods**, **parasitoid wasps** (Hymenoptera, primarily superfamilies Evanioidea, Ichneumonoidea, Chalcidoidea, and Proctotrupoidea), **predatory arachnids** (Aranae, predatory Acari, and Phalangida), **moths** (representing Lepidopetera, primarily Geometridae and Microlepidoptera; nectar feeders, pollinators, and folivores), **a chamise-feeding moth species** (Geometridae), **fluid-feeding herbivores** (Homoptera and herbivorous Heteroptera), **springtails** (Collembola; litter-dwelling fungivore/detritivores), **ants** (Formicidae; a variety of feeding strategies, often omnivorous). The chamise-feeding moth species collected in the chambers was analyzed separately from other moths because they comprised a very large proportion of some of the group samples, particularly at higher CO_2 concentrations. In contrast, no chamise-feeding moths were collected in the FACE study. Predatory arachnids and chamise moths were the only two groups analyzed in the chamber study that were not also analyzed in the FACE study, due to absence or extreme rarity in collections.

Statistical Analysis

Abundance of individuals/trap was calculated by averaging all collections within a year. A general linear regression model (SYSTAT version 8, copyright SPSS 1998) was used to analyze the response of arthropod abundance to CO_2 treatment in the chambers. All collections within a year were averaged together because individual graphical analyses revealed generally consistent patterns among months. Combining data across dates effectively increased sampling intensity and strengthened effect signals. Log transformations of data (log or log of x+1) were performed prior to analyses when data were not normally distributed. P values less than 0.05 were considered statistically significant. When only one (mean of less than 0.1 per trap) or no chamise-feeding moths were collected in a chamber within a year, data from that chamber was excluded from analysis, assuming that too few (if any) individuals occupied the chamber to permit a population-level response. It was not possible to test for statistically significant differences in mean arthropod abundance between CO_2 treatments in the FACE study because the FACE ring was not replicated and traps within a ring-plot were not independent (Hurlbert 1984).

Results

Atmospheric CO_2 concentrations appear to have more strongly affected arthropod abundances in the CO_2 LT chambers in 1998, during the high precipitation El Niño season, than during the dry 1997 season (Figs. 1-8). Correlations between arthropod abundances were less likely to be due to chance in 1998 than in 1997 for all groups, although only 2 relationships were significant (Table 1). Total arthropod abundance and parasitic wasp abundance were negatively correlated with CO_2 concentration in 1998

(Figs. 1b and 2b, Table 1). Fluid-feeding herbivores, ants, and arachnid predators were also negatively correlated with elevated CO_2 in 1998 (Figs. 3b, 4b, and 5b), but the relationships were not statistically significant (Table 1). In contrast, abundance of the chamise-feeding moth species, when present, appeared to increase dramatically with elevated CO_2, rising from a mean of 0.6 to 6.8 moths/chamber (Table 1, Fig. 6), and abundance of all other moths (excluding the chamise-feeding moth) also displayed a positive, but non-significant correlation with elevated CO_2 (Table 1, Fig. 7b). The only groups that did not display linear relationships between abundance and CO_2 concentration were springtails (Table 1, Fig. 8b) and predatory arachnids (Table 1, Fig 5b), although only the springtail relationship was significant. Springtail and predatory arachnid patterns suggested curvilinear relationships, with peak abundance near 450-550 ppmv, and lowest abundance at 750 ppmv (Table 1, Figs. 5b and 8b).

In agreement with the chamber data, all slopes of relationships between arthropod group abundance and CO_2 concentration in the ring plot study (when analyzed) were in the same direction as found in the chamber study (Figs. 1-4, Figs. 7 and 8). Spatial distribution of ant abundance in the FACE ring did not appear to be correlated, and ant abundance was not correlated with fluid-feeder abundance.

Discussion

Abundance of parasitic wasps was lower under elevated CO_2 as predicted, however, moth abundance appeared to have increased, not the effect suggested by earlier organismal studies. Mean fluid-feeder abundance decreased with elevated CO_2 as expected, but the relationship in this study was not statistically significantly. The

increased chamise-feeding moth abundance at elevated CO_2 may reflect a response simlar to that of larvae of the common blue butterfly (*Polyommatus icarus*; Goverde et al. 1999). At elevated CO_2, common blue butterfly larvae exhibited increased consumption of elevated CO_2-grown plant material, more efficient conversion of ingested material, faster development, and higher weight, which should increase population growth rate. However, Goverde *et al.* (1999) attributed their atypical results to high foliar nitrogen concentration in the nitrogen fertilized, nitrogen-fixing *Lotus corniculatus* host plants, and chamise does not fix nitrogen, nor were our plots fertilized, so it is not clear why this species apparently benefited from elevated CO_2 exposure. Perhaps direct exposure of larvae also contributed to positive moth performance, as indicated by Caulfield and Bunce' (1994) study with beet armyworms. They specifically designed an experiment to test for interaction between direct exposure of insects to and plant growth under elevated CO_2. The most interesting result was that armyworm survival was enhanced on sugarbeet, but only for larvae exposed to elevated CO_2 and fed elevated CO_2 grown plants. Larvae not exposed to both manipulated atmosphere and foliage were unaffected.

Our results also reinforce the cautionary observation that effects of elevated CO_2 can appear strong and consistent for individual species or taxa in laboratory studies, but may be reversed or buffered in natural systems (reviewed by Koch and Mooney 1996, Korner and Bazzaz 1996, Weiner 1996). It is not surprising that the chamise-feeding moth response was not predicted by the majority of previous lepidopteran study results, and it is not the first effect of elevated CO_2 at a greater hierarchical level found to be

opposite of predictions based on responses at lower levels (Loehle 1995, Weiner 1996). For example, short-term leaf responses, like increased photosynthetic rates, have not always resulted in increased whole-plant biomass accumulation (Koch and Mooney 1996). Our study also suggests that while plant biomass probably increased with elevated CO_2 (Oechel *et al.* 1997), arthropod biomass, in fact, may have. The implications of this with respect to ecosystem dynamics are critical, since declining arthropod populations could reduce nutrient cycling and energy flow to higher trophic levels.

The curvilinear relationship with elevated CO_2 that we observed in springtails (1998), is similar to the relationship between fungal abundance and CO_2 level seen in the same chambers (M. Allen pers. comm.) Changes in predatory arachnid, springtail, and fungal abundance may be directly related, since fungi are the primary food source of springtails, and spiders and mites are prominant predators in the litter and soil (Hopkin 1997). This curvilinear springtail-CO_2 relationship provides an explaination for the seemingly contradictory results of Jones *et al.* (1998) and Klironomous *et al.* (1997). Jones *et al.* (1998) recorded higher springtail abundance at approximately 550 ppmv CO_2 compared to ambient (approximately 350 ppmv), and Klironomous *et al.* (1997) recorded lower springtail abundance at 700 ppmv CO_2 compared to ambient. Both sets of results were in agreement with the curvilinear springtail-CO_2 relationship recorded here in 1998 (Fig. 8b). The relationship observed here should serve as a cautionary reminder regarding the assumption of linear relationships. Most elevated CO_2 studies to date have compared only one ambient CO_2 treatment (approx. 360 ppmv), to only one elevated CO_2 treatment (anywhere from 550 to 750 ppmv), which are within the segment of our

springtail response curve where abundance changes from an increasing trend to a decreasing one.

Some variability in results may also be due to experimental methodology. The difference in CO_2 effect between years could be due to time (cumulative CO_2 effect on plants and/or arthropods), climatic factors (an interaction with the CO_2 effect), or both. Increased arthropod abundance during a wetter, more productive season likely increased capture rate in 1998, strengthening effect signals. Weaker support for arthropod CO_2 effects in the FACE study than in the chamber study can be explained, in part, by a difference in methods. The FACE study had only two CO_2 treatments with a difference of approximately 200 ppmv, while chamber treatments included six CO_2 levels spanning 500 ppmv. Collections made over a shorter time period in FACE study traps than in the chamber traps (24 hours as opposed to two weeks) resulting in lower numbers of arthropods collected/trap simply due to a difference in trapping methods.

Another possible methodological effect may have been reflected in chamise-feeding moth results. Chamise-feeding moths were not collected in the FACE study, but they were so abundant in one chamber that they entirely defoliated the chamise. The absence of chamise-feeding moths from FACE study collections indicates the outbreak-level abundance of that species in the chambers was possibly the result of an interaction between structural effects of the chmbers and CO_2 level. Similar outbreaks (not correlated with CO_2 level) unique to the chambers were noted in two species of scale insect (Hemiptera: Homoptera) in the field. It is also possible that the lack of an enclosing structure may have resulted in differential movement of mobile arthropod

groups in or out of the FACE ring, further increasing the differences in abundance caused by CO_2 level compared to the chambers.

Our results reinforce Weiner's (1996) emphasis that, given the complexity of natural systems, there is a great need for *in situ*, "whole ecosystem," studies to more accurately predict future regional ecosystem states. However, our results also indicate that greater replication within and across CO_2 levels will be needed in FACE designs to achieve the power required to detect these type of effects on arthropod populations. The most promising future research avenues suggested by this study are those leading to understanding higher-level arthropod-mediated ecological functions in general, and specific effects related to parasitic wasps, moths, and springtails.

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Table 1. Regression statistics for arthropod abundance vs. CO_2 concentrations in chambers (n = 12 chambers at 6 CO_2 levels; see text for details). Polynomial regressions are denoted by an *.

Arthropod Group	1997 r^2	1997 slope	1997 P	1998 r^2	1998 slope	1998 P
Total arthropods (log 98)	6.7%	- 7.42E-4	0.418	44.2%	- 1.05E-3	0.018
Parasitic wasps (log 98)	4.9%	- 1.55E-3	0.490	51.3%	- 8.22E-4	0.009
Fluid-feeding herbivores (log)	0.2%	9.92E-5	0.894	21.3%	- 1.51E-3	0.131
Ants (log)	5.5%	- 3.38E-4	0.465	22.6%	- 8.84E-4	0.118
Arachnid predators	7.2%	- 7.42E-4	0.397	8.2%	- 1.05E-3	0.366
	-	-	-	39.7%*	-	0.103*
Chamise-feeding moths	-	-	-	98.2%	1.25E-2	0.006
Other moths (log)	1.3%	-1.31E-4	0.729	10.4%	2.33E-4	0.306
Springtails	7.3%	- 1.05E-3	0.395	11.5%	-7.88E-4	0.281
	-	-	-	70.0%*	-	0.004*

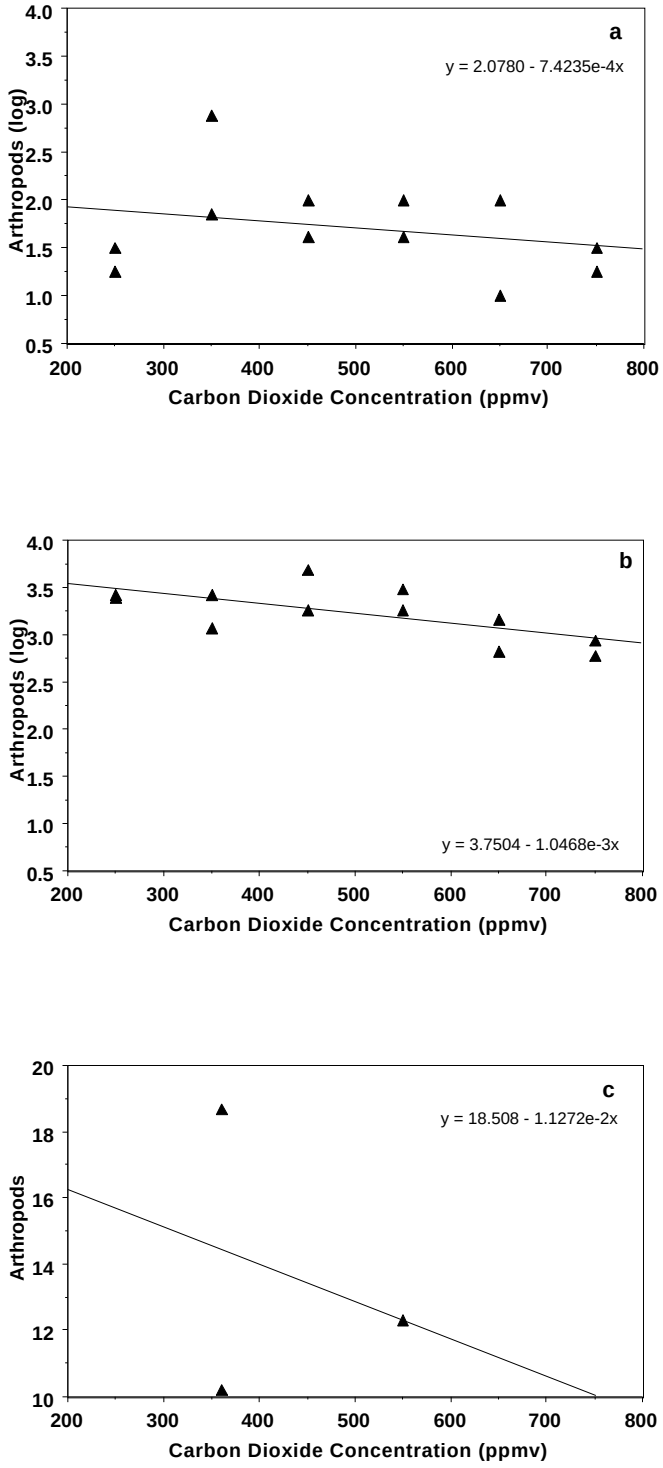

Figure 1. Arthropod abundance in CO_2LT chambers, April-September, a) 1997 and b) 1998, and c) in ring plots, May-June, 1998. Data are averages of 5 collection periods for each chamber (n=15 traps), and 4 collection periods for each ring plot (n=120 traps).

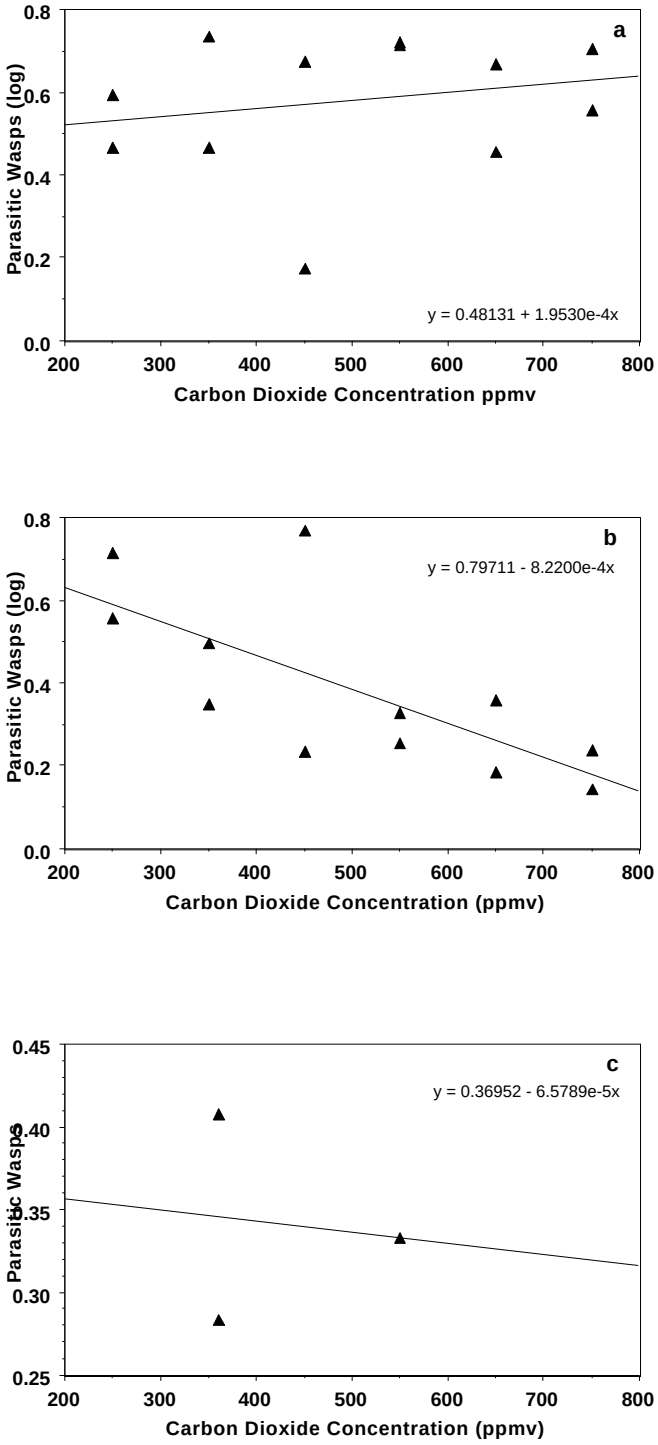

Figure 2. Parasitic wasp abundance in CO_2LT chambers, April-September, a) 1997 and b) 1998, and c) in ring plots, May-June, 1998. Data are averages of 5 collection periods for each chamber (n=15 traps), and 4 collection periods for each ring plot (n=120 traps).

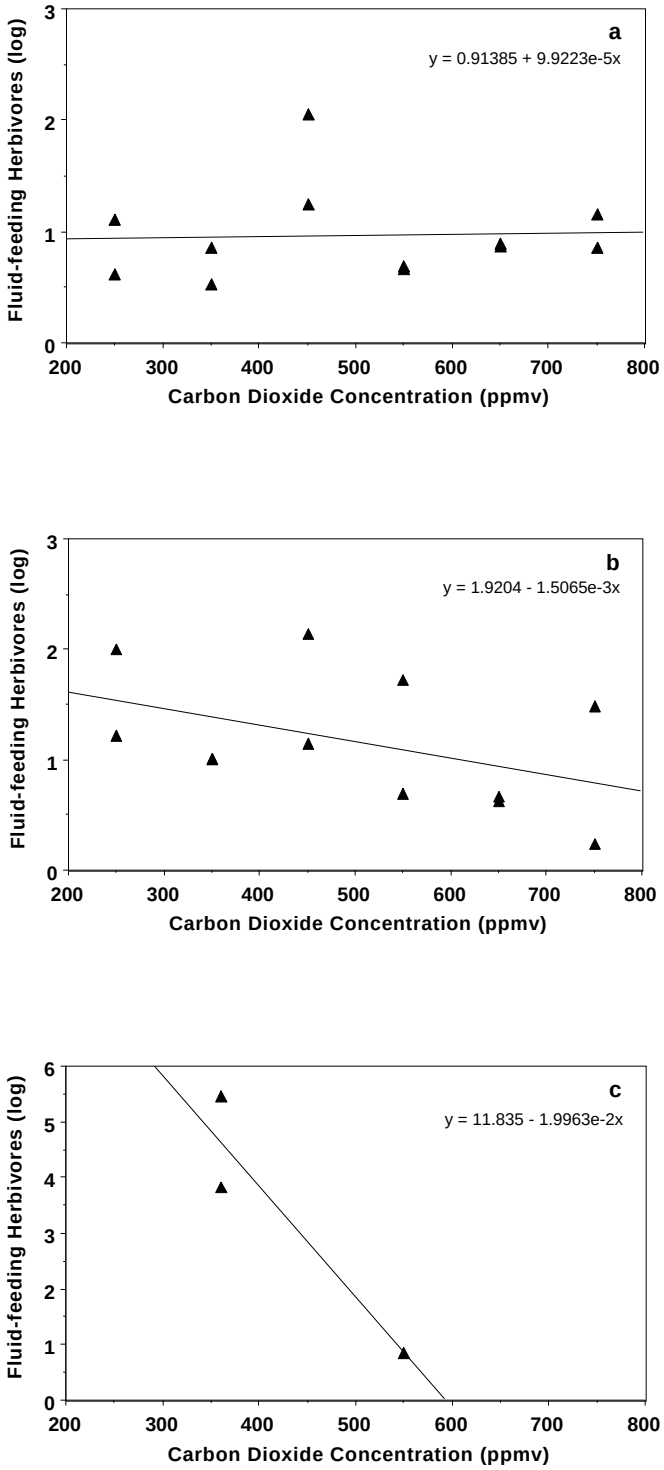

Figure 3. Fluid-feeder abundance in CO_2LT chambers, April-September, a) 1997 and b) 1998, and c) in ring plots, May-June, 1998. Data are averages of 5 collection periods for each chamber (n=15 traps), and 4 collection periods for each ring plot (n=120 traps).

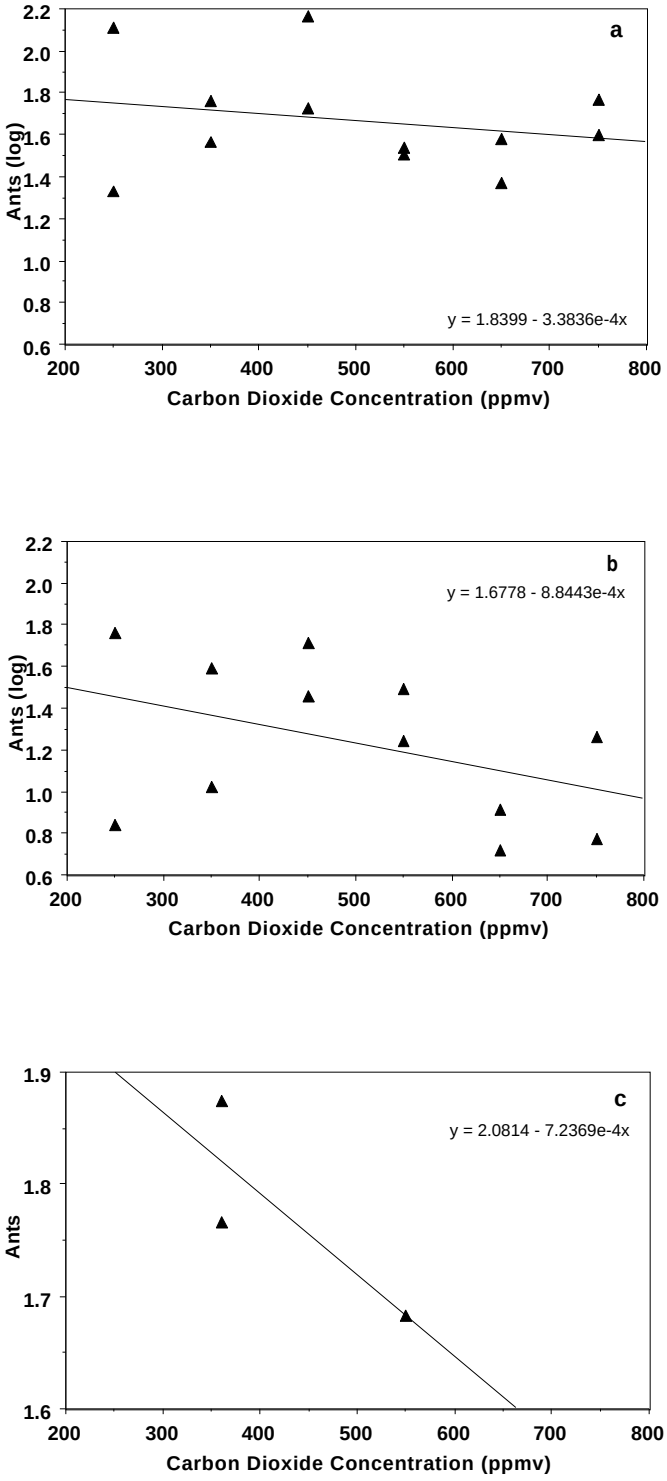

Figure 4. Ant abundance in CO_2LT chambers, April-September, a) 1997 and b) 1998, and c) in ring plots, May-June, 1998. Data are averages of 5 collection periods for each chamber (n=15 traps), and 4 collection periods for each ring plot (n=120 traps).

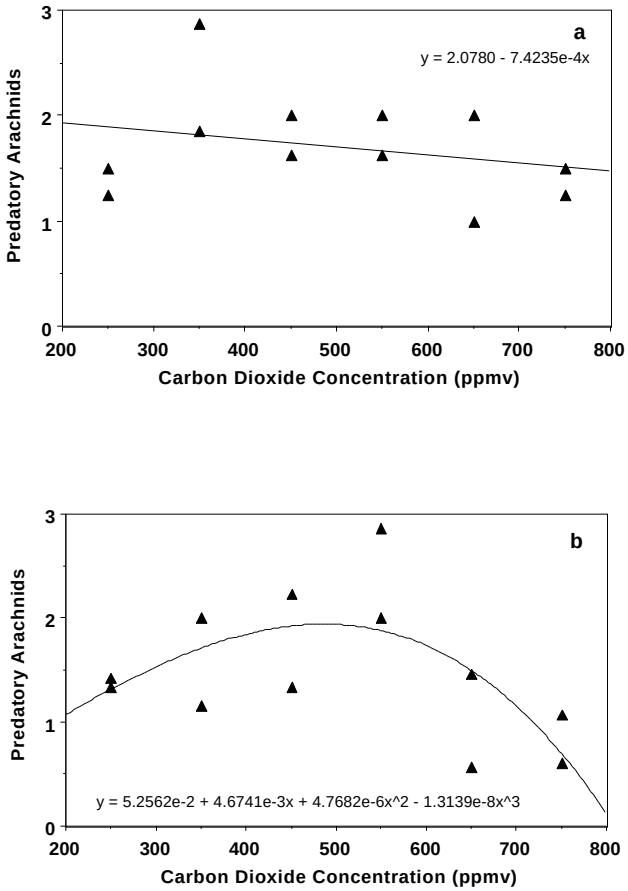

Figure 5. Predatory Arachnid abundance in CO_2LT chambers, April-September, a) 1997 and b) 1998. Data are averages of 5 collection periods for each chamber (n=15 traps).

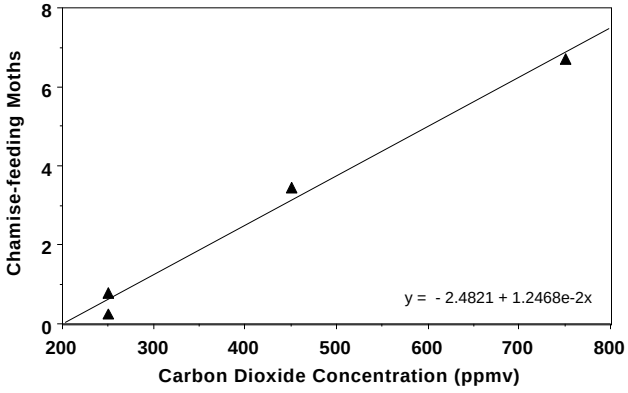

Figure 6. Chamise-feeding moth abundance in CO_2LT chambers, April-September, 1998. Data are averages of 5 collection periods for each chamber (n=15 traps).

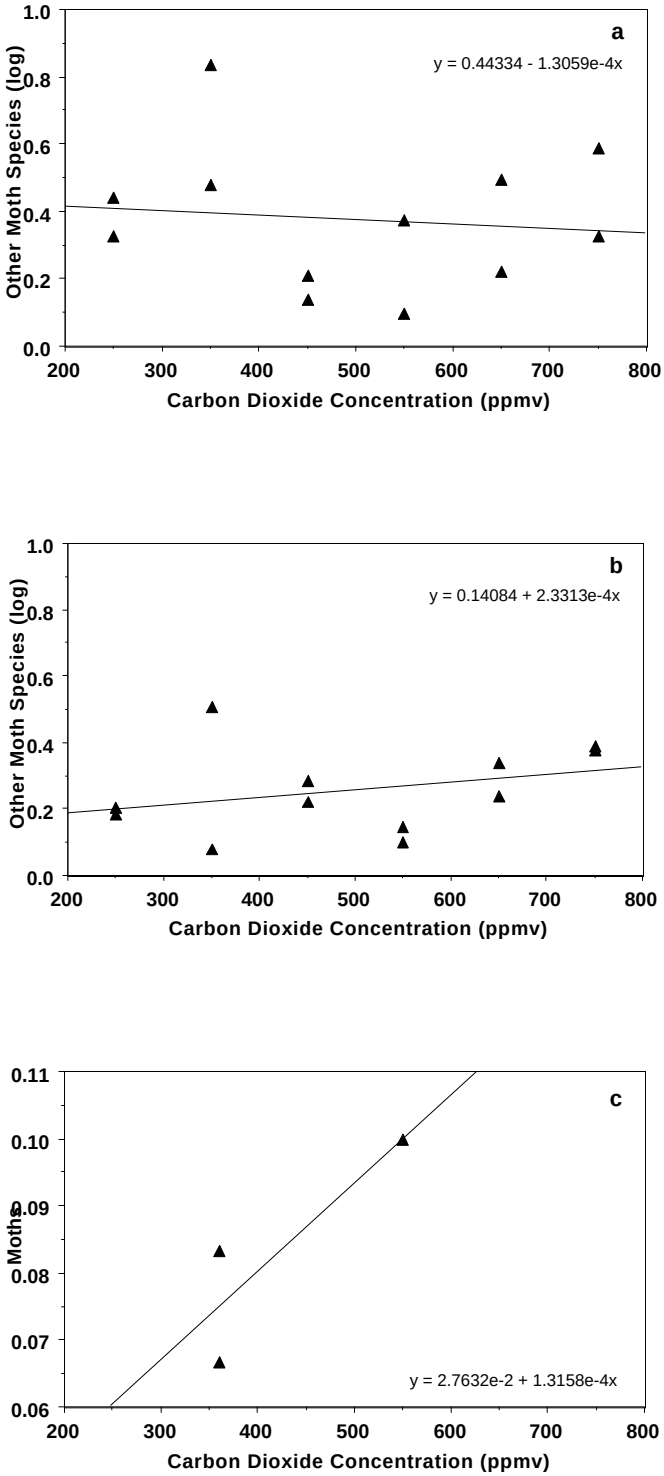

Figure 7. Other moth abundance in CO_2LT chambers, April-September, a) 1997 and b) 1998, and c) in ring plots, May-June, 1998. Data are averages of 5 collection periods for each chamber (n=15 traps), and 4 collection periods for each ring plot (n=120 traps).

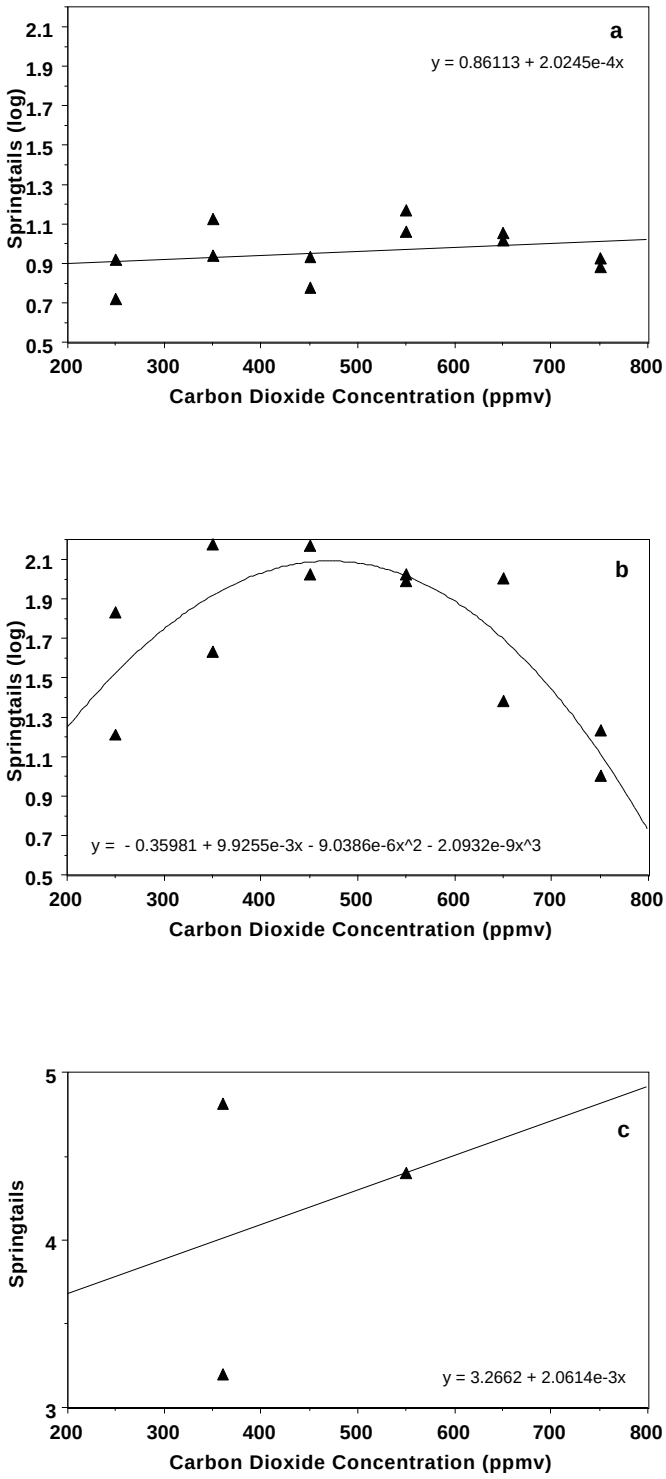

Figure 8. Springtail abundance in CO_2LT chambers, April-September, a) 1997 and b) 1998, and c) in ring plots, May-June, 1998. Data are averages of 5 collection periods for each chamber (n=15 traps), and 4 collection periods for each ring plot (n=120 traps).

The relationship between atmospheric CO_2 concentration and arthropod diversity in chaparral, and a model of the relationship between CO_2 and succession.

Alison Eileen Williams

Introduction

Background

An increase in overall plant productivity and other ecological effects of elevated atmospheric CO_2 (DeLucia *et al.* 1999, Mellilo *et al.* 1995, Oechel *et al.* 1997) should alter species diversity of the chaparral community. In particular, elevated CO_2 should increase growth rates of plant roots (Arnone *et al.* 2000, Mikan *et al.* 2000, Van Ginkel *et al.* 2000) and shoots (Oechel *et al.* 1995, IPCC 1997). Although the effects of elevated CO_2 on arthropod species diversity and composition in chaparral has not been studied, plant species diversity has been found to increase in grassland communities. Three year exposure of a grassland community to elevated atmospheric CO_2 led to greater plant species richness and heterogeneity (species diversity) (Potvin and Vasseur 1997). Specifically, growth of dicots was stimulated more than monocots under elevated CO_2, and complete coexistence of early and late successional species was enhanced. A similar pattern was observed in a Kansas prairie, where abundance of C3 grasses declined and C3 forbs increased, while C4 species abundance remained unchanged (Owensby *et al.* 1993). Decreased dominance among herbacious plant types and increased growth rate of woody plants should increase insect habitat heterogeneity, both in architectural growth forms and host-plant species diversity, and therefore increase insect species diversity (Southwood *et al.* 1979, Strong *et al.* 1984, Lawton 1983).

Conceptual Succession-CO_2-Diversity Model and Hypothesis

Earlier results suggest that arthropod and plant diversity should first increase after fire, and then decrease as shrub biomass accumulates. It is known that plant species diversity is lowest in chaparral when shrub biomass reaches its maximum (Force 1981). Furthermore, studies of insect family and plant species diversity in two chaparral communities found increases in diversity during the first three years after fire (Force 1981, Mills 1985). Mills (1985) studied post-fire insect succession in the same chamise chaparral community studied here, and found that insect family diversity (Simpson's index) almost doubled the second year after fire. In a chaparral succession model (Fig. 1) we can assume that maximum arthropod diversity and habitat heterogeneity is achieved at approximately half way to maximum canopy closure, when intermediate shrub biomass and the mix of early and late successional species has maximized the structural complexity and species diversity of the vegetation. This model is analogous to the commonly observed intermediate disturbance pattern (Sousa 1979, Petraitis *et al.* 1989); early successional stage (typical of frequently disturbed communities) results in low community diversity, intermediate successional stage (typical of communities experiencing intermediate disturbance frequencies) results in high community diversity, and late successional stage (typical of infrequently disturbed communities) again results in lower community diversity.

Research suggests that CO_2 enrichment will increase the rate of canopy closure, generally speeding ecological succession (see Swank and Oechel 1991. All else being equal, the relationship between diversity and CO_2 level should be affected by the difference in shrub biomass accumulation caused by increased CO_2 levels. *Adenostoma fasciculatum*

(chamise shrub) growth and physiology measurements taken concurrent to this study (shoot growth, net primary productivity; Y. Cheng, unpublished data) indicate that the difference in annual shrub growth caused by 100 ppmv elevated CO_2 is less than would be expected over a two year period at a fixed CO_2 level. Maximum shrub biomass in chaparral is generally reached in 20-30 years (Reid 1985, Black 1987), and the chamise chaparral community in this study was in its fifth year of postfire succession, putting the ambient (360 ppmv) system approximately one quarter of the way to maximum canopy closure, and probably half way to maximum habitat heterogeneity and arthropod species diversity (Fig. 1). Using conservative estimates of 20 years to maximum shrub biomass, and a two year growth differences caused by 100 ppmv elevated CO_2, we can a hypothesis regarding the slope of the CO_2-diversity relationship in our study (Fig. 1). Therefore, the chaparral succession model predicts diversity at the time our data were collected should increase as CO_2 levels rise from 250 ppmv to 750 ppmv (Fig. 1; as in grasslands, Potvin and Vasseur 1997, Owensby *et al.* 1993). Thus, we can use the results of this study to test the model prediction that in the chaparral system and post-fire period studied here, insect diversity and atmospheric CO_2 concentration will be positively correlated.

Although specific predictions are difficult to make, we also expected to find shifts in the dominance of arthropod groups among atmospheric CO_2 treatments and between experimental designs (below). Elevated CO_2 has been found to affect the relative abundance of aphid (Homoptera), springtail (Collembola), and soil fungal species (Klironomous *et al.* 1997, Jones *et al.* 1998). Design differences between CO_2 enrichment technologies, particularly enclosure of plots, should also alter relative abundance of arthropod taxons. For example, in the FACE ring, flying insects may be either attracted or

repelled by elevated CO_2 or its effect on the plant community, and in the chambers, restricted movement of arthropod species could have produced founder effects. Other structural effects of the chambers, such as the lack of precipitation on foliage (collected precipitation is redistributed at ground-level), may also have affected arthropods differently than in the open ring plots.

Methods

Experimental design and arthropod collection and identification methods were as described in Chapter 1. Diversity refers to how many species there are (richness) and their abundance relative to others (heterogeneity) (Hurlbert 1971, Force 1981, Magurran 1988, Krebs 1989). For example, a collection would be considered more diverse than another if the number of individuals was more evenly distributed among species, even if both collections contain the same number of species. Diversity was measured in this study using rarefaction. Rarefaction produces a species richness value that adjusts for sample size bias, and also accounts for the relative abundance (evenness or dominance) of species (Hurlbert 1971, Smith and Grassle 1977). The rarefaction index at $m = 2$ is algebraically equivalent to 1 – 1/Simpson's index of diversity (Smith and Grassle 1977). Rarefaction calculations were performed using the modified Coleman function (Brewer and Williamson 1994):

$$s_m = S - \mathrm{S}\,(1 - m/N)^{n}\,i$$

where S = the number of species in the collection, N = the total number of individuals in the collection, n_i = the number of individuals in the i^{th} species in the collection, m = the sample

size of individuals (in this study m = the smallest sample size, 125), and s_m = the expected number of species in m. Linear regression (SYSTAT version 8, copyright SPSS 1998) was used to analyze the diversity-CO_2 relationship.

Results and Discussion

Arthropod abundance and diversity

Unlike arthropod abundance (data presented in Chapter 1) and absolute species richness (richness practically mirrored abundance), diversity (s_m) did not decrease with increasing CO_2 levels (Fig. 2). Although the positive relationship between arthropod diversity and CO_2 was not statistically significant (Fig. 2, $F=2.56$, $P=0.14$, $r^2=0.20$), it was consistent with the model-generated hypothesis that elevated CO_2 increases arthropod species diversity in the early post-fire chaparral community. However, if elevated CO_2 speeds succession as hypothesized, this positive relationship may be ephemeral, and elevated CO_2 could begin to decrease plant diversity and increase dominance of shrub species 8-10 years after fire, compared to lower CO_2 levels (see top axis of Fig. 1).

Relative abundance of arthropod groups in the chambers and ring plots at corresponding CO_2 concentrations were similar (Fig. 3), with some minor differences. Consistently higher parasitic wasp abundance in the open ring plots compared to closed chambers (Fig. 3), and the lack of chamise-feeding moths in the FACE ring (data presented in Chapter 1), indicated that chamber enclosure did affect the population sizes of some species. Comparison of the relative abundance of arthropod groups collected between the FACE ring and CO_2LT chambers indicates that our results may be generally applicable to the natural, non-enclosed community.

Conceptual Model Predictions

Although it is perhaps premature to speculate just how elevated CO_2 might affect the complex functioning of the chaparral community, there is no doubt it will alter community composition. If fire (or other disturbance) frequency is not similarly increased, faster succession would result in less total area of early successional habitat within the chaparral community at any point in time. Also, unless annual seed production and/or seedling establishment were also increased, annual plant species would not be able to build as large a viable seed bank prior to canopy closure, possibly reducing population densities during subsequent post-burn succession events. All else being equal, the species-area curve (Gleason 1922, Williams 1943) predicts that lower abundance of hostplants and reduced habitat area would result in reduced arthropod diversity. Insect species that rely on early successional annual hostplants or nectar sources should also be negatively affected by a decrease in the total area of early-successional habitat, because there would be a decreased number of immigrants for recolonization. Thus, while community diversity may be increased by elevated atmospheric CO_2 during the early post-fire period in chaparral communities in the future, peak diversity at mid-succession should not be higher, and could even be lower, than it would have been under lower CO_2 levels, and that seral stage may be shorter lived (Fig. 1). This succession hypothesis further illustrates why it is unwise to assume a simple, static, linear relationship between CO_2 concentration and ecological response.

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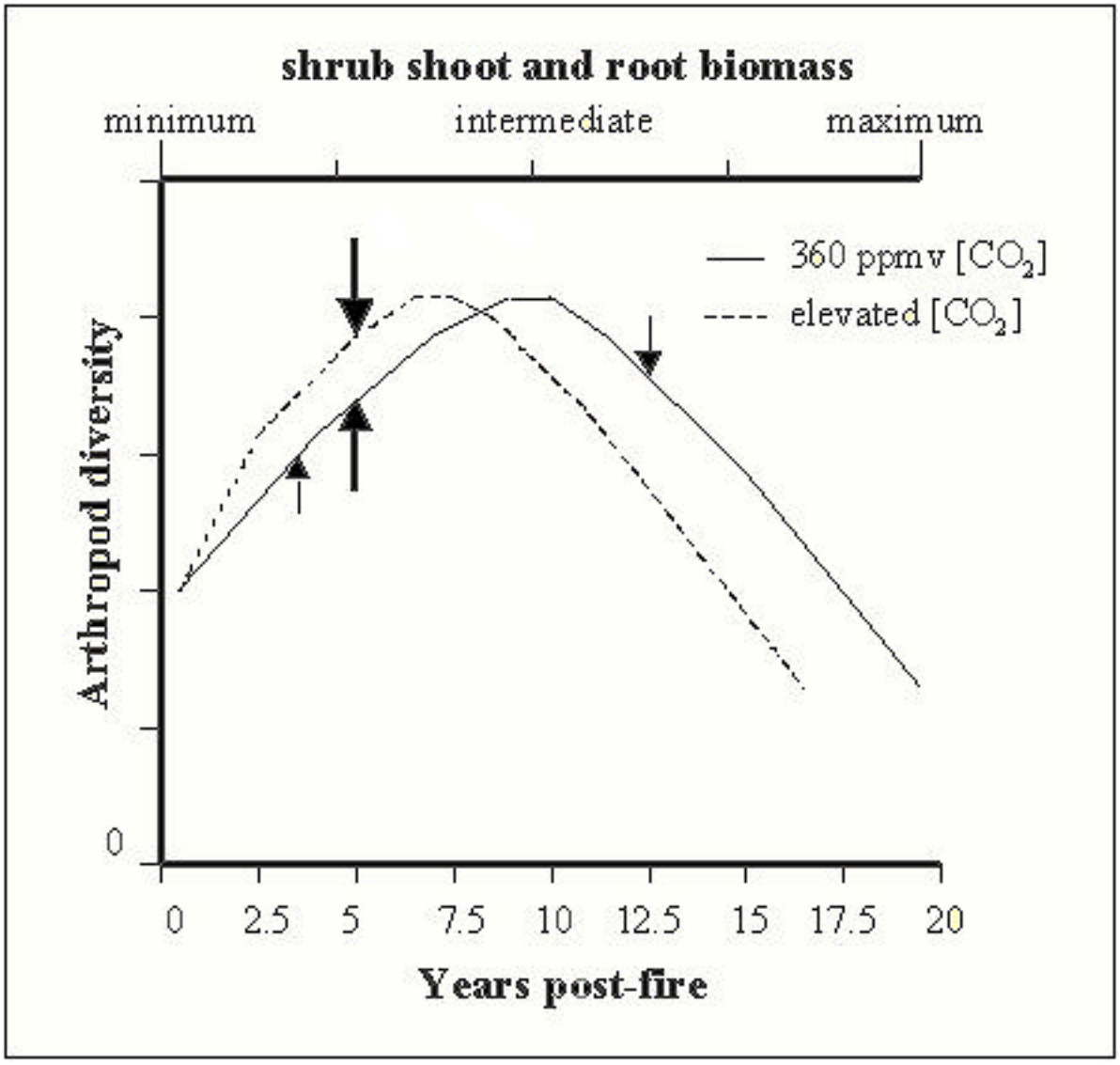


Figure 1. Conceptual succession-CO_2-diversity model for chaparral. Large arrows indicate predicted arthropod diversity levels during this study (5 years after fire). Small arrows indicate predicted arthropod diversity levels on the ambient (360 ppmv) curve in the lowest (250 ppmv) and highest (750 ppmv) CO_2 levels in CO_2LT chambers, if the change in plant growth and succession caused by 100 ppmv CO_2 (the difference among chambers) were equivalent to 2 years ambient growth.

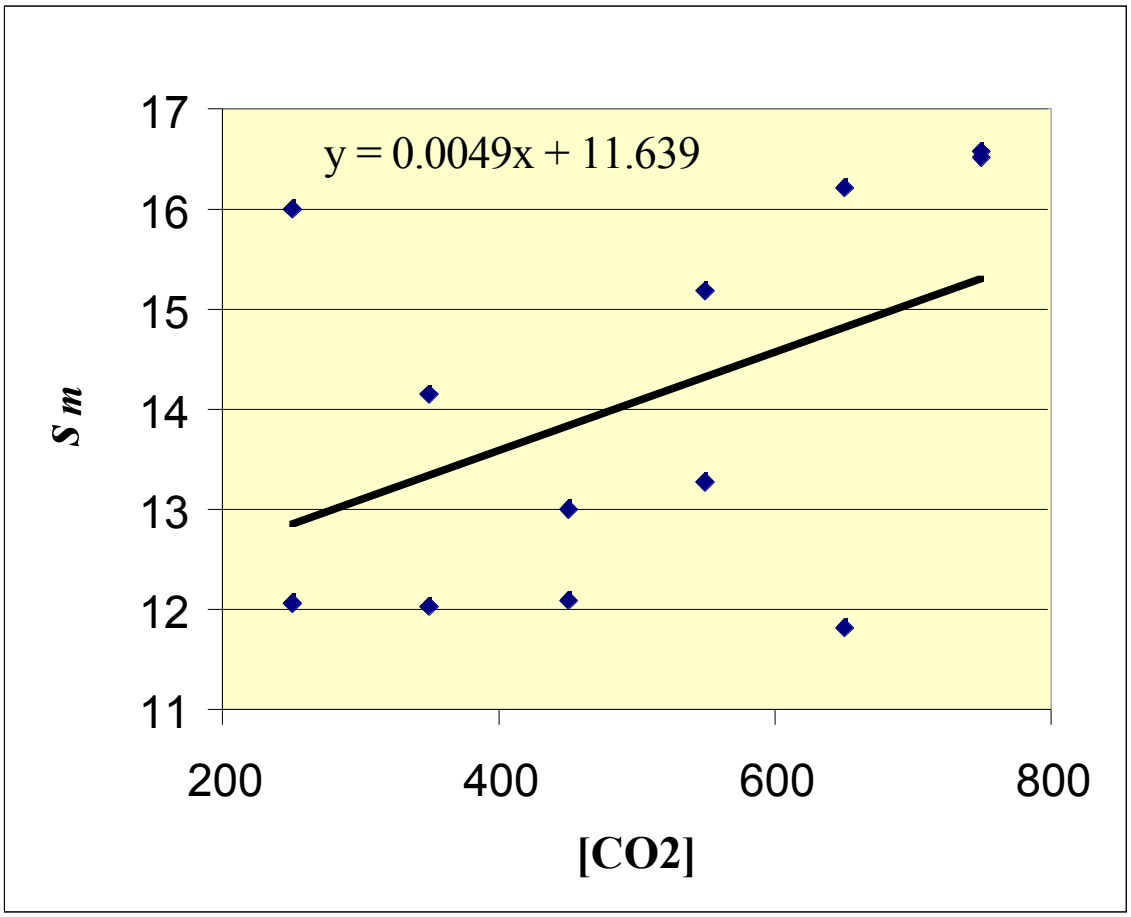
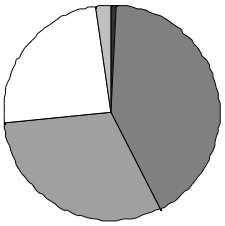


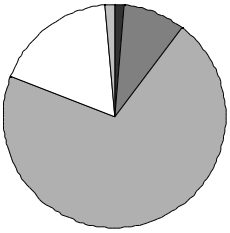
Figure 2. Arthropod species diversity in CO_2 LT chambers, April-October 1998. Each data point represents expected sample species richness (s_m = modified Coleman's function rarefaction value, a species diversity index) at smallest sample size (m=125), averaged across 5 dates for each chamber at each CO_2 level.

Figure 3. Relative abundance of arthropod groups from the FACE ring (550 ppmv CO_2), ambient ring plots (approximatly 360 ppmv), and CO_2LT chambers, at 250, 350, 550, and 750 ppmv CO_2 in 1998. Values represent a proportion of the total collection for each group, calculated using average individuals per trap for each treatment method.

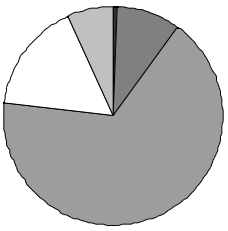
250 ppmv CO_2LT chambers



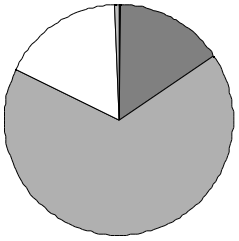
350 ppmv CO_2LT chamber



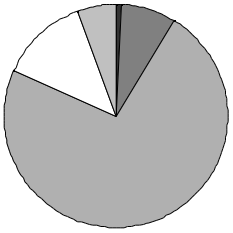
Ambient ring plots



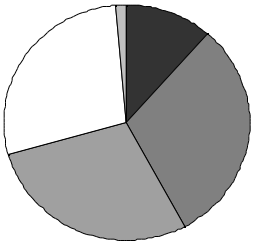
550 ppmv CO2LT chamber

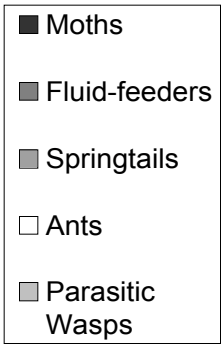


550 ppmv FACE ring



750 ppmv CO2LT chamber





Effects of elevated atmospheric CO_2 and hostplant density on psyllid abundance

Alison Eileen Williams

Introduction

Herbivory, particularly by bark lice (Hemiptera: Homoptera: Psyllidae), has the potential to play a large part in regulating the post-fire abundance of key shrub species in the chaparral community (Mills 1985, Moreno and Oechel 1992). *Ceanothus greggii* is a common shrub species in chamise chaparral, and must regrow from seed after fire (Moreno and Oechel 1992). Two studies of the post-fire relationship between *C. greggii* and its herbivores in the same chaparral system studied here found that psyllids reduced survivorship and decreased the relative abundance of *C. greggii*. In 1982 and 1983 (Mills 1985), 73% of naturally-growing *C. greggii* seedlings surveyed at Sky Oaks Biological Field Station had psyllid infestations, and insecticide-treated seedlings survived significantly better than untreated seedlings (Mills 1985). Although none of the seedlings appeared to have died as a direct result of psyllid damage, 15% of the infested seedlings died from drought stress the following year. None of the non-infested seedlings died. Psyllids also significantly reduced the mean growth rate of infested seedlings, and appeared to contribute to reduced competitive ability of *C. greggii* seedlings compared to chamise seedlings (Mills 1985). In a second study (Moreno and Oechel 1992), 51.4% of *C. Greggii* seedlings appeared to be infested, and infested seedlings suffered significantly higher mortality during the second season after fire than uninfested seedlings. Seedling mortality in the second season was 30.0%, and of those that died, the ratio of infested to uninfested shrubs was 3:1.

Psyllid abundance, like that of other herbivorous insects, may be affected by elevated atmospheric CO_2 levels (Bezemer and Jones 1998, Coviella and Trumble 1999). Although there have been few studies of CO_2 effects on insects and mites that feed on cell contents like psyllids, food consumption and population densities increased with elevated CO_2 in the two studies where whole-cell feeders were studied (reviewed by Bezemer and Jones 1998).

Hostplant density is another factor that might affect psyllid density. In addition to basic nutritional quality effects, high densities of host plants, or more resources, should allow greater population densities of psyllids to be maintained at any given site. Since evidence suggests that elevated CO_2 may beneficially affect plant populations via increased water use efficiency and growth rates (Schortmeyer *et al.* 1999, Thomas *et al.* 2000), this could also lead to further enhanced psyllid population densities. However, if psyllid density continues to increase under elevated CO_2, higher herbivore load might cause increased *C. greggii* mortality (Mills 1985, Moreno and Oechel 1992). Until the relative effects of such complex interactions are better understood, we cannot predict the ultimate effects of elevated CO_2 on this shrub. The major objectives of this study are to compare densities of *C. greggii* shrubs growing under ambient (360 ppmv) and elevated (550 ppmv) atmospheric CO_2 levels, and identify possible effects of elevated CO_2 and shrub densities on psyllid densities.

Methods

Psyllid Abundance

To address the objectives, psyllid abundance was compared in 15m diameter open plots that differed in atmospheric CO_2 concentrations and *C. greggii* densities. These plots

were located within the 5-year post-burn chamise chaparral community at San Diego State University's Sky Oaks Biological Field Station described in Chapter 1. In June 1999, all live *C. greggii* shrubs were counted in the FACE ring plot (550 ppmv CO_2; 15 m diameter) and 2 adjacent ambient CO_2 plots (360 ppmv CO_2; 15 m diameter), including one with a similar ring structure. Ambient plots were located approximately 10 m north (ambient north plot), and 20 m south (ambient control ring) of the FACE ring.

Psyllid densities were measured for two seasons using whitefly trap cards (Surefire© brand) over 24 hours periods. In June 1999, traps were placed the center of 12 haphazardly selected *C. greggii* shrubs per plot located in the FACE ring and one ambient plot. In May 2000, 16 shrubs per plot were selected for trap placement in the control ring (ambient [CO_2]), the FACE ring (high [CO_2]), and the north plot (ambient [CO_2]). The three plots varied in *C. greggii* density, with the north plot density > the FACE ring density > the control ring density. Shrubs in the low-density plot appeared to be smaller than those in the high density plot, based on visual inspection. Although location of shrubs was haphazardly selected, shrubs with minimum dimensions of 30 cm at the widest diameter and 25 cm at the widest diameter at a right angle to the first were selected for trap placement. Shrub dimensions were then used to calculate psyllid density per plant volume. Unfortunately, shrub measurements were not collected in 2000, so psyllid densities could not be adjusted for differences in shrub volume in that year.

Data Analysis

Ellipsoidal volume of shrubs was estimated using the oblate spheroid formula: $4/3p(a/2)^2(b/2)$, where a is the widest diameter, and b is the widest diameter at right angles to it. To achieve a normal distribution, psyllid per shrub were transformed using

log (x + 1) prior to analyses. To compare number of psyllids collected per shrub in FACE vs. the ambient north plot in 1999, a Student's t-test was used. To compare psyllid densities among FACE and the two ambient plots in 2000, an ANOVA was performed, followed by a Tukey's multiple-comparison post-hoc test when significant differences among means were detected (SYSTAT version 8, copyright SPSS 1998). Two types of psyllids were identified from traps in 2000: a species of psyllid that appeared to feed exclusively on *C. greggii* (hereafter referred to as lilac psyllids; probably responsible for much of the *C. greggii* damage observed in earlier studies), and all other psyllid species. Analyses of psyllid densities in 2000 were run on each psyllid group seperately and results compared.

Results

C. greggii densities were highly variable among sites. The mean density of *C. greggi* in the FACE plot (229 shrubs; 550 ppm CO_2) was intermediate to the mean density of the ambient plots. (north plot = 443 and control ring = 76 shrubs The ambient north plot had approximately twice as many *C. greggii* shrubs as the FACE plot and the ambient control ring plot had only about 30% of the density found in the FACE plot (Table 1).

In both 1999 and 2000, mean psyllid densities per shrub were higher (but not statistically significant) on shrubs growing in the FACE plot compared to ambient control plots (Table 1). In June 1999, the mean density of psyllids collected per *C.greggii* shrub was higher in the FACE compared to the ambient north plot (Table 1; $t = 1.88$, $P = 0.068$), and when values were adjusted for plant volume using psyllids/cm^3 ellipsoidal shrub volume, the difference between means became significant (Table 1; $t = 3.42$, $P < 0.01$). In 2000 the density of lilac psyllids per shrub in the FACE was higher than in the control ring plot but not significantly higher than in the ambient north plot (Figure 1; ANOVA $F = 5.69$,

$P < 0.01$; post-hoc test: FACE vs. control ring, $P < 0.01$, FACE vs. north plot, $P = 0.77$, control ring vs. north plot, $P = 0.04$). In 2000, mean densities of psyllid species other than lilac psyllids also tended to be highest in the FACE plot, but were not significantly different from either ambient plot (Figure 2; ANOVA $F = 1.22$, $P = 0.31$).

Table 1. *Ceanothus greggii* and psyllid densities in 15 m diameter post-burn chamise chaparral ring plots at Sky Oaks Biological Field Station. The FACE ring was an elevated CO_2 treatment plot (mean CO_2 concentration = 550 ppmv), other plots were ambient CO_2 concentrations (approximately 360 ppmv). Psyllid densities are presented as means with standard deviations in parentheses. N = 12 traps per 15 m diameter plot for 1999 and 16 traps per plot for 2000. Data were log transformed for analyses, but non-transformed values are also given below. Significantly different values are represented by different subscripts.

	FACE ring (Elevated CO_2)	ambient north plot	ambient control ring
***C. greggii* shrubs/plot**	229	443	76
Psyllids/ ellipsoidal shrub volume dm^3 (1999)	0.043 (0.028)^a	0.012 (0.013)^b	-
Psyllids/shrub			
1999	12.58 (5.96)	8.33 (4.48)	-
All psyllids			

2000			
Lilac psyllids	3.44 (2.37)[a]	2.81 (1.80)[a]	1.56 (1.83)[b]
All other psyllids	7.25 (6.33)	5.62 (3.34)	4.63 (2.78)

Discussion

Our hypothesis that psyllid density would increase with elevated CO_2 was supported in 1999 by the difference in densities between the FACE ring and the north plot when shrub volume was considered (Table 1). If the effect was similar to that in 1999, a similar adjustment to data collected in 2000 might have produced a greater difference in psylllid densities among elevated and ambient CO_2 treatments (Table 1).

This study demonstrated that, at least in some cases, psyllid density increased with elevated CO_2, and apparently also with increasing plant density. Results suggested that CO_2 level and plant density may produce additive effects. That is, both variables might be positively correlated with psyllid density for different. If plant density explains part of the difference in psyllid densities among plots, had plant densities been equal, psyllid densities probably would have been even higher in the elevated CO_2 FACE plot compared to the ambient north plot. Our hypotheses that higher hostplant density should correspond with higher psyllid density was supported by the result that lilac psyllid density was almost double under 6 times greater plant density in the ambient N plot compared to the control ring plot (Table 1). The heterogeneous distribution of *C. greggii* among the 15 m diameter plots (Table 1) indicates that other environmental factors such as soil moisture and nutrients

or random events affected post-fire seedling establishment and survival more strongly than atmospheric CO_2 concentration.

Elevated CO_2 appears to have affected psyllids (Fig. 1) differently in this study than it did most fluid-feeding herbivores in the CO_2LT chambers (data presented in Chapter 1). Although not statistically significant, total fluid feeding herbivore abundance was lower under elevated CO_2 in the CO_2LT chambers and FACE ring in 1998, but this trend may have been weakened by inclusion of whole-cell feeding psyllids. The difference between the Chapter 1 fluid-feeder results, and these results for psyllids in this study may be explained by several factors. Perhaps cell content feeding resulted in different physiological responses than phloem feeding and similar folivores, (abundance data presented in Chapter 1). It is also possible that the apparent psyllid population increase with elevated CO_2 may have been mediated by the physiology of *C.greggii*.

Mills (1985) hypothesized that higher foliar water content of *C. greggii* could account for higher insect density and damage compared to *Adenostoma fasciculatum*. Although it has not been consistently observed, foliar water content increased with elevated CO_2 in soybeans and limabeans, and may result from increased water use efficiency when water availability is limited (Lincoln et al. 1993). Therefore, higher water content of foliage may partly explain higher psyllid densities found in this study under elevated CO_2. Another contributing factor may be that *C. greggii* are nitrogen-fixing shrubs, as decreased N content is characteristic of elevated CO_2-grown foliage (Lincoln *et al.* 1993, Bezemer and Jones 1998) and might limit herbivore growth to a greater extent on other hostplant species. Nitrogen-fixing hostplants were one explanation for Goverde *et al.'s* (1999) positive results with common blue butterfly larvae. Thus, the apparent difference between

the response of psyllids in this study compared to all fluid-feeding herbivores in the chambers could have been due to differences among hostplants, insect feeding methods, or both.

Higher psyllid density per shrub volume in the FACE ring in 1999, and a similar (but not significant) pattern in 2000 indicates that psyllid densities may increase under future elevated atmospheric CO_2 levels, leading to increased psyllid infestations of *C. greggii*. Increased population densities are also in general agreement with previous whole-cell feeder results (reviewed by Bezemer and Jones 1998). If psyllid densities do increase, *C. greggii* density could be reduced in the chamise chaparral community (Mills 1985, Moreno and Oechel 1992). While this conclusion is limited to one type of herbivore and one shrub species, *C. greggii* is a co-dominant member of this ecosystem, and its reduction should have major implications for ecosystem dynamics.

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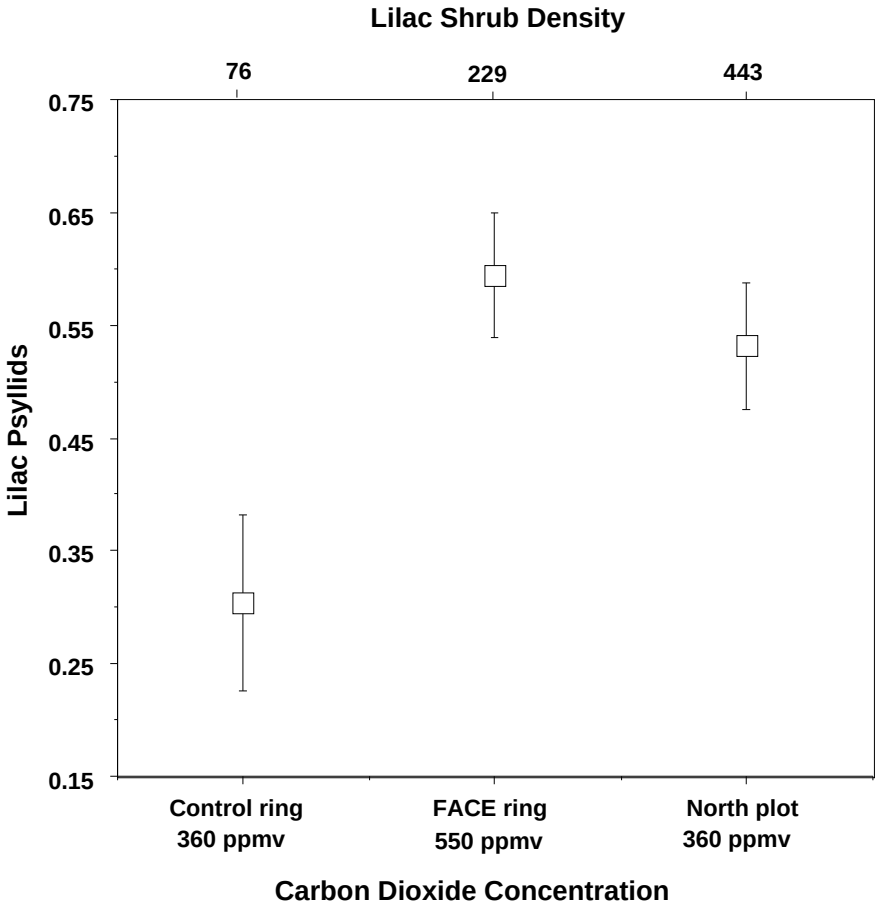

Figure 1. Psyllid abundance per trap in 2000 (log transformed) of the species observed feeding specifically on *Ceanothus greggii*. Lilac shrub density refers to *Ceanothus greggii* shrubs per 15m-diameter ring plot.

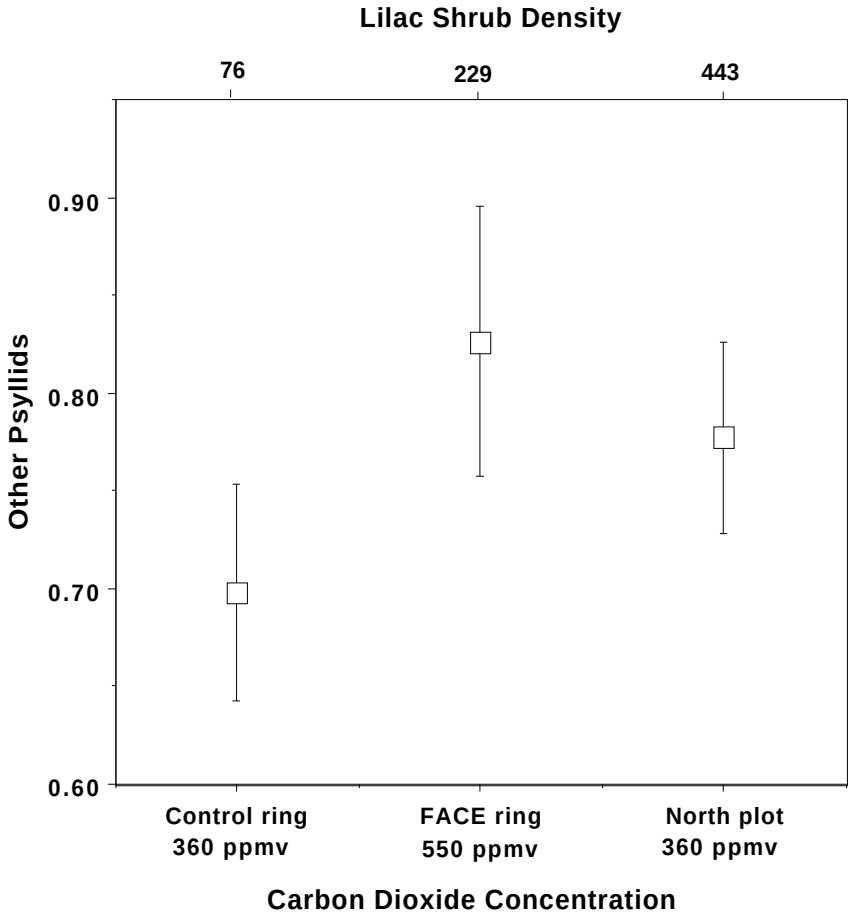

Figure 2. Psyllid abundance per trapin 2000 (log transformed) for all species other than the one known to feed specifically on *Ceanothus greggii*. Lilac shrub density refers to *Ceanothus greggii* shrubs per 15m diameter ring plot.

Effects of lifelong [CO_2] enrichment on carboxylation and light utilization of Quercus pubescens Willd. examined with gas exchange, biochemistry and optical techniques

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ABSTRACT

Lifelong exposure to elevated concentrations of atmospheric CO_2 may enhance carbon assimilation of trees with unlimited rooting volume and consequently may reduce requirements for photoprotective pigments. In early summer we examined effects of elevated [CO_2] on carboxylation and light utilization of mature Quercus pubescens trees growing under chronic [CO_2] enrichment at two CO_2 springs and control sites in Italy. Net photosynthesis was enhanced 36-77%. There was no evidence of photosynthetic downregulation early in the growing season when sink demand presumably was greatest. Specifically, maximum assimilation at saturating [CO_2], electron transport capacity, and Rubisco content, activity and carboxylation capacity were not significantly different in trees growing at the CO_2 springs and their respective control sites. Foliar biochemical content, NDVI (leaf reflectance index of chlorophyll pigments), and photochemical efficiency of

PSII (ΔF/Fm') also were not significantly affected by [CO_2] enrichment except that starch content and ΔF/Fm' tended to be higher at one spring (42% and 15%, respectively). Contrary to expectation, prolonged elevation of [CO_2] did not reduce xanthophyll cycle pigment pools or alter midday PRI values (leaf reflectance index of xanthophyll cycle pigments), despite the enhancement of carbon assimilation. However, both these pigments and PRI were well correlated with electron transport capacity.

Key-words: CO_2 springs, Photochemical Reflectance Index, Quercus pubescens, xanthophyll cycle pigments

INTRODUCTION

Trees account for up to 70% of terrestrial atmospheric carbon fixation (Meyer & Turner 1992; Melilo *et al.* 1993) and thus may have a significant impact on rapidly rising concentrations of atmospheric carbon dioxide (Harmon, Ferrell & Franklin 1990; Walker & Kasting 1992). Carbon uptake is often stimulated by short-term exposure to elevated [CO_2]; however, the photosynthetic response of trees over longer time scales varies with carbon sink demand (Griffin & Seemann 1996). When sink demand is low due to genetic or environmental limitations, photosynthetic capacity is often decreased, or *downregulated*, through biochemical, physiological and morphological feedback mechanisms (Long & Drake 1991; Stitt 1991; Sage 1994; Griffin & Seemann 1996). Photosynthetic downregulation has been observed for older foliage and late in the growing season (e.g., Mousseau 1993; Tissue, Thomas & Strain 1993; Lewis, Tissue & Strain 1996; Rey & Jarvis 1998; Spunda *et al.* 1998; Turnbull *et al.* 1998; Tissue, Griffin & Ball 1999) but is seldom reported for current-year foliage and when resources are readily available (e.g.,

Idso, Kimball & Allen 1991; Gunderson, Norby & Wullschleger 1993; Idso *et al.* 1995; Hogan *et al.* 1997; Turnbull *et al.* 1998). However, these and most other [CO_2] enrichment studies were conducted on seedlings or young trees under controlled conditions and with less than ten years of CO_2 fumigation (Saxe, Ellsworth & Heath 1998; Norby *et al*. 1999; Medlyn *et al*. 1999). Thus they may not describe the physiological responses of more mature trees exposed to chronic [CO_2] enrichment and natural fluctuations in other resources (Eamus & Jarvis 1989; Lee & Jarvis 1995).

Carbon assimilation is a primary sink for light energy captured by photosynthetic pigments. If carbon assimilation rates are increased with long-term [CO_2] enrichment (and irradiance is held constant), more captured light energy will be utilized photochemically, and photochemical efficiency of photosystem two (PSII) may be increased. To the extent that PSII photochemical efficiency is linked to carbon assimilation, we might expect susceptibility to photoinhibitory damage and investment in photoprotective xanthophyll cycle pigments to be reduced with long-term [CO_2] enrichment. Xanthophyll cycle pigments accept excess light energy from excited chlorophyll molecules of PSII and dissipate it harmlessly as heat (Frank *et al.* 1994).

Only a few studies have linked PSII photochemical efficiency and xanthophyll cycle pigment pools with carbon assimilation, particularly in the context of elevated [CO_2]. Under full midday sunlight, leaves with low rates of photosynthesis had greater total and/or de-epoxidized (photoprotective) pools of xanthophyll cycle pigment than leaves with higher photosynthetic rates (Thayer & Björkman 1990; Gamon, Serrano & Surfus 1997). Barták, Nijs & Impens (1996) and Spunda *et al.* (1998) reported that simultaneous measurements

of PSII photochemical efficiency and carbon assimilation varied together with both short-term (minutes) and long-term (six months and four seasons) exposure to [CO_2] enrichment. PSII photochemical efficiency and an optical estimate of xanthophyll cycle pigment activity were also linked with carbon assimilation of plants briefly exposed to sub-ambient CO_2 concentrations (Gamon *et al.* 1997). However, we know of only one investigation that examined the long-term effects of [CO_2] enrichment on these photoprotective pigments (Hogan *et al.* 1997). This investigation reported no elevated-[CO_2] effect on content and activity of xanthophyll cycle pigments of tree seedlings.

The goal of our study was to examine the effect of long-term [CO_2] enrichment on foliar carbon assimilation and photoprotective pigment pools of mature Quercus pubescens Willd. trees growing naturally at CO_2 springs in central Italy. Carbon dioxide springs release CO_2 from geological sources (Miglietta *et al*. 1995) and present a rare opportunity to study *in situ* the long-term adjustment of mature long-lived species to lifelong atmospheric [CO_2] enrichment. For example, a recent study in Iceland reported significant physiological downregulation of a perennial grass species growing near a CO_2 spring thought to be active for more than 100 years (Cook *et al.* 1998). However, CO_2 springs suffer from high spatial and temporal variability in CO_2 concentration, presence of confounding environmental factors such as sulfur emission, and lack of true replicates (Miglietta *et al.* 1993a). We addressed the replication problem by examining the response of Q. pubescens trees at two CO_2 springs and nearby control sites. Additionally, although both springs in this study produce low levels of H_2S and SO_2 (11-22 ppb and 2-16 ppb, respectively; Schulte *et al.* 1998), no damage was apparent at either site or at another spring

with more than 100 times greater concentration of these trace gases (Badiani *et al.* 1993; Miglietta *et al.* 1993b).

To investigate elevated-[CO_2] effects on carboxylation and light utilization of Q. pubescens, we examined gas exchange rates, a biochemical model of photosynthesis, chlorophyll fluorescence of PSII, and foliar biochemical composition of trees at the CO_2 springs and control sites. Additionally, we examined the use of two leaf reflectance indices - the Photochemical Reflectance Index (PRI; a measure of xanthophyll cycle pigment activity; Peñuelas, Filella & Gamon 1995; Gamon *et al.* 1997; Peñuelas *et al.* 1997; Gamon & Surfus 1999) and a modified Normalized Difference Vegetation Index (NDVI; a measure of chlorophyll content; Gitelson & Merzylak 1994; Gamon & Surfus 1999) - to detect variations in pigment content and carbon assimilation. These reflectance indices are of particular interest because they can be measured quickly, non-destructively, and *in situ* and may be useful for scaling the physiological response to [CO_2] enrichment from leaf to canopy to stand levels (cf. Gamon & Qiu 1999).

MATERIALS AND METHODS

Site and species description

Measurements were made on mature Q. pubescens trees growing in forests near two CO_2 springs (Bossoleto and Laiatico) in Tuscany, Italy. Trees at these springs were coppiced 40-50 years ago and have likely been exposed throughout their lives to [CO_2] enrichment (Miglietta *et al.* 1993a; Hättenschwiler *et al.* 1997a). The Bossoleto spring has been described by Körner & Miglietta (1994), van Gardingen *et al.* (1995) and Schulte *et al.* (1998). This spring is near Siena, Italy (43˚ 17’ N, 11˚ 36’ E) and is characterized by a Mediterranean forest dominated by Q. ilex L. and Q. pubescens. CO_2 is discharged from

several vents that occur in a large circular crater (approximately 80 m circumference, 20 m deep). Quercus trees grow on the steep surrounding slopes. A control site (Bossoleto control) with similar species, soil composition and slope orientation (A. Raschi, *unpublished data*) was located 4 km northeast of the spring. The Laiatico spring is approximately 70 km east of the Bossoleto spring. It is within the village of Laiatico near the city of Volterra, Italy (43˚ 26' N, 10˚ 42' E) and has been described in Hättenschwiler *et al.* (1997a) and Schulte *et al.* (1998). A large vent and several neighboring small ones occur along a stream on a gentle north-facing slope. CO_2 flows downslope due to the topography and northeast tending winds (C. Stylinski, *unpublished data*). This spring is covered by a Mediterranean forest dominated in the upper canopy by Q. ilex, Q. pubescens, Q. cerris L., Fraxinus ornus L., and Arbutus unedo L. A control site (Laiatico control) was located in an area with similar species, soil composition and slope orientation (Raiesi 1998a, b) approximately 150 m southeast and upslope from the vent.

Atmospheric [CO_2] has been measured at both springs with infrared gas analyzers and $\Delta^{14}C$ analysis (long-term average of ^{14}C-depleted CO_2 from geological sources that is incorporated into plant tissues). Data from these methods agree and indicate that average daytime atmospheric [CO_2] in the study areas is 590 to 700 µmol mol^{-1} with short-term variations between 450 and 800 µmol mol^{-1} (Körner & Miglietta 1994; Hättenschwiler *et al.* 1997a; Schulze *et al.* 1998). Using a portable infrared gas analyzer (IRGA, EGM-1, PP Systems; Hitchin, UK) placed near the study trees, we monitored atmospheric [CO_2] at the spring and control sites every minute from midmorning (starting between 9:00 and 11:00 a.m.) until midafternoon (ending between 4:00 to 4:30 p.m.) concurrently with physiological measurements.

Physiological measurements were made on three Laiatico and four Bossoleto Q. pubescens trees of similar size growing near each CO_2 spring and control site between late May and mid June 1997. Some measurements were also made on an additional tree at the Bossoleto spring site and control site. Q. pubescens is a winter-deciduous species that can produce multiple leaf flushes. Leaf ontogeny and age can affect physiological responses to elevated [CO_2] (Griffin & Seemann 1996; Turnbull *et al.* 1998). To minimize these effects, measurements of the oldest cohort were made on leaves approximately 1 to 1.5 m above the ground.

Photosynthesis, PSII photochemical efficiency of light-adapted leaves, and activity of xanthophyll cycle pigments are strongly influenced by incident irradiance. Consequently, we attempted to standardize light conditions by selecting leaves on the outer part of the tree crown that received photosynthetic photon flux density (PPFD) greater than 1500 μmol m^{-2} sec^{-1} during our measurement period. Incident PPFD, fluorescence, reflectance, and tissue samples for pigment and ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) analyses were collected from the same subset of leaves. Each leaf was measured and harvested within several minutes, and sampling of each tree was completed within an hour. Sampling at each study site was conducted over several days. Average incident PPFD (±SEM; μmol m^{-2} s^{-1}) for trees at each site was as follows: 1863±43 and 1830±40 (Bossoleto spring and control, respectively) and 1631±17 and 1882±62 (Laiatico spring and control, respectively). Gas exchange measurements required more time (10 to 30 minutes) than these techniques (one to several minutes) and could not be measured concurrently with optical measurements. Instead, rates of gas exchange were collected within one to two

weeks of optical measurements under saturating light (2000 µmol m^{-2} sec^{-1} PPFD) using an internal light source.

Gas exchange

Curves of carbon assimilation response to internal [CO_2] (A/C_i) were measured *in situ* with a gas exchange system (Li-Cor model 6400; Lincoln, NE) equipped with a CO_2 control module. Measurements were made on one to two leaves of each tree under steady-state conditions and at a range of external [CO_2] between 50 and 2200 µmol mol^{-1}. Data were recorded after leaves acclimated to each [CO_2] and when the coefficient of variation between the sample and reference CO_2 analyzers was <3% (5 minutes on average). Temperature was maintained close to ambient air values (27–30 ˚C) with Peltier thermoelectric coolers. Leaf water vapor pressure deficit was maintained with desiccant between 2.1-3.7 kPa, and the range was less than 1 kPa for each response curve.

Using the A/C_i curves and a biochemical model of photosynthesis, we calculated parameters that potentially limit photosynthesis under elevated [CO_2]. We used Photosyn Assistant software (v 1.1, Dundee Scientific; Dundee, Scotland, UK) that applies the biochemical model described by von Caemmerer and Farquhar (1981), Sharkey (1985), Harley and Sharkey (1991), and Harley *et al.* (1992). This model calculates maximum carboxylation rate by Rubisco (V_{cmax}) and ribulose 1,5-bisphophate (RuBP) regeneration capacity regulated by electron transport under saturating light (J_{max}). We did not report the rate of triose phosphate utilization by starch and sucrose synthesis because our A/C_i curves did not appear to saturate (Wullschleger 1993). Maximum carbon assimilation (A_{max}) was

determined from A/C_i curves using net assimilation rates measured at 2200 µmol mol^{-1} $[CO_2]$.

At the Bossoleto control and spring sites only, carbon assimilation and stomatal conductance were also measured on four to five leaves per tree at external $[CO_2]$ of 350 and 600 µmol mol^{-1} and at saturating PPFD (2000 µmol m^{-2} sec^{-1}). These reciprocal measurements were used at the control and spring sites to determine net carbon assimilation rates at approximate growth $[CO_2]$ (350 and 600 µmol mol^{-1}, respectively). At the Laiatico control and spring sites, carbon assimilation and conductance at growth $[CO_2]$ were determined from A/C_i curves using measurements at 350 or 600 µmol mol^{-1}, respectively.

Reflectance and fluorescence

As described above, five to ten leaves were selected from each tree at both study sites for reflectance and fluorescence measurements. Leaf reflectance was measured on the adaxial side with a portable reflectometer (Unispec, PP Systems; Haverhill, MA). The reflectometer consists of a leaf clamp and bifurcated fiber optic cable attached to a light source and to a narrow-waveband spectroradiometer (Gamon & Surfus 1999). Leaf reflectance was determined from leaf radiance divided by radiance of a 99%-reflective white standard (Spectralon, Labsphere Inc.; North Dutton**,** NH). Spectral (wavelength) calibration was checked daily with a mercury-argon lamp and remained stable over the sampling period. The Photochemical Reflectance Index (PRI) was calculated as $(R_{531} - R_{570}) / (R_{531} + R_{570})$, where R_{531} and R_{570} are reflectance of the xanthophyll and reference wavebands, respectively (Gamon *et al.* 1997). The reference waveband largely normalizes for reflectance variations due to chloroplast movement and content of other pigments. The

Normalized Difference Reflectance Index (NDVI) was calculated as (R_{750}– R_{705}) / (R_{750} + R_{705}), where R_{750} and R_{705} are reflectance of the near-infrared region and the edge of red (chlorophyll) absorption wavebands, respectively (Gitelson & Merzylak 1994; Gamon & Surfus 1999).

Chlorophyll fluorescence of PSII was recorded immediately after reflectance measurements on the same portion of the leaf using a modulated fluorometer (OS-500, OptiScience; Tyngsboro, MA). Photochemical efficiency of PSII was calculated as ΔF/Fm' or (Fm' – F) / Fm', where Fm' is the maximal fluorescence during light saturation and F is the level of steady-state fluorescence of light-adapted leaves (Genty, Briantais & Baker 1989).

Foliar chemical analyses

At the Bossoleto spring and control sites only, disks were punched from leaves measured for reflectance and fluorescence (one leaf per tree). Disks were immediately immersed in liquid nitrogen and stored at –80˚C until biochemical analysis. Chlorophyll, carotenoid and xanthophyll cycle pigments were extracted and analyzed with high-performance liquid chromatography (HPLC; model LC-10AS with detector SPD-10AV, Shimadzu; Kyoto, Japan) according to Thayer & Björkman (1990). Xanthophyll cycle pigments were expressed as total pool size (violaxanthin + antheraxanthin + zeaxanthin) and pool size in the de-epoxidized state (0.5 * antheraxanthin + zeaxanthin) / (violaxanthin + antheraxanthin + zeaxanthin).

From each tree, five of the leaves measured optically were also harvested, immediately frozen and stored at –80˚C for Rubisco analysis. Content, total activity (fully

activated) and activation state (ratio of initial to total activity) of Rubisco were determined as described by Tissue *et al*. (1993).

Additional leaves were collected from each tree after reflectance and fluorescence measurements at the Bossoleto site for content of total nitrogen, total carbon, sugar and starch. Within three hours of harvesting, leaves were dried in a microwave oven for five minutes (Körner & Miglietta 1994). Prior to chemical analysis, the leaves were redried in a conventional oven at 70˚C to a constant mass and ground in a Wiley mill with 60-mesh screen. Content of total nitrogen and of total carbon were determined with an elemental analyzer (NSC 2500, Fison Instruments, Milan, Italy). Sugar content and starch content were determined as described by Gaines (1973). Nonstructural carbohydrates (TNC) were calculated as the sum of sugar and starch.

Statistical Analysis

Student's *t*-tests for populations with unequal variance were used to compare spring and control site variables (Systat v.8, SPSS, Inc.). Correlations between PRI, pigment content and electron transport capacity were examined with the Pearson correlation analysis (Systat v.8 SPSS, Inc.). Percentage differences between spring and control sites were calculated as [(spring site – control site) / control site] * 100. Other values are presented as mean ± one standard error of the mean (SEM).

RESULTS

Average daily atmospheric CO_2 concentrations during our sampling period were 368 µmol mol^{-1} (±3, n=5 days) and 664 µmol mol^{-1} (±70, n=6 days) at the Bossoleto control and spring sites, respectively, and 353 µmol mol^{-1} (n=1 day) and 830 µmol mol^{-1}

(±21, n=2 days) at the Laiatico control and spring sites, respectively. These values agree with other estimates of [CO_2] at the Bossoleto and Laiatico sites (Körner & Miglietta 1994; Hättenschwiler *et al.* 1997a; Schulte *et al.* 1998). In our study, IRGA measurements at the Laiatico spring likely overestimated [CO_2] available to the study trees because the IRGA was several meters closer to the vents than were the study trees. Minute-to-minute [CO_2] fluctuations were large (up to 10,000 µmol mol^{-1}) at both CO_2 springs but did not appear to affect physiological measurements (data not shown). For example, the standard deviation values for ΔF/Fm' for each tree (typically measured every few minutes for 30 to 90 minutes) were relatively small and similar in magnitude at the spring and control sites (≤0.07).

Net carbon assimilation at growth [CO_2] was 77% higher at the Bossoleto spring (P=0.008) and 36% higher at the Laiatico spring (P=0.096) than at their respective control sites (Table 1). Stomatal conductance was not significantly different at these sites. At both the Bossoleto and Laiatico study sites, no photosynthetic downregulation was evident from modeled biochemical parameters and gas exchange measurements. Specifically, Rubisco carboxylation capacity (V_{cmax}), electron transport capacity (J_{max}), and maximum carbon assimilation (A_{max}) were not significantly different for the two springs and their respective control sites (Fig. 1). Similarly, photosynthetic rates measured at a common CO_2 concentration (350 or 600 µmol mol^{-1}) were not significantly different between trees at the Bossoleto control and spring sites (350 µmol mol^{-1}, P=0.78 and 600 µmol mol^{-1}, P=0.19; not shown).

Variations in leaf chemistry at the Bossoleto study site were consistent with photosynthetic trends (Table 2). Total nitrogen content and Rubisco content, activity, and

activation state were not significantly different between the Bossoleto spring and control sites. Although Rubisco was not measured at the Laiatico study site, V_{cmax} correlates with Rubisco content and activity (Sage 1994; r^2=0.91 and 0.87 in this study). The lack of an elevated-[CO_2] effect on V_{cmax} suggests there was no difference between Rubisco pools of leaves at the Laiatico spring and control sites. Content of chlorophyll, carotenoids, and xanthophyll cycle pigments (expressed per leaf area or per chlorophyll content) and de-epoxidization state of xanthophyll cycle pigments also did not differ between the Bossoleto spring and control sites. Sugar and TNC content and carbon-to-nitrogen ratios were similar for plants growing at the Bossoleto spring and control sites. Only starch content differed notably between these sites, measuring higher at the CO_2 spring (42%, P=0.10).

The degree of multiple scattering of light through the mesophyll layer of a leaf determines reflectance in the near-infrared wavebands (700-1000 nm) (Gausman 1985). By this measure there was no apparent effect of [CO_2] enrichment on leaf spectra of Q. pubescens (data not shown), suggesting that leaf thickness was not altered by growth at high [CO_2]. Additionally and in agreement with the HPLC analysis, leaf NDVI for Q. pubescens trees was not significantly different at the Bossoleto and Laiatico study sites, indicating no difference in chlorophyll content (Fig. 2; also see Table 2). Despite enhanced carbon assimilation at growth [CO_2], PRI also did not differ significantly between spring and control trees at these sites, indicating no difference in xanthophyll cycle pigment levels. Likewise, ΔF/Fm' was not significantly different at the Laiatico spring and control sites and was only 15% higher at the Bossoleto spring site compared to the control site (P=0.09).

When data from the Bossoleto spring and control sites were combined, total content of xanthophyll cycle pigments was well correlated with PRI (r^2=0.75, Fig. 3), suggesting

that this *in situ* optical measurement (normalized for incident light) is a valid surrogate for these pigments. A strong relationship between PRI and xanthophyll cycle pigments has also been observed for several other species (Peñuelas *et al.* 1994; Gamon *et al.* 1997; Gamon & Surfus 1999; Stylinski 2000). Total content of xanthophyll cycle pigments was also well correlated with J_{max} (r^2=0.92). When Laiatico and Bossoleto data were combined, PRI also correlated with J_{max} (overall r^2=0.79, P<0.001). A single modeled value (unfilled symbol, Fig. 3) was treated as an outlier and excluded from the correlation analyses. Note that we were not able to measure A/C_i curves and leaf reflectance on the same leaves or the same dates, which may have led to this discrepancy.

DISCUSSION

In early summer, net carbon assimilation was stimulated 36% and 77% for mature Quercus pubescens trees growing in native soils and with lifelong [CO_2] enrichment at two CO_2 springs compared to nearby control sites. By contrast, stomatal conductance did not differ between the spring and control sites. These findings agree with many other tree studies investigating exposure to [CO_2] enrichment (see reviews by Curtis & Wang 1998, Saxe *et al*. 1998, Norby *et al.* 1999 and Medlyn *et al*. 1999). If maintained, enhanced leaf photosynthetic rates at the CO_2 springs could increase carbon sequestering and productivity of whole tree canopies. Furthermore, because trees contribute significantly to terrestrial carbon fixation (Meyer & Turner 1992; Melilo *et al.* 1993), higher carbon acquisition by Q. pubescens and other species could slow the rise in atmospheric [CO_2]. However, elevated-[CO_2] effects on respiratory loss of carbon and on higher-level plant attributes (e.g., canopy leaf area) must also be examined to predict the response of whole trees and stands to [CO_2] enrichment (Saxe *et al*. 1998; Norby *et al*. 1999).

Photosynthetic downregulation was not observed for Q. pubescens trees growing at the CO_2 springs early in the growing season, when sink demand likely was greatest. When downregulation occurs, maximum carbon assimilation (A_{max}), electron transport capacity (J_{max}), and Rubisco content, activity, and carboxylation capacity (V_{cmax}) are typically lower in plants growing under elevated [CO_2] than in those growing under ambient [CO_2] (Sage, Sharkey & Seemann 1989; Stitt 1991; Sage 1994). In our study, these variables were not significantly different between trees growing at the two CO_2 springs and their respective control sites. The absence of photosynthetic downregulation in current-year leaves has been observed in other studies of trees with unlimited rooting volume that were exposed to elevated [CO_2] (e.g., Idso *et al.* 1991, 1995; Gunderson *et al.* 1993; Teskey 1995; Scarascia-Mugnozza *et al*. 1996; Hogan *et al.* 1997; Turnbull *et al.* 1998). These studies include initial investigations at the Bossoleto spring and control sites, which found no difference in photosynthetic capacity in early summer for Q. pubescens (van Gardingen *et al.* 1997; Tognetti *et al.* 1998). At another Italian CO_2 spring, Jones *et al.* (1995) also reported no photosynthetic downregulation of A. unedo trees in early summer.

These findings on current-year foliage (typically measured at the beginning of the growing season) contrast with well-documented diminished photosynthetic capacity under high [CO_2] of older leaves and of leaves under less than optimal environmental conditions (e.g., Mousseau 1993; Tissue *et al*. 1993, 1997, 1999; Lewis *et al*. 1996; Rey & Jarvis 1998; Spunda *et al*. 1998; Turnbull *et al.* 1998; also see review by Medlyn *et al*. 1999). For example, A_{max} did not differ for new *Q. ilex* leaves but was downregulated for one-year-old leaves at the Laiatico CO_2 spring compared to a control site (C. Cario *et al*. *unpublished*

data). Similarly, photosynthetic capacity of Q. pubescens at the CO_2 springs may be lower in the summer and autumn months, when sink demand is reduced.

Q. pubescens at the Bossoleto CO_2 spring had no foliar sugar accumulation and only moderate starch accumulation, indicating that these trees have an active carbon sink within or near the leaves and that the leaves can effectively translocate surplus assimilates (Saxe *et al.* 1998). Higher rates of leaf isoprene emission by Q. pubescens at the Bossoleto spring may also limit carbohydrate accumulation in the leaves (Tognetti *et al.* 1998). Trees growing at the CO_2 springs also did not have lower foliar nitrogen and/or Rubisco (estimated by wet chemistry or V_{cmax}), possibly due to larger soil total nitrogen pools and higher nitrogen mineralization rates at the CO_2 springs (Raiesi 1998a, b). In general, these biochemical results agree with other studies of perennial species growing at Italian CO_2 springs (Körner & Miglietta 1994; Bettarini *et al.* 1995; Jones *et al.* 1995; Miglietta *et al.* 1995).

Increased biomass may provide a sink for the surplus carbon fixed by trees at Italian CO_2 springs. Raiesi (1998a, b) attributed higher carbon and nitrogen pools (g m^{-2}) in the soil at the Laiatico spring to enhanced net primary productivity of the surrounding vegetation. Additionally, Hättenschwiler *et al.* (1997a) noted higher radial stem width in young Q. ilex trees at the Bossoleto and Laiatico CO_2 springs compared to control trees. By contrast, Hättenschwiler *et al.* (1997b) found lower leaf area per unit branch biomass for Q. ilex at the Bossoleto spring. However, these branch data do not necessarily apply to Q. pubescens or scale up to the whole tree. Indeed, many other studies have reported no change or an increase in leaf area index with elevated [CO_2] (e.g., Tissue *et al.* 1997; also see reviews by Saxe *et al.* 1998 and Norby *et al.* 1999).

Given similar irradiance and absorptance, we hypothesized that enhanced carbon assimilation rates with elevated [CO_2] would reduce content and activity of xanthophyll cycle pigments (as measured optically *in situ* or by wet chemistry) and improve PSII photochemical efficiency (ΔF/Fm'). However, with the exception of a small increase in ΔF/Fm' at the Bossoleto spring site, these variables did not differ between the spring and control sites, despite the significant stimulation of net photosynthesis at growth [CO_2]. This pattern was also observed in June 1997 for mature Q. ilex trees exposed to [CO_2] two times greater than ambient levels for more than seven years in open-top chambers in a central Italy forest (C. Stylinski, G. Matteucci & P. De Angelis, *unpublished data*; also see Scarascia-Mugnozza *et al.* 1996). Furthermore, Hogan *et al.* (1997) reported higher photosynthetic rates at growth [CO_2] and no change in total and de-epoxidized pools of xanthophyll cycle pigments and ΔF/Fm' (normalized for incident light) of Nothofagus fusca (Hook F.) Oersted and Pinus radiata D. Don seedlings grown with elevated [CO_2] compared to those grown at ambient [CO_2]. However, studies of other woody species have recorded higher and lower ΔF/Fm' with long-term [CO_2] enrichment (see review by Saxe *et al.* 1998). Inconsistent trends are also reported for maximum PSII photochemical efficiency for woody species under high [CO_2] (e.g., Jones *et al.* 1995; Faria *et al.* 1996; Scarascia-Mugnozza *et al*. 1996; Hogan *et al.* 1997; Spunda *et al.* 1998). Additionally, antioxidants – another photoprotective mechanism – are reportedly both higher and lower with [CO_2] enrichment (Badiani *et al.* 1993; Schwanz *et al*. 1996; Polle *et al.* 1997; Schwanz & Polle 1998). Overall, these contradictory effects of elevated [CO_2] on xanthophyll cycle pigments, antioxidants and PSII photochemical efficiency suggest that we do not have a clear understanding of the response of photoprotective mechanisms to [CO_2] enrichment.

Leaf age and developmental history and resource availability certainly influence these responses (Saxe *et al.* 1998), particularly of xanthophyll cycle pigments (Demmig-Adams & Adams 1996; Gamon *et al*. 1997; Gamon & Surfus 1999), and some of these could have been complicating factors in our study.

While others have reported a link between xanthophyll cycle pigments and photosynthesis (both measured under full midday sunlight; Thayer & Björkman 1990; Gamon *et al.* 1997), our results demonstrate that xanthophyll cycle pigments and thus PRI are poor indicators of carbon assimilation at growth [CO_2] for plants growing under long-term [CO_2] enrichment. However, these pigments and PRI were well correlated with J_{max} of Q. pubescens. This relationship with J_{max} occurs because downregulation of electron transport capacity is associated with a rise in non-photochemical quenching of chlorophyll fluorescence (Weis & Berry 1987); xanthophyll cycle pigments provide a form of non-photochemical quenching (Demmig-Adams & Adams 1992; Chaumont, Morot-Gaudry & Foyer 1995). This relationship may be useful for other investigations and deserves further exploration.

In conclusion, photosynthesis of mature Q. pubescens trees with unlimited rooting volume was not downregulated with lifetime exposure to elevated [CO_2], at least early in the growing season when resources were readily available. Despite enhanced carbon assimilation rates at the CO_2 springs, investments in photoprotective xanthophyll cycle pigment were not reduced. Although PSII photochemical efficiency of light-adapted leaves was slightly higher at one CO_2 spring, the capacity of electron transport was not affected by [CO_2] enrichment at either study site. Both PRI and the total pool of xanthophyll cycle pigments were well correlated with electron transport capacity of Q. pubescens. However,

neither variable was a useful indicator of carbon assimilation at growth [CO_2] for plants under long-term [CO_2] enrichment.

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Table 1: Gas exchange rates of Quercus pubescens leaves measured at growth [CO_2] at the Laiatico and Bossoleto study sites. Growth [CO_2] was 350 µmol mol^{-1} for control sites and 600 µmol mol^{-1} for spring sites. Values are means ± SEM and were determined either from A/C_i curves (Laiatico; n=3) or from gas exchange measurements made at 350 or 600 µmol mol^{-1} (Bossoleto; n=4). *P*-values are from *t*-tests.

Gas exchange at growth [CO_2]	Control site	Spring site	*P*-value
Laiatico			
Assimilation (µmol m^{-2} s^{-1})	11.5 ± 1.6	15.6 ± 0.6	0.096
Conductance (mol m^{-2} s^{-1})	0.17 ± 0.03	0.15 ± 0.03	0.559
Bossoleto			
Assimilation (µmol m^{-2} s^{-1})	11.1 ± 0.9	19.7 ± 1.7	0.008
Conductance (mol m^{-2} s^{-1})	0.17 ± 0.02	0.16 ± 0.02	0.820

Table 2: Leaf chemistry of Quercus pubescens trees growing at the Bossoleto CO_2 spring and control site. Values are means ± SEM (n=4). *P*-values are from *t*-tests. [V+A+Z] is total xanthophyll cycle pigments. De-epoxidization state is the proportion of pigments in the photoprotective state. TNC is total nonstructural carbohydrates.

	Control site	Spring site	*P*-value
Nitrogen			
Total nitrogen (%)	2.0 ± 0.1	2.1 ± 0.1	0.64
Rubisco content (mg m^{-2})	1088 ± 88	1186 ± 36	0.36
Rubisco activity (μmol m^{-2} s^{-1})	45.3 ± 3.9	50.9 ± 0.7	0.25
Rubisco activation state (%)	69.2 ± 0.6	68.9 ± 1.2	0.84
Pigments			
Chlorophyll (μmol m^{-2})	432 ± 60	375 ± 66	0.55
Carotenoid (μmol m^{-2})	191 ± 19	157 ± 19	0.26
Chlorophyll / Carotenoid	2.2 ± 0.1	2.3 ± 0.1	0.60
[V+A+Z] (μmol m^{-2})	38 ± 3	31 ± 3	0.22
[V+A+Z] / Chlorophyll	0.1 ± 0.0	0.1 ± 0.0	0.85
De-epoxidation state	0.5 ± 0.1	0.5 ± 0.1	1.00
Carbon			
TNC (%)	21.4 ± 1.9	25.4 ± 2.0	0.20
Total sugars (%)	18.1 ± 1.7	21.2 ± 1.8	0.25

	Control site	Spring site	*P*-value
Starch (%)	3.6 ± 0.2	5.1 ± 0.7	0.10
Carbon / Nitrogen	26.4 ± 1.6	25.1 ± 0.9	0.52

FIGURE LEGENDS

Figure 1: Modeled biochemical parameters based on analysis of A/C_i response curves for Quercus pubescens trees growing at two CO_2 springs and control sites. Values are mean ± SEM (n=4 at Bossoleto and n=3 at Laiatico). *P*-values are from *t*-tests.

Figure 2: NDVI (a measure of chlorophyll content), PRI (a measure of xanthophyll cycle pigment activity) and PSII photochemical efficiency of light-adapted leaves (ΔF/Fm') measured at growth CO_2 concentration for Quercus pubescens trees growing at the two CO_2 springs and control sites. Values are mean ± SEM (n=4 at Bossoleto and n=3 at Laiatico). *P*-values are from *t*-tests.

Figure 3: Correlations between PRI, total pool of xanthophyll cycle pigments (normalized by chlorophyll) and J_{max} (modeled parameter of electron transport capacity) of Quercus pubescens trees. The top and left graphs are of Q. pubescens at the Bossoleto site. The right graph includes both the Bossoleto (circles, solid line) and Laiatico (triangles, dashed line) sites. The unfilled symbol was treated as an outlier and not included in correlation analyses (see text for details).

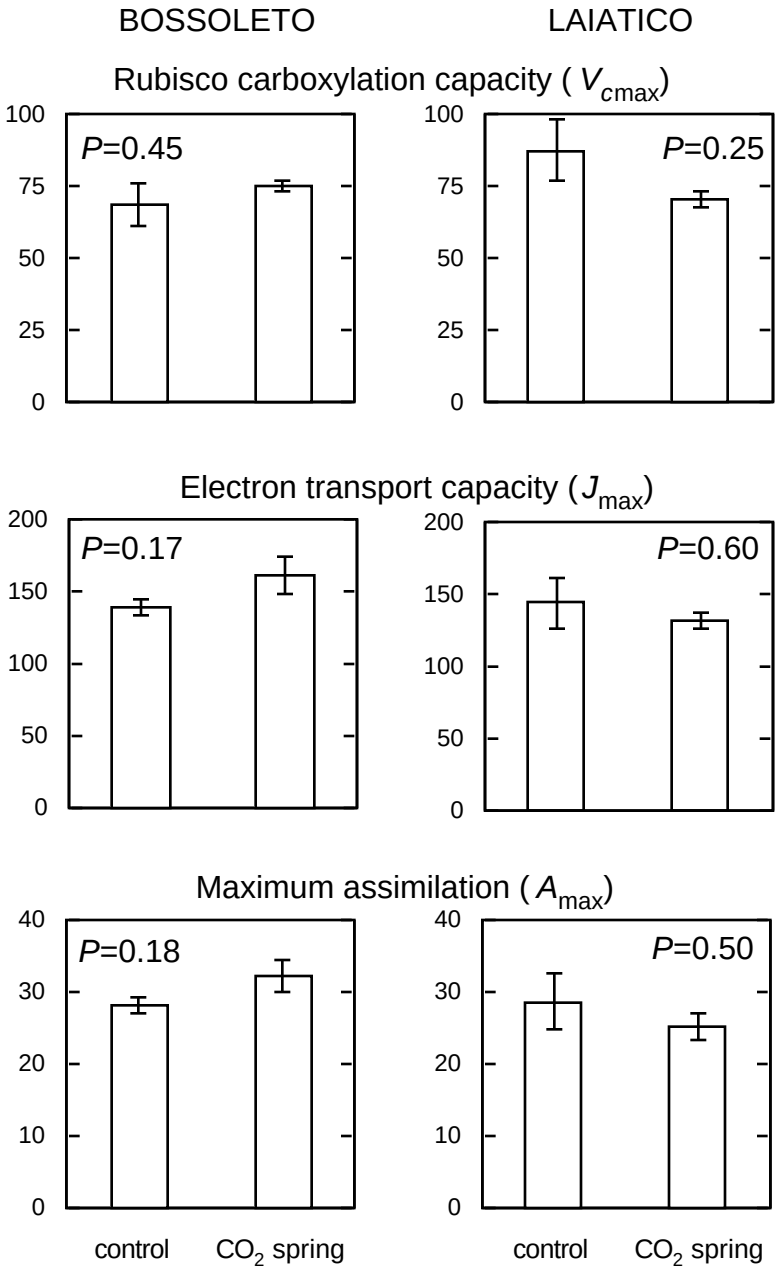
BOSSOLETO
LAIATICO
Rubisco carboxylation capacity (V_{cmax})
P=0.45
P=0.25
Electron transport capacity (J_{max})
P=0.17
P=0.60
Maximum assimilation (A_{max})
P=0.18
P=0.50
100
75
50
25
0
200
150
40
30
20
10
control
CO_2 spring

Figure 1

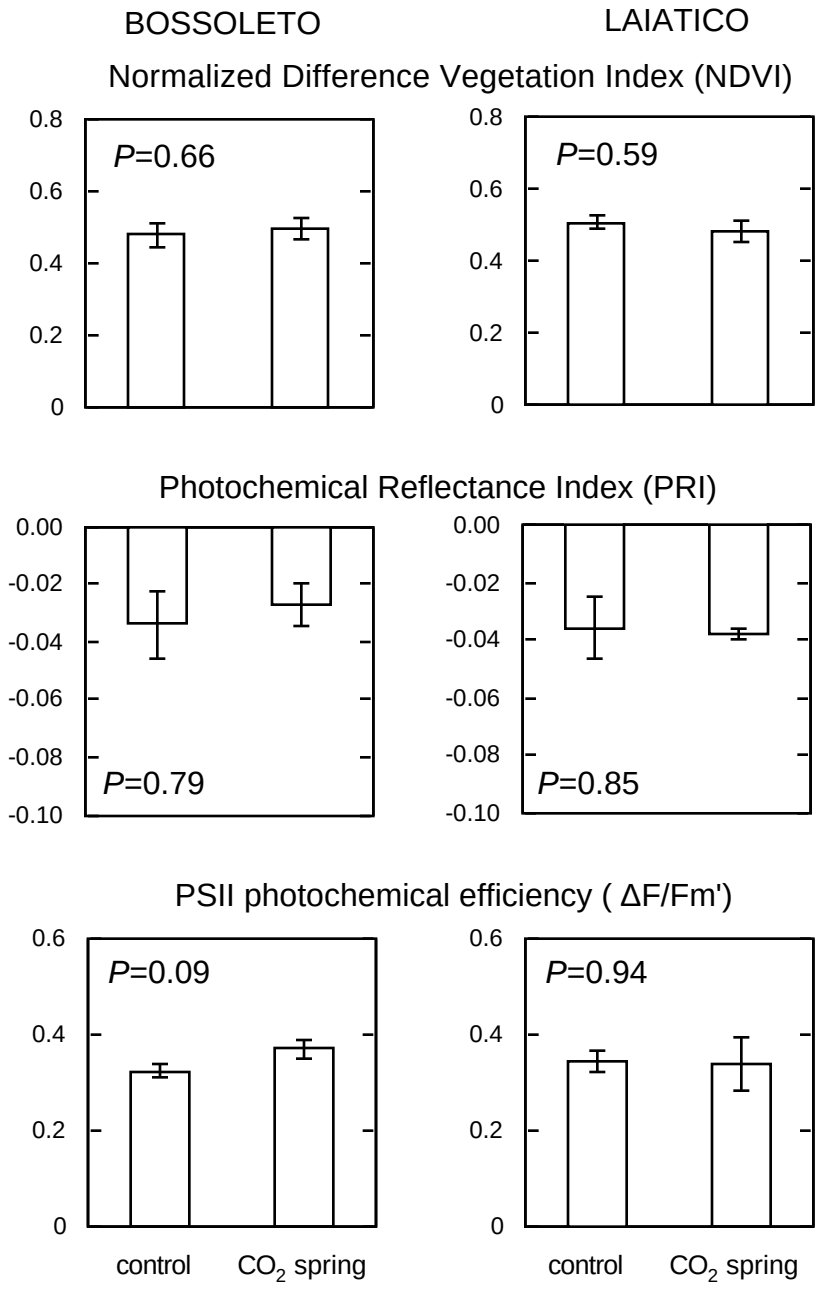
BOSSOLETO
LAIATICO
Normalized Difference Vegetation Index (NDVI)
P=0.66
P=0.59
0.8
0.6
0.4
0.2
0
Photochemical Reflectance Index (PRI)
P=0.79
P=0.85
0.00
-0.02
-0.04
-0.06
-0.08
-0.10
PSII photochemical efficiency (ΔF/Fm')
P=0.09
P=0.94
control
CO2 spring

Figure 2

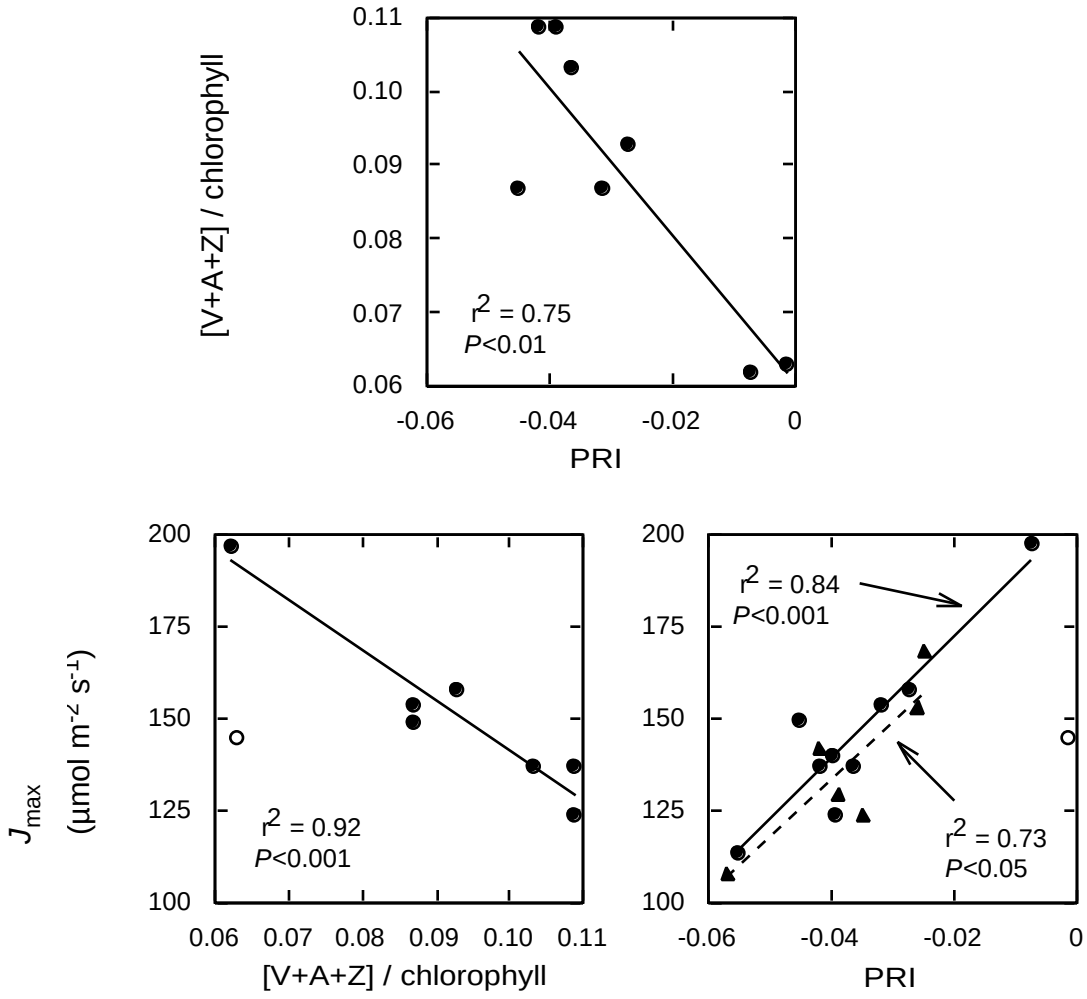
[V+A+Z] / chlorophyll
0.11
0.10
0.09
0.08
0.07
0.06
r2 = 0.75
P<0.01
-0.06
-0.04
-0.02
0
PRI
Jmax
(µmol m-2 s-1)
200
175
150
125
100
r2 = 0.92
P<0.001
0.06
0.07
0.08
0.09
0.10
0.11
[V+A+Z] / chlorophyll
r2 = 0.84
P<0.001
r2 = 0.73
P<0.05
-0.06
-0.04
-0.02
0
PRI

Figure 3