Root growth and function of three Mojave Desert grasses in response to elevated atmospheric CO_2 concentration

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SUMMARY

Root growth and physiological responses to elevated CO_2 were investigated for three important Mojave Desert grasses: the C_3 perennial *Achnatherum hymenoides*, the C_4 perennial *Pleuraphis rigida* and the C_3 annual *Bromus madritensis* ssp. *rubens*. Seeds of each species were grown at ambient (360 µl l^{-1}) or elevated (1000 µl l^{-1}) CO_2 in a glasshouse and harvested at three phenological stages: vegetative, anthesis and seed fill. Because *P. rigida* did not flower during the course of this study, harvests for this species represent three vegetative stages. Primary productivity was increased in both C_3 grasses in response to elevated CO_2 (40 and 19% for *A. hymenoides* and *B. rubens*, respectively), but root biomass increased only in the C_3 perennial grass. Neither above-ground nor below-ground biomass of the C_4 perennial grass was significantly affected by the CO_2 treatment. Elevated CO_2 did not significantly affect root surface area for any species. Total plant nitrogen was also not statistically different between CO_2 treatments for any species, indicating no enhanced uptake of N under elevated CO_2. Physiological uptake capacities for NO_3 and NH_4 were not affected by the CO_2 treatment during the second harvest; measurements were not made for the first harvest. However, at the third harvest uptake capacity was significantly decreased in response to elevated CO_2 for at least one N form in each species. NO_3 uptake rates were lower in *A. hymenoides* and *P. rigida*, and NH_4 uptake rates were lower in *B. rubens* at elevated CO_2. Nitrogen uptake on a whole root-system basis ($NO_3 + NH_4$ uptake capacity × root biomass) was influenced positively by elevated CO_2 only for *A. hymenoides* after anthesis. These results suggest that elevated CO_2 may result in a competitive advantage for *A. hymenoides* relative to species that do not increase root-system N uptake capacity. Root respiration measurements normalized to 20°C were not significantly affected by the CO_2 treatment. However, specific root respiration was significantly correlated with either root C:N ratio or root water content when all data per species were included within a simple regression model. The results of this study provide little evidence for up-regulation of root physiology in response to elevated CO_2 and indicate that root biomass responses to CO_2 are species-specific.

Key words: elevated CO_2, root growth, root respiration, water relations, nitrogen uptake, *Bromus madritensis* ssp. *rubens*, *Achnatherum hymenoides*, *Pleuraphis rigida*.

INTRODUCTION

Hot deserts, such as the Mojave Desert in southwestern North America, are predicted to be among the most sensitive ecosystems to rising atmospheric CO_2 concentration (Strain & Bazzaz, 1983). Studies

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in a variety of ecosystems have measured increased assimilation rate but decreased stomatal conductance under elevated atmospheric CO_2, which increases water-use efficiency at the leaf level (Koch & Mooney, 1996). Increased water-use efficiency is especially important in arid ecosystems, and models that incorporate effects of elevated CO_2 on water-use efficiency predict that deserts will have among the

largest relative increase in net primary production (Melillo *et al.*, 1993). However, deserts are both water- and nutrient-limited ecosystems (Smith *et al.*, 1997), and it is not certain how long plants can sustain a positive response to CO_2 without concomitant increases in the availability and/or acquisition of growth-limiting nutrients. Thus understanding the interaction between rising atmospheric CO_2 concentration and factors that may affect the availability and uptake of other resources, such as water and nutrients, has been recognized as a key element in predicting plant and ecosystem responses to global change (Bazzaz, 1990).

Increased water and nutrient acquisition in response to elevated CO_2 may occur through increases in carbon allocation below ground, which in turn can be utilized to grow larger root systems. Increased root biomass, greater root growth and length, and changes in root distribution occurred concomitantly with increased atmospheric CO_2 concentrations in experiments with different C_3 species, including crop plants (Prior *et al.*, 1994), woody species (Ceulemans & Mousseau, 1994; Norby, 1994), and Great Basin grasses (Smith *et al.*, 1987). In a survey of 150 observations of plant responses to elevated CO_2, Rogers *et al.* (1994) found that 87% of the species increased absolute production of roots. The increase in response to elevated CO_2 can be large for fine roots: more than twofold during a multi-year study of *Citrus aurantinum* (Idso & Kimball, 1992) and one- to twofold in a multi-year study of *Pinus ponderosa* (Tingey *et al.*, 1996). Increases in root distribution may be important in terms of plant resource acquisition, particularly if biomass is not allocated into tap roots or other highly suberized components of the root system that are not involved in water and nutrient uptake (Berntson & Woodward, 1992; Stulen & Den Hertog, 1993). Elevated CO_2 can also result in more highly branched root systems, which permit plants to explore larger soil volumes (Norby, 1994; Rogers *et al.*, 1994).

In addition to the size and architecture of root systems, elevated CO_2 may also influence root physiological uptake capacity. Root uptake and assimilation of NO_3 and NH_4 are energy-requiring processes (Bloom *et al.*, 1992; Lambers, 1996), and because elevated CO_2 can enhance the supply of root respiratory substrates (Tschaplinski *et al.*, 1993; BassiriRad *et al.*, 1996b), metabolically regulated processes such as root N uptake are expected to be stimulated under elevated CO_2. However, recent studies of NO_3 and NH_4 uptake in response to elevated atmospheric CO_2 have shown surprising and sometimes conflicting results. Root uptake capacity for NO_3 actually decreased significantly in response to CO_2 enrichment in *Larrea tridentata* (BassiriRad *et al.*, 1997) as well as in annual grass and forb species (Jackson & Reynolds, 1996). However, NO_3 uptake rates more than doubled in

Bouteloua eriopoda and were unaffected in *Prosopis glandulosa* (BassiriRad *et al.*, 1997). For NH_4 uptake, no evidence of up-regulation in response to elevated CO_2 has yet been documented (BassiriRad *et al.*, 1996a,b; Jackson & Reynolds, 1996)

Studies of responses of desert vegetation to elevated CO_2 have been largely confined to cacti and other succulents (Nobel & Hartsock, 1986; Palta & Nobel, 1989). There are few studies on shrubs, grasses and annuals, which dominate most North American deserts (e.g. Drennan & Nobel, 1996; Huxman *et al.*, 1998). In the present study, the importance of altered root growth and function were investigated following long-term exposure of three Mojave Desert grass species: *Achnatherum hymenoides* (a C_3 perennial), *Bromus madritensis* ssp. *rubens* (an introduced C_3 annual), and *Pleuraphis rigida* (a C_4 perennial) to ambient (~ 360 µl l^{-1}) or elevated (~ 1000 µl l^{-1}) atmospheric CO_2 concentration. These species were selected to represent the major functional types among grasses in the Mojave Desert: C_3 versus C_4, and annual versus perennial. The objective was to assess the relative importance of root growth, root respiration and physiological uptake capacity in determining plant N uptake responses to elevated CO_2.

MATERIALS AND METHODS

In February 1997, seeds of red brome (*Bromus madritensis* ssp. *rubens*) (L.) Husnot, Indian ricegrass (*Achnatherum hymenoides* (Roemer and Schultes) Barkworth; previously known as *Oryzopsis hymenoides* (Roemer and Schultes) Ricker), and galleta grass (*Pleuraphis rigida* Thurber; formerly *Hilaria rigida* Scribner) were planted in monoculture in 1 m tall $\times$ 0.15 m diameter PVC pots. A homogeneous sand with approx. 2 µg g^{-1} NO_3 and 10 µg g^{-1} NH_4 was used for a potting medium. Pots were placed in two adjacent glasshouses in the Fritz Went Glasshouse Facility at the University of Nevada, Reno, USA. One glasshouse was maintained at ambient atmospheric CO_2 concentration (~ 360 µl l^{-1}) and the other at elevated concentration (~ 1000 µl l^{-1}). We used 1000 µl l^{-1} CO_2 in order to maximize the potential for CO_2 response. Within each CO_2 treatment room, 18 pots of each species were thinned to a density of 15 (*B. rubens*), eight (*A. hymenoides*) and five (*P. rigida*) plants per pot, 1 wk after planting. These plant densities are similar to tiller densities found at the Nevada Desert FACE Facility (Jordan *et al.*, 1999). Pots were watered twice a week to maintain soil water content near pot capacity. No additional nutrients were added, and no evidence of nutrient deficiencies was noted during the study. Plants were grown under natural irradiance and 28/20°C day/night thermoperiod.

All above-ground tissues and whole-root systems from six replicate pots of each species per CO_2

Table 1. *Number of days since planting and phenological stage corresponding to each harvest for* Achnatherum hymenoides, Bromus madritensis *spp*. rubens *and* Pleuraphis rigida

	A. hymenoides		B. rubens		P. rigida	
Harvest date	No. of days	Phenological stage	No. of days	Phenological stage	No. of days	Phenological stage
1	45	Vegetative	27	Vegetative	80	Vegetative
2	71	Anthesis	55	Anthesis	122	Vegetative
3	108	Seed fill	85	Seed fill	161	Vegetative

Initiation of phenological stages was similar between CO_2 treatments.

treatment were harvested on three sampling dates corresponding to different shoot phenologies: vegetative, anthesis and seed fill. *Pleuraphis rigida* never initiated flowering, thus harvest dates for this species represent three vegetative stages. Initiation of different phenological stages was very similar between the CO_2 treatments, but different among the three species. Table 1 summarizes the number of days from planting to each harvest for the three species.

On each sample date, a 0.2×1 m section of PVC was cut from the side of each pot, and root samples were removed from depths between 0.10 and 0.80 m for physiological measurements. Harvest of roots from each CO_2 treatment was alternated to prevent masking of treatment effects by inherent diurnal variation of physiological parameters. Fine root subsamples (< 1 mm in diameter) were washed in deionized water to remove adsorbed soil and organic matter, blotted dry with paper towels, and weighed to obtain fresh weights before analysis. All remaining roots in each pot were collected by rinsing the soil within the pots through a fine mesh (0.6 mm). The rinsed roots were blotted dry with paper towels and fresh weights recorded. The roots were then wrapped in moist paper towels, placed in plastic bags and stored in a cold room at 4°C. Surface area measurements were made on dyed roots (Congo red) which were placed in a single layer between sheets of clear acetate. Images of the roots were collected using a scanner attached to a PC. A DOS-based computer program (ROOT 32 Version 3.1; Ryan Dotson, Desert Research Institute, Reno, NV) was used to convert the number of pixels associated with a given image to surface area estimates (cm²). On completion of surface area measurements, roots were oven-dried at 45°C to a constant mass, weighed, and their N content determined with an elemental CHN analyser (Perkin-Elmer 2400 Elemental Analyzer, Norwalk, CT, USA). Nitrogen uptake rate was calculated as the ratio of the at-harvest plant N content to at-harvest root dry mass. Root biomass, surface area and N measurements are expressed per individual plant rather than per pot, to account for random mortality and subsequent differences in numbers of individuals within pots of a given species and treatment.

Above-ground tissues were sorted by tissue type (leaves, culms, inflorescences and dead), dried in a convection oven at 45°C to a constant mass, and weighed for dry mass. As with root tissue, the N content of above-ground tissues was determined with an elemental CHN analyser.

NO_3 and NH_4 uptake kinetics

During the second and third harvests for each species, approx. 100 mg (f. wt) of fine root subsamples collected from the middle section of the pots (depths between 0.2 and 0.6 m) were placed in empty tea bags and equilibrated for 20 min in 0.5 mM $CaCl_2$ at the assay temperature of 20°C. The roots were then placed into solutions containing either 250 μM $^{15}NH_4Cl$ or 250 μM $K^{15}NO_3$ for 30 min. Solutions of 250 μM were selected because NO_3 concentrations in Mojave Desert soils range from ~ 230 μM in intershrub areas to ~ 350 μM beneath shrubs to depths of 0.55 m (Rundel & Gibson, 1996), based on an assumed gravimetric soil water content of 10%. All solutions were well mixed and aerated, adjusted to pH 6, and contained 0.01 M sucrose as an energy source and 0.5 mM $CaCl_2$ for membrane integrity (Jackson *et al.*, 1990). After incubation each sample was rinsed in several solutions of 1 mM KCl at 5°C to remove any ^{15}N adsorbed to the root surfaces. Roots were then oven-dried at 45°C, ground and analysed for ^{15}N content and percentage N by mass spectrometry. The nutrient uptake assay was completed < 1 h after harvesting roots in order to minimize the effects of root excision on NO_3 and NH_4 uptake (Bloom & Caldwell, 1988). Physiological rates of N uptake are expressed on a root dry mass basis (μM g^{-1} h^{-1}). Whole-root-system flux rates were obtained by multiplying physiological fluxes by root-system biomass and are expressed on an individual plant basis.

Root respiration

CO_2 efflux rates were measured with an LI-6200 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA) on ~ 200 mg (f. wt) of fine root

Table 2. *Mean* (n = 6) *whole-plant and root dry weights, root : shoot ratios and root surface areas, all expressed on an individual plant basis, and analysis of variance* P *values for* Achnatherum hymenoides, Bromus madritensis *spp.* rubens *and* Pleuraphis rigida *grown at 360 or 1000 μl l^{-1} CO$_2$ concentration and harvested at three different phenology stages*

		A. hymenoides		B. rubens		P. rigida	
	Date	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
Plant d. wt (g)	1	0.25 (0.04)	0.35 (0.03)	0.20 (0.04)	0.28 (0.03)	0.64 (0.13)	0.40 (0.14)
	2	0.69 (0.08)	0.92 (0.06)	0.62 (0.06)	0.69 (0.04)	1.60 (0.56)	1.59 (0.56)
	3	0.99 (0.08)	1.39 (0.12)	0.80 (0.07)	0.95 (0.06)	2.01 (0.12)	1.30 (0.27)
CO$_2$		<0.001		0.030		0.243	
Date		<0.001		<0.001		0.003	
Date × CO$_2$		0.129		0.689		0.583	
Root d. wt (g)	1	0.10 (0.01)	0.11 (0.01)	0.09 (0.02)	0.13 (0.01)	0.30 (0.06)	0.16 (0.07)
	2	0.29 (0.04)	0.39 (0.03)	0.26 (0.02)	0.24 (0.02)	0.92 (0.40)	0.99 (0.37)
	3	0.39 (0.02)	0.52 (0.04)	0.24 (0.02)	0.24 (0.02)	1.20 (0.10)	0.71 (0.15)
CO$_2$		<0.001		0.799		0.228	
Date		<0.001		<0.001		0.002	
Date × CO$_2$		0.098		0.300		0.423	
R : S ratio (g g^{-1})	1	0.69 (0.04)	0.48 (0.02)	0.84 (0.04)	0.95 (0.10)	0.93 (0.14)	0.96 (0.28)
	2	0.78 (0.10)	0.75 (0.08)	0.86 (0.17)	0.55 (0.04)	1.28 (0.31)	1.50 (0.23)
	3	0.70 (0.08)	0.60 (0.04)	0.45 (0.05)	0.33 (0.03)	1.47 (0.12)	1.24 (0.09)
CO$_2$		0.050		0.150		0.965	
Date		0.040		<0.001		0.097	
Date × CO$_2$		0.416		0.078		0.580	
Root surface area (dm²)	1	0.71 (0.10)	0.44 (0.03)	0.54 (0.08)	0.74 (0.12)	1.36 (0.29)	0.26 (0.09)
	2	1.90 (0.27)	2.81 (0.29)	1.80 (0.19)	1.77 (0.14)	3.26 (1.17)	4.60 (2.1)
	3	3.56 (0.62)	3.83 (0.28)	2.17 (0.17)	2.06 (0.29)	4.02 (0.31)	2.23 (0.43)
CO$_2$		0.265		0.910		0.174	
Date		<0.001		<0.001		0.001	
Date × CO$_2$		0.219		0.654		0.186	

SE in parentheses.

subsamples excavated from the upper 0.2 m of the soil and a deepest horizon (0.6–0.8 m). The instrument was programmed so that each observation took 60 s after an initial 5-min adjustment period at the set temperature; longer equilibrium times did not affect CO$_2$ efflux rates. Carbon dioxide concentration within the 0.25 l cuvette was kept near that of the ambient air (between 350 and 400 μl l^{-1}) to minimize potential effects of diffusion through minor leaks. Care was taken to minimize root exposure to light by covering the cuvette with a black cloth. In addition, a piece of moistened tissue paper was kept inside the cuvette to prevent dehydration of the roots by maintaining a constant humidity near saturation, and to reduce errors that can result from changing partial pressure of water in the sample cell of the instrument (Rakonczay *et al.*, 1997). Following respiration measurements, root samples were dried, weighed and ground, and their C and N contents determined with an elemental CHN analyser. Respiration rates were expressed on the basis of root dry mass, and normalized to 20°C assuming a temperature coefficient (Q$_{10}$) of 2.0 (Amthor, 1991), as:

$$R = R_{20} \times Q_{10}^{(t-20)/10}$$

(*R*, the specific respiration rate (nmol CO$_2$ g^{-1} s^{-1}); R_{20}, respiration rate at 20°C; Q_{10}, rate of change of

respiration per 10°C change in temperature; *t*, respiration-chamber air temperature).

Root osmotic potential and total nonstructural carbohydrate concentrations

Root osmotic potential (MPa) and nonstructural carbohydrate concentrations (mg g^{-1}) were determined on subsamples of fine roots (~200 mg f. wt) collected concurrently with root respiration measurements. For each species × depth × CO$_2$ treatment combination, half of the root subsample was immediately transferred into 1 ml syringes and kept frozen in liquid N until further analysis in the laboratory. Osmotic potentials were measured on 10 μl of the sap expressed from thawed syringes using a calibrated vapour pressure osmometer (Wescor 5500, Logan, UT, USA). Soluble simple sugars and starch concentrations for samples collected during the second and third harvest of each species were determined enzymatically on the other half of frozen root subsamples, as described by Hendrix (1993), and expressed as root d. wt; no carbohydrate analyses were performed on roots from the first harvest. Soil water content (g water per g soil) and leaf predawn water potential (Ψ$_w$, MPa) were determined using a standard gravimetric method and a Scholander-type pressure chamber, respectively.

Table 3. *Mean* (n = 6) *soil water content* (*SWC*), *leaf pre-dawn water potential* (Ψ_w), *root water content* (*RWC*), *osmotic potential* (π) *and analysis of variance* P *values for* Achnatherum hymenoides, Bromus madritensis *spp.* rubens *and* Pleuraphis rigida *grown at 360 or 1000 $\mu l\ l^{-1}$ CO_2 concentration and harvested at three different phenology stages*

Depth	Date	A. hymenoides Ambient	A. hymenoides Elevated	B. rubens Ambient	B. rubens Elevated	P. rigida Ambient	P. rigida Elevated
SWC ($\times 10^{-2}$ g g^{-1}							
$\sim$0.2 m	1	6.9 (0.8)	7.9 (0.2)	n/a	n/a	9.7 (0.1)	9.9 (0.4)
	2	8.4 (0.5)	9.4 (0.2)	3.1 (0.7)	4.6 (0.9)	10.1 (0.5)	10.1 (0.6)
	3	7.4 (0.5)	10.0 (0.3)	7.0 (0.5)	8.0 (0.3)	10.6 (0.7)	10.9 (0.2)
$\sim$0.7 m	1	9.0 (0.8)	10.1 (1.0)	n/a	n/a	10.7 (0.5)	11.5 (0.5)
	2	7.3 (1.2)	9.3 (1.9)	4.8 (0.9)	7.1 (1.5)	9.3 (0.3)	11.8 (0.9)
	3	5.0 (1.3)	8.8 (1.5)	6.7 (1.5)	9.4 (1.0)	10.7 (0.4)	12.8 (0.6)
CO$_2$		0.002		0.013		0.001	
Date		0.483		<0.001		0.021	
Depth		0.887		0.072		0.002	
CO$_2$ × Date		0.030		0.296		0.528	
Ψ_w (MPa)	1	−0.22 (0.03)	−0.23 (0.03)	−0.38 (0.05)	−0.27 (0.01)	−0.18 (0.03)	−0.20 (0.04)
	2	−0.62 (0.09)	−0.42 (0.03)	−0.73 (0.18)	−0.71 (0.09)	−0.33 (0.06)	−0.28 (0.05)
	3	−0.45 (0.02)	−0.24 (0.02)	n/a	n/a	−0.26 (0.06)	−0.27 (0.03)
CO$_2$		<0.001		0.494		0.836	
Date		<0.001		<0.001		0.072	
CO$_2$ × Date		0.033		0.657		0.729	
RWC ($\times 10^{-2}$ g g^{-1})							
$\sim$0.2 m	1	4.6 (0.8)	5.6 (0.8)	5.8 (0.8)	6.1 (0.9)	3.7 (0.5)	5.0 (0.6)
	2	2.9 (0.3)	3.5 (0.2)	3.1 (0.6)	3.6 (0.2)	2.2 (0.2)	4.3 (0.7)
	3	2.8 (0.3)	3.0 (0.1)	4.2 (0.5)	5.6 (0.5)	2.5 (0.2)	2.3 (0.2)
$\sim$0.7 m	1	12.8 (0.9)	13.1 (0.8)	10.5 (1.7)	11.8 (1.5)	6.4 (1.5)	7.2 (1.7)
	2	6.8 (0.7)	5.5 (0.7)	5.4 (1.1)	4.9 (0.8)	5.1 (0.8)	6.4 (0.4)
	3	2.6 (0.8)	4.2 (0.8)	4.5 (0.7)	6.3 (0.5)	3.8 (0.4)	4.5 (0.7)
CO$_2$		0.269		0.138		0.017	
Date		<0.001		<0.001		0.001	
Depth		<0.001		<0.001		<0.001	
CO$_2$ × Date		0.371		0.490		0.256	
Root π (MPa)							
$\sim$0.2 m	1	−0.45 (0.03)	−0.46 (0.02)	−0.94 (0.08)	−0.80 (0.04)	−0.52 (0.08)	−0.61 (0.05)
	2	−0.33 (0.01)	−0.34 (0.01)	−0.82 (0.10)	−0.58 (0.02)	−0.32 (0.01)	−0.34 (0.04)
	3	−0.37 (0.02)	−0.28 (0.01)	−0.70 (0.05)	−0.41 (0.05)	−0.41 (0.10)	−0.41 (0.06)
$\sim$0.7 m	1	−0.45 (0.03)	−0.51 (0.02)	−0.97 (0.11)	−0.86 (0.11)	−0.78 (0.05)	−0.88 (0.13)
	2	−0.43 (0.04)	−0.41 (0.04)	−0.92 (0.10)	−0.81 (0.02)	−0.34 (0.04)	−0.54 (0.17)
	3	−0.77 (0.12)	−0.43 (0.02)	−0.97 (0.12)	−0.47 (0.07)	−0.42 (0.04)	−0.49 (0.04)
CO$_2$		0.016		<0.001		0.251	
Date		0.006		<0.001		<0.001	
Depth		<0.001		0.008		<0.001	
CO$_2$ × Date		<0.001		0.047		0.778	

Soil and root water parameters were measured at two soil depths. n/a, data not available; SE in parentheses.

Statistical analysis

Statistical analyses were performed with SAS software (SAS Institute, Inc., Cary, NC, USA). Two-way ANOVA was performed to test the main effects of CO_2 concentration and sample period on physiological NO_3 and NH_4 uptake rates and whole-root-system N fluxes for each species. Three-way ANOVA was performed on remaining measured and calculated variables to test the main effects of CO_2 concentration, depth and sample period for each species. The assumptions of equal variance and normality were tested by plots of residuals against predicted values, as well as normality curves. Data were transformed as needed until the Shapiro–Wilk test statistic, normal probability plots and stem leaf plots (Cody & Smith, 1991) indicated normally distributed data. Differences were considered significant at $P < 0.05$.

RESULTS

Plant biomass and root growth

Elevated atmospheric CO_2 concentration substantially increased total plant biomass in the two C_3 grasses *A. hymenoides* and *B. rubens*, but not in the C_4 grass *P. rigida* (Table 2). At the final harvest date, plants of *A. hymenoides* and *B. rubens* were larger in response to CO_2 enrichment by 40 and 19%,
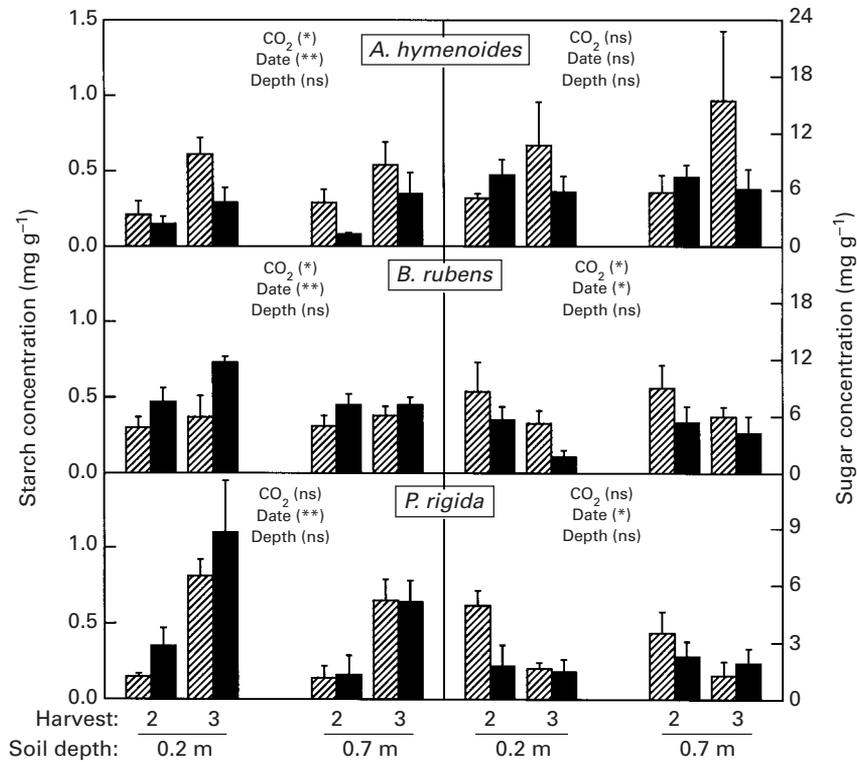
Fig. 1. Mean ($n = 6$, ± 1 SE) starch (left panels) and simple sugar concentrations (right panels) for roots of *Achnatherum hymenoides*, *Bromus madritensis* ssp. *rubens* and *Pleuraphis rigida* grown at either ambient CO_2 (hatched bars) or elevated CO_2 (solid bars) and harvested at two different shoot phenologies and two soil depths. Three-way ANOVA results for date, CO_2 and depth are represented by: ns, not significant; *, $P < 0.05$; **, $P < 0.01$.

respectively. However, the elevated CO_2 treatment significantly increased root production only in *A. hymenoides* (Table 2). Root biomass increased significantly over time for both C_3 species under each CO_2 treatment (Table 2). This same pattern occurred for *P. rigida*, except that root biomass did not significantly increase between the second and third sample dates (Table 2). No plants from any treatment appeared to be pot-bound, as there were few roots against the walls of the PVC pots.

The response of root:shoot (R:S) ratio to elevated CO_2 was variable among species and shoot phenology (Table 2). For *A. hymenoides*, R:S ratio was lower in response to elevated CO_2 at each harvest date, whereas for *B. rubens* and *P. rigida*, elevated CO_2 did not significantly affect R:S ratio (Table 2). Root surface area tended to increase over time for each species, but was not significantly affected by the CO_2 treatment for any species (Table 2).

Root water relations and nonstructural carbohydrate concentrations

Despite identical irrigation regimes, average soil water content for plants grown under elevated CO_2 were 1–4 percentage points higher than for plants grown under ambient CO_2 (Table 3); this difference was more pronounced for the two C_3 grasses than for *P. rigida*. In addition, leaf predawn water potential

(Ψ_w) was significantly less negative under elevated CO_2 for *A. hymenoides* during the second and third sample periods, and for *B. rubens* during the first sample period. No significant CO_2 effect occurred for Ψ_w of *B. rubens* during the second sample period. Due to leaf senescence and subsequently limited fresh leaf material, Ψ_w measurements for *B. rubens* were not taken during the third sample period. For *P. rigida*, Ψ_w was not significantly effected by the CO_2 treatment throughout the study (Table 3). Root water contents were significantly higher under elevated CO_2 only for *P. rigida*. However for all species and both CO_2 treatments, deeper roots (0.6–0.8 m) had higher water contents than shallow roots (0.1–0.3 m) (Table 3). Root solute potentials (π) under elevated CO_2 were higher throughout the experiment for *B. rubens* and at the third harvest for *A. hymenoides*, especially for the deeper roots. The root solute potential of *P. rigida* was not affected significantly by the CO_2 treatment (Table 3).

Nonstructural carbohydrate concentrations were affected differentially among species by CO_2 enrichment. For *A. hymenoides*, root starch concentrations decreased significantly in plants grown at elevated CO_2 for each sample date and soil depth, but simple sugar concentrations were not affected significantly by the CO_2 treatment (Fig. 1). In contrast, *B. rubens* roots exhibited higher starch but lower simple sugar concentrations in plants grown

Table 4. *Mean* (n = 6) *whole-plant N content, plant and root N concentrations, N uptake rate (NUR), all expressed on an individual plant basis, and analysis of variance* P *values for* Achnatherum hymenoides, Bromus madritensis spp. rubens *and* Pleuraphis rigida *grown at 360 or 1000 μl l^{-1} CO_2 concentration and harvested at three different phenology stages*

	Date	A. hymenoides		B. rubens		P. rigida	
		Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
Plant N content (mg N)	1	3.7 (0.6)	4.2 (0.4)	2.7 (0.4)	2.9 (0.3)	7.3 (1.8)	5.2 (2.0)
	2	5.6 (1.0)	6.1 (0.6)	4.1 (0.3)	3.8 (0.2)	11.0 (5.1)	12.2 (3.2)
	3	6.0 (0.6)	7.1 (1.0)	5.0 (0.5)	5.0 (0.4)	9.4 (0.5)	8.3 (1.1)
CO_2		0.281		0.911		0.768	
Date		0.003		<0.001		0.179	
Date × CO_2		0.908		0.791		0.832	
Plant [N] (mg N g^{-1} d.wt)	1	14.2 (0.7)	12.0 (0.4)	13.9 (0.9)	10.8 (0.5)	11.2 (1.1)	11.1 (2.6)
	2	8.1 (0.8)	6.5 (0.3)	6.7 (0.2)	5.6 (0.4)	5.4 (0.7)	5.9 (0.4)
	3	6.2 (0.4)	5.0 (0.3)	5.9 (0.5)	5.2 (0.2)	4.4 (0.2)	4.5 (0.5)
CO_2		<0.001		<0.001		0.883	
Date		<0.001		<0.001		<0.001	
Date × CO_2		0.593		0.052		0.976	
Root [N] (mg N g^{-1} d.wt)	1	7.6 (0.5)	7.8 (0.8)	9.2 (0.7)	9.1 (0.8)	6.2 (0.5)	4.9 (0.7)
	2	7.0 (0.6)	6.6 (0.3)	6.0 (0.3)	5.4 (0.4)	5.1 (0.8)	5.6 (0.4)
	3	6.7 (0.4)	5.4 (0.3)	5.9 (0.2)	5.4 (0.5)	4.3 (0.2)	4.4 (0.5)
CO_2		0.256		0.328		0.591	
Date		0.015		<0.001		0.122	
Date × CO_2		0.357		0.895		0.273	
NUR (mg total N g^{-1} root d.wt)	1	35.4 (2.8)	37.3 (2.6)	30.7 (2.1)	23.4 (2.3)	25.0 (4.1)	49.6 (31.0)
	2	21.4 (5.4)	14.9 (0.6)	16.1 (1.6)	9.9 (1.1)	9.2 (1.5)	9.6 (0.1)
	3	15.3 (0.8)	13.4 (1.2)	20.0 (2.6)	21.5 (1.4)	7.3 (0.5)	8.7 (1.6)
CO_2		0.362		0.013		0.443	
Date		<0.001		<0.001		0.068	
Date × CO_2		0.353		0.060		0.590	

SE in parentheses.

under elevated CO_2. For *P. rigida*, no significant CO_2 effect was found on nonstructural carbohydrate concentrations, although simple sugar concentrations of CO_2-enriched roots were lower on the second sample date (Fig. 1). For both perennial species, starch concentrations significantly increased over time at both soil depths, suggesting a shift in below-ground reserve allocation over the course of this study. *Bromus madritensis* ssp. *rubens* also showed a slight, significant increase in starch allocation over time at the 0.2 m depth.

Root nitrogen uptake

Whole-plant N content was not modified by CO_2 enrichment for any species (Table 4). However, whole-plant N content increased significantly over time in *A. hymenoides* and *B. rubens* (Table 4). Plant N concentrations were significantly decreased in both C_3 grasses, but not in *P. rigida* in response to CO_2 enrichment (Table 4). For all species, the CO_2 treatment did not affect root N concentrations, and both plant and root N concentrations decreased throughout the course of plant growth (Table 4).

Neither NO_3 nor NH_4 uptake capacities were affected significantly by CO_2 enrichment during the second harvest for any species (Fig. 2); measure-ments were not performed for the first harvest. At the third harvest, the CO_2 treatment significantly decreased uptake rates for at least one N form for each species. For *A. hymenoides* and *P. rigida*, NO_3 uptake rates were lower for plants grown at elevated CO_2. For *B. rubens* and *P. rigida*, NH_4 uptake rates were lower for plants grown under elevated CO_2 (Fig. 2). Whole-root-system N uptake rates (NH_4 plus NO_3 uptake rates × root biomass) were also not affected significantly by CO_2 enrichment during the second harvest for any species (Fig. 3). However during the third harvest, whole-root-system N uptake rates were significantly increased for *A. hymenoides* and significantly decreased for *B. rubens* and *P. rigida* (Fig. 3). Because the physiological capacities for NO_3 uptake were only 30–48% of those for NH_4 uptake, NH_4 probably accounted for most of the N uptake in these grasses.

Root respiration rates

Elevated atmospheric CO_2 had no significant effect on root respiration normalized to 20°C and expressed on a dry weight basis for any species (R_{20}), regardless of date or depth (Table 5). However, R_{20} showed a marked negative time effect for all species × treatment combinations. This decline during on-
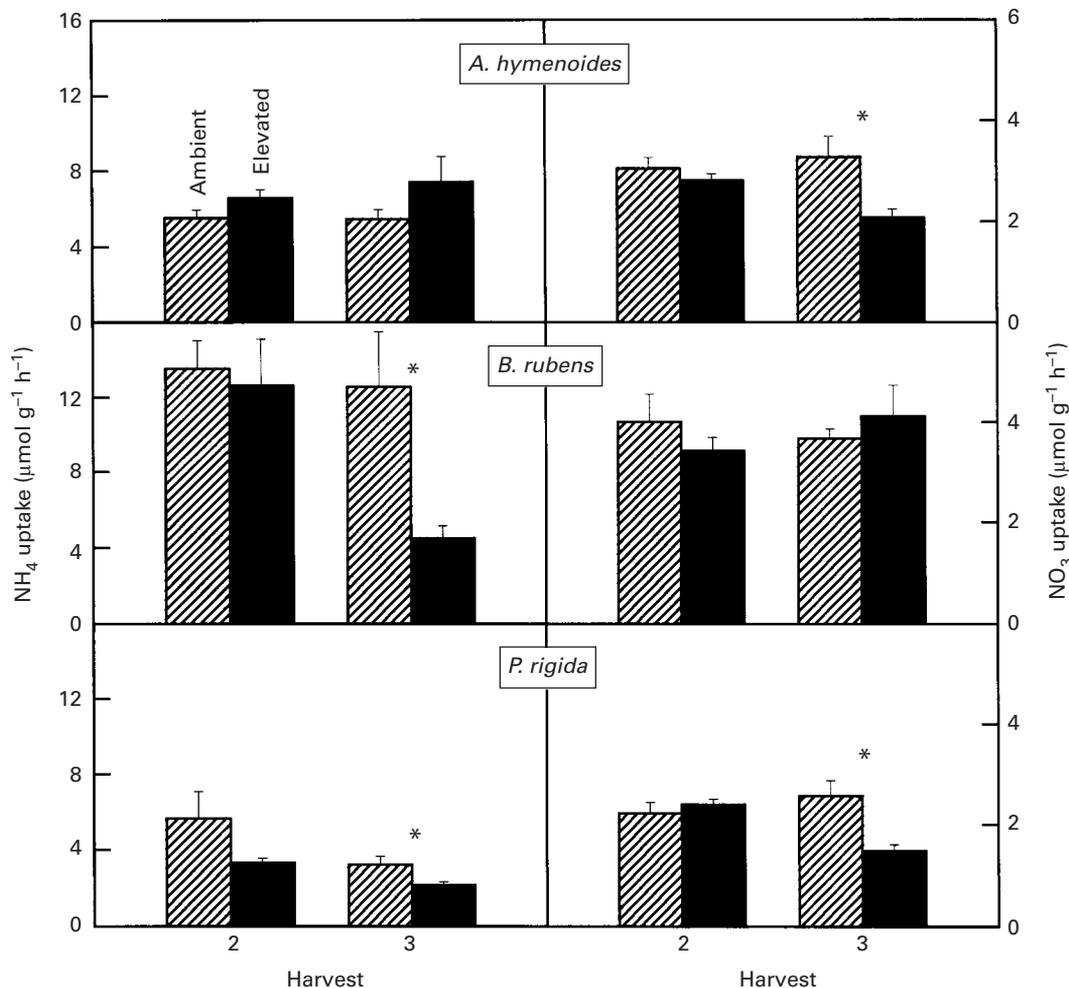
Fig. 2. Mean ($n = 6$, ± 1 SE) NO_3 and NH_4 uptake rates for *Achnatherum hymenoides*, *Bromus madritensis* ssp. *rubens* and *Pleuraphis rigida* grown at either ambient (hatched bars) or elevated CO_2 (solid bars). Measurements were made during two different shoot phenologies. Asterisk indicates a significant CO_2 effect ($P < 0.05$).

togeny was slightly greater for roots collected at the 0.6–0.8 m depth than for roots collected at the 0.1–0.3 m depth (Table 5). Rates of efflux of CO_2 in the deepest roots of *B. rubens*, *P. rigida* and *A. hymenoides* grown at ambient CO_2 concentration reached 14.4, 11.9 and 8.3 nmol CO_2 g^{-1} s^{-1}, respectively, on the final sample date (Table 5), which are within the range of rates reported in literature. In addition, R_{20} was significantly correlated with either root C:N ratio or root water content, when all data for a given species were included in a simple regression model (Fig. 4).

DISCUSSION

Primary productivity of both the C_3 grasses *A. hymenoides* and *B. rubens* increased in response to elevated atmospheric CO_2 concentration, but not for the C_4 grass *P. rigida* (Table 2). These results are consistent with the general pattern, reported in the literature, that C_3 species are more responsive to CO_2 enrichment than C_4 species (Poorter, 1993). Improved water-use efficiency from CO_2-induced stomatal closure (L. A. DeFalco *et al.*, unpublished)

and maintenance of favourable water conditions (Table 2) probably contributed to enhanced C_3 growth at elevated CO_2 (Tyree & Alexander, 1993; Morgan *et al.*, 1998).

Although total plant biomass increased in both C_3 grasses in response to elevated CO_2, root production increased only in *A. hymenoides*. Differences in allocation patterns between the two C_3 grasses are probably related to different life-history strategies. *Bromus madritensis* ssp. *rubens* is a short-lived species whose life-history strategy is defined by the production of many offspring at the expense of belowground allocation (Huxman *et al.*, 1998). In contrast, the perennial *A. hymenoides* exhibits a larger root system that increases the absolute potential for nutrient acquisition and potentially serves an important storage function (Hunt *et al.*, 1991). Surprisingly, our nonstructural carbohydrate measurements do not agree with observed root allocation patterns because starch concentrations were unexpectedly lower in *A. hymenoides* and higher in *B. rubens* in response to elevated CO_2 (Fig. 1). However fructans, which may serve as an important storage carbohydrate in grasses (Baxter *et al.*, 1997), were
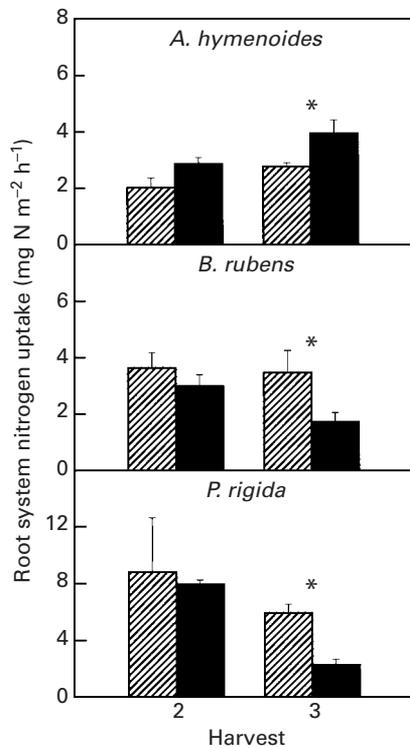
Fig. 3. Whole-root-system rates of N uptake (expressed as soil surface area) for *Achnatherum hymenoides*, *Bromus madritensis* ssp. *rubens* and *Pleuraphis rigida* grown at either ambient (hatched bars) or elevated CO$_2$ (solid bars) and harvested at two different shoot phenologies. Root system rates of N uptake are obtained by summing physiological fluxes for NO$_3$ and NH$_4$ and multiplying them by root biomass. Error bars represent SE.

not analysed in the present study. Therefore the effect of CO$_2$ treatment on fructan concentrations in these grasses remains an open question.

Whole-plant N concentrations of *A. hymenoides* and *B. rubens* expressed per unit d. wt were significantly decreased in response to elevated CO$_2$

(Table 4), which is a common observation when plants are compared at a given phenological stage but not at similar sizes (Conroy, 1992; Coleman *et al.*, 1993). This reduction in total N concentration in the C$_3$ grasses arose largely from increased shoot growth (L. A. DeFalco *et al.*, unpublished) rather than from changes in whole-plant N uptake (Table 4). Our results do not support our initial hypothesis that physiological rates of N uptake would increase with elevated CO$_2$. Rather, NO$_3$ and NH$_4$ uptake rates were either unaffected by CO$_2$, or decreased for at least one N form after anthesis or during late vegetative growth in all species (Fig. 2). The only positive N uptake-capacity response to elevated CO$_2$ was on a whole-root-system basis (NO$_3$ + NH$_4$ physiological uptake capacity × root biomass) for *A. hymenoides* after anthesis (Fig. 3). This increase in N uptake capacity for *A. hymenoides* was primarily due to increased root biomass and occurred despite a decreased NO$_3$ uptake capacity. Unfortunately, increased root system N uptake capacity for *A. hymenoides* did not translate to a significant increase in plant N content ($P = 0.28$; Table 4). Nevertheless, greater root-system N uptake capacity for *A. hymenoides* may provide a competitive advantage for this species relative to species for which either root growth and function are unaffected by CO$_2$, or N uptake is down-regulated in response to elevated CO$_2$, such as *B. rubens* and *P. rigida* in this study (Fig. 3). For all species, NO$_3$ uptake rates were only 30–48% of NH$_4$ uptake rates, indicating that NH$_4$ probably accounted for most of the N uptake by these grasses. Jackson & Reynolds (1996) reported similar findings for annual grass and forb species in a California grassland.

Negative effects of CO$_2$ on N uptake capacities have been reported for several species. In the desert shrub *Larrea tridentata*, root uptake capacity for

Table 5. *Mean* (n = 6) *specific root respiration normalized at 20°C (nmol CO$_2$ g^{-1} root d. wt s^{-1}) and analysis of variance* P *values for* Achnatherum hymenoides, Bromus madritensis *spp.* rubens *and* Pleuraphis rigida *grown at 360 or 1000 μl l^{-1} CO$_2$ concentration and harvested at three different phenology stages at two soil depths*

Depth (m)	Date	A. hymenoides		B. rubens		P. rigida	
		Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
∼0.2	1	35.4 (5.6)	38.4 (3.2)	105.3 (15.1)	127.5 (11.7)	21.9 (4.2)	33.6 (7.8)
	2	16.6 (1.9)	12.7 (0.7)	18.8 (4.9)	17.5 (2.4)	7.6 (1.1)	10.5 (3.6)
	3	7.2 (1.6)	5.6 (0.7)	10.9 (2.6)	11.0 (2.1)	6.7 (3.1)	7.5 (1.9)
∼0.7	1	72.2 (6.0)	89.5 (8.5)	191.1 (36.4)	237.7 (55.9)	35.4 (5.2)	25.5 (3.7)
	2	28.2 (3.7)	28.3 (4.1)	38.1 (10.4)	21.8 (3.1)	15.1 (3.6)	18.6 (2.5)
	3	8.3 (1.9)	11.5 (2.1)	14.4 (1.2)	16.1 (4.8)	11.9 (2.8)	13.9 (4.0)
CO$_2$		0.194		0.254		0.480	
Date		<0.001		<0.001		<0.001	
Depth		<0.001		<0.001		0.036	
CO$_2$ × Date		0.094		0.090		0.929	
CO$_2$ × Depth		0.102		0.811		0.196	
Date × depth		<0.001		<0.001		0.726	
CO$_2$ × date × depth		0.597		0.604		0.134	

SE in parentheses.
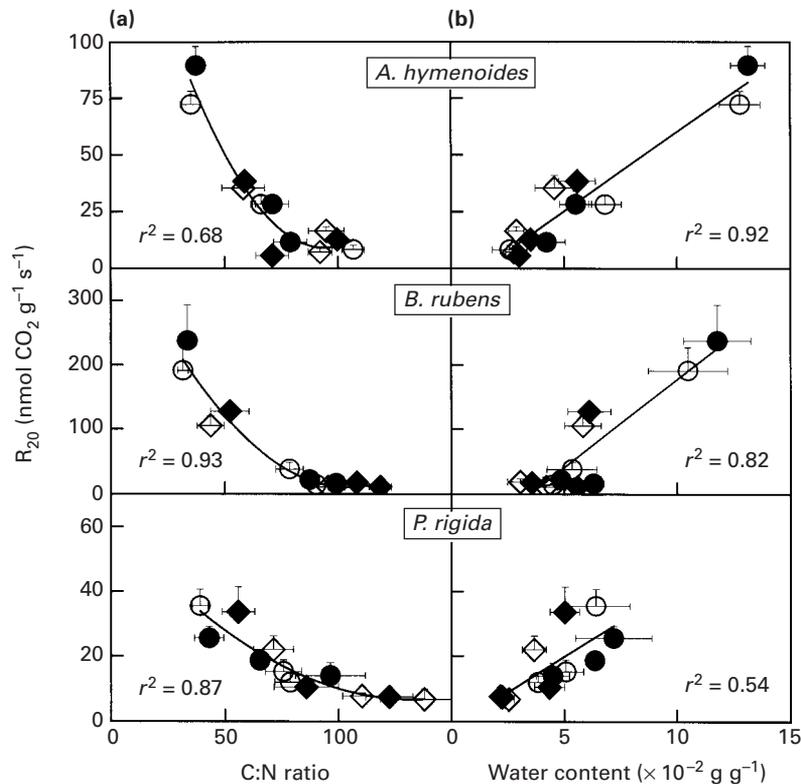
Fig. 4. Mean ($n = 6$, ± 1 SE) specific root respiration at 20°C versus (a) root C:N ratio and (b) root water content for *Achnatherum hymenoides*, *Bromus madritensis* ssp. *rubens* and *Pleuraphis rigida* grown at either ambient (open symbols) or elevated CO_2 (solid symbols) and harvested at three different shoot phenologies and at a soil depth of either 0.2 m (diamonds) or 0.7 m (circles). All CO_2 efflux measurements were made at ambient CO_2 concentration and normalized to 20°C assuming a Q_{10} value of 2.

NO_3 decreased by 55% in response to CO_2 enrichment (BassiriRad *et al.*, 1997) and NO_3 uptake capacities decreased 28% overall in annual grass and forb species (Jackson & Reynolds, 1996). However, other species show no effect or increased uptake capacity in response to elevated CO_2 (BassiriRad *et al.*, 1996a, 1997; Jackson & Reynolds, 1996). Bassiri-Rad *et al.* (1996b) suggest that the discrepancy between their studies may have been due to differences in soil fertility, because N sources and concentrations can greatly affect N uptake (Raab & Terry, 1995). In our study, shoot phenology also appeared to influence the effect of elevated CO_2 on N uptake capacity, because elevated CO_2 affected N uptake only after anthesis in the two C_3 species and late in vegetative growth for the C_4 species. Although the explanation for this phenologically related down-regulation of N uptake capacity is not known, a potential feedback mechanism is that elevated CO_2 causes increases in low-quality substrate release into the rhizosphere, and subsequently leads to increased N sequestration by microbial populations (Diaz *et al.*, 1993; Zak *et al.*, 1993). No attempt to validate this hypothesis was made in our study.

Elevated CO_2 had no significant effect on specific root respiration (R_{20}) for any species (Table 5). Similar results have been reported for *Plantago lanceolata* (Den Hertog *et al.*, 1993), *Vigna radiata*

and *Helianthus annuus* (Gifford *et al.*, 1985). In addition, Poorter *et al.* (1992) found that prolonged exposure to atmospheric CO_2 concentration did not affect root respiration as long as plants were grown at an optimum supply of nutrients and water. However, a reduction in R_{20} is a more common response in the literature (Lambers *et al.*, 1996) and has been partially attributed to reduced construction and maintenance costs of roots under elevated CO_2 (Ryan, 1991; Wullschleger *et al.*, 1994). In the present study, R_{20} expressed on a dry weight basis decreased significantly over time, irrespective of species or CO_2 treatment (Table 5). Decreases in R_{20} during ontogeny may be due to decreases in the amount and activity of respiratory enzymes and corresponding increases in cellulose and starch concentrations with root age (Lambers *et al.*, 1996). Increases in root starch concentrations over time for each species in this study are consistent with this hypothesis.

In conclusion, this study provides the first data on root growth and function responses of three important Mojave Desert grasses, *A. hymenoides*, *P. rigida* and *B. rubens*, to long-term CO_2 enrichment. Primary productivity of the two C_3 grasses increased in response to elevated CO_2, but root production increased only in *A. hymenoides*, which is also the only species for which whole-root-system N uptake

capacity increased in response to elevated CO_2. Combined changes in physiological N uptake capacity and root growth may result in a shift in the competitive balance among these Mojave Desert species as global CO_2 enrichment increases. Determining how factors such as water and nutrient limitation, common in the Mojave Desert, will influence these responses requires further investigation.

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