

Growth and nitrogen uptake in an experimental community of annuals exposed to elevated atmospheric CO₂

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Abstract

Rising levels of atmospheric CO₂ may alter patterns of plant biomass production. These changes will be dependent on the ability of plants to acquire sufficient nutrients to maintain enhanced growth. Species-specific differences in responsiveness to CO₂ may lead to changes in plant community composition and biodiversity. Differences in species-level growth responses to CO₂ may be, in a large part, driven by differences in the ability to acquire nutrients. To understand the mechanisms of how elevated CO₂ leads to changes in community-level productivity, we need to study the growth responses and patterns of nutrient acquisition for each of the species that comprise the community.

In this paper, we present a study of how elevated CO₂ affects community-level and species-level patterns of nitrogen uptake and biomass production. As an experimental system we use experimental communities of 11 co-occurring annuals common to disturbed seasonal grasslands in south-western U.S.A. We established experimental communities with approximately even numbers of each species in three different atmospheric CO₂ concentrations (375, 550, and 700 ppm). We maintained these communities for 1, 1.5, and 2 months at which times we applied a ¹⁵N tracer (¹⁵NH₄¹⁵NO₃) to quantify the nitrogen uptake and then measured plant biomass, nitrogen content, and nitrogen uptake rates for the entire communities as well as for each species.

Overall, community-level responses to elevated CO₂ were consistent with the majority of other studies of individual- and multispecies assemblages, where elevated CO₂ leads to enhanced biomass production early on, but this enhancement declines through time. In contrast, the responses of the individual species within the communities was highly variable, showing the full range of responses from positive to negative. Due to the large variation in size between the different species, community-level responses were generally determined by the responses of only one or a few species. Thus, while several of the smaller species showed trends of increased biomass and nitrogen uptake in elevated CO₂ at the end of the experiment, community-level patterns showed a decrease in these parameters due to the significant reduction in biomass and nitrogen content in the single largest species.

The relationship between enhancement of nitrogen uptake and biomass production in elevated CO₂ was highly significant for both 550 ppm and 700 ppm CO₂. This relationship strongly suggests that the ability of plants to increase nitrogen uptake (through changes in physiology, morphology, architecture, or mycorrhizal symbionts) may be an important determinant of which species in a community will be able to respond to increased CO₂ levels with increased biomass production. The fact that the most dominant species within the community showed reduced enhancement and the smaller species showed increased enhancement suggest that through time, elevated CO₂ may lead to significant changes in community composition.

At the community level, nitrogen uptake rates relative to plant nitrogen content were invariable between the three different CO₂ levels at each harvest. This was in contrast to significant reductions in total plant nitrogen uptake and nitrogen uptake relative to

total plant biomass. These patterns support the hypothesis that plant nitrogen uptake is largely regulated by physiological activity, assuming that physiological activity is controlled by nitrogen content and thus protein and enzyme content.

Keywords: annual plants, biodiversity, community composition, elevated carbon dioxide, competition, nitrogen uptake

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Introduction

Future environmental change has the potential of leading to changes in patterns productivity and biodiversity in terrestrial plant communities (Lubchenko *et al.* 1991; Walker & Steffen 1996). One aspect of environmental change that has received a great deal of attention in recent years is rising CO₂ concentrations in the atmosphere. Increasing concentrations of CO₂ have the potential of leading to global climatic change through the greenhouse effect (Houghton *et al.* 1996). In addition, elevated CO₂ directly affects plant function, and may lead to different growth responses for different species leading to changes in community composition and biodiversity (Bazzaz 1990; Bazzaz *et al.* 1996; Körner & Bazzaz 1996).

One of the challenges in predicting the potential effects of elevated CO₂ on the productivity and structure of plant communities is that the response of communities of multiple species has not been successfully predicted on the basis of the growth responses of individual species (Bazzaz & Carlson 1984; Bazzaz & McConnaughay 1992; Körner 1996; Körner & Bazzaz 1996). This difficulty results from elevated CO₂ leading to different effects for different species (e.g. Hunt *et al.* 1991; Poorter 1993; Wullschlegel *et al.* 1995). Recent studies have documented that growth response of plants to an elevated CO₂ environment can itself be significantly changed (often reduced) when grown in competing populations relative to growing in the absence of neighbours (Wayne & Bazzaz 1995).

The ability of plants to maintain growth enhancement in elevated CO₂ environments is largely dependent on an adequate supply of nutrients (Bazzaz 1990; Field *et al.* 1992). Recent studies have emphasized that elevated CO₂ concentrations can lead to direct and indirect effects on the ability of plants to acquire nutrients from the soil (Norby 1994; Berntson & Bazzaz 1996). Elevated CO₂ can lead to changes in the architecture, morphology, and size of plant root systems (Berntson & Woodward 1992; Rogers *et al.* 1992, 1994; Berntson 1994), nutrient uptake kinetics (Bassirirad *et al.* 1996a,b; Jackson & Reynolds 1996), and mycorrhizal symbioses (O'Neill 1994; Godbold & Berntson 1997). Together, these direct effects of elevated

CO₂ on plant ability to acquire nutrients from the soil suggest that plant ability to acquire nutrients in elevated CO₂ environments will be enhanced, but these effects are known to be variable between different species within a single community (e.g. O'Neill & Norby 1988; Berntson 1996). However, changes in the availability of nutrients in the soil (either increases or decreases) may also control plant ability to acquire nutrients in elevated CO₂ environments (Hungate *et al.* 1997a,b; Berntson & Bazzaz 1997b, 1998). Because these direct and indirect effects on plant ability to acquire nutrients may be synchronous, we should focus on integrated patterns of resource acquisition and not any one of these factors alone (Berntson & Bazzaz 1996).

The impact of future environmental change on the species composition, productivity, and nutrient cycling of terrestrial communities may be significant (Huston 1994; Vitousek 1994; Körner 1995; Wedin & Tilman 1996). The differential impact of elevated CO₂ on different species within mixed-species communities and thus the resultant change in community structure may itself lead to changes in patterns of productivity (Naeem *et al.* 1994; Tilman & Downing 1994; Körner 1995; Wedin & Tilman 1996). Within communities of interacting plants, individual species can show the full range of responses to elevated CO₂, from decreases to increases in biomass (Chiariello & Field 1996; Roy *et al.* 1996). Ecosystem-level responses to elevated CO₂ are a function of both diversity, which may be influenced by CO₂ levels, and the impact of CO₂ on plant physiology, phenology, growth, and allocation (Naeem *et al.* 1996).

Most studies examining community/ecosystem-level responses have assessed net change after a fixed period of time for a single elevated CO₂ treatment relative to an ambient CO₂ control. This may lead to incomplete conclusions regarding the effects of elevated CO₂ as its impact is known to vary through ontogeny (Bazzaz 1993; Coleman *et al.* 1994; Berntson & Bazzaz 1997a), especially if effects of competition are magnified (Weiner 1996). In addition there is growing evidence that the effects of elevated CO₂ are nonlinear with increasing concentrations (Ackerly & Bazzaz 1995). If these effects vary by species, then the conclusions we draw regarding the effects of

Table 1. List of species names, families, species codes, and results of germination trials used to determine seed sowing densities within experimental communities.

Species	Authority	Common Name	Family	Code	% Germination
<i>Aster bigelovii</i>	Gray	Purple aster	Compositae	AB	30
<i>Aster tanacetifolius</i>	H.B.K.	Tahoka daisy	Compositae	AT	30
<i>Coreopsis tinctoria</i>	Nutt.	Palms coreopsis	Compositae	CT	80
<i>Gaillardia pulchella</i>	Foug.	Blanket flower	Compositae	GP	42
<i>Verbesina encilioides</i>	(Cav.) A. Gray	Golden crownbeard	Compositae	VE	45
<i>Monarda citriodora</i>	Cerv. ex Lag.	Lemon mint	Labitae	MC	25
<i>Linum grandiflorum</i>	Desf. cv. <i>rubrum</i> Vilm.	Scarlet flax	Linaceae	LR	70
<i>Mentzelia lindleyi</i>	Torrey & A. Gray	Lindley's blazing star	Loasaceae	ML	14
<i>Eschscholzia caespitosa</i>	Benth.	Pastel poppy	Papaveraceae	EC	50
<i>E. mexicana</i>	Greene	Mexican gold poppy	Papaveraceae	EM	15
<i>Phlox drummondii</i>	Hooker	Drummond's phlox	Polemoniaceae	PD	32

elevated CO₂ on individual species responses or community structure and function will be highly dependent upon the time during community ontogeny at which measurements are made (Coleman *et al.* 1994) and the particular CO₂ concentrations used. If responses to rising CO₂ are nonlinear, extrapolation of the effects of CO₂ using only control (ambient) concentrations and a single experimental increase are likely to be misleading.

In this study, we document how elevated CO₂ affects community-level and species-level patterns of plant biomass production and nitrogen uptake and content in experimental communities of 11 co-occurring annuals common to seasonal grasslands in south-western U.S.A. Using this model system, we test the following hypotheses:

- elevated CO₂ leads to changes in net productivity as well as species composition;
- the responses of all species within a community are equally important for determining community-level responses to elevated CO₂;
- growth responses to elevated CO₂ for the different species are related to patterns of nitrogen acquisition.

We decided to use a model community consisting of a large number of fast-growing annual species to address these hypotheses because such experimental systems allow us to study species-rich systems, with high levels of replication which can be logistically difficult and extremely expensive to implement in the field (Lawton 1995, 1996).

Materials and methods

Selection of species for experimental communities

We established experimental communities of 11 co-occurring, fast-growing annuals (see Table 1) common to seasonal grasslands in south-western U.S.A. We selected

this assemblage of species because they represent a variety of morphologies (from rosettes to erect plants with leaf shapes that vary from highly dissected to complete margins), phenologies, and families. However, these species are all fast-growing, commonly occupy disturbed habitats (e.g. along roadsides), germinate after the first significant rains in the late fall, flower and set seed by late spring or summer, and complete their life cycles in less than one growing season (Wills & Irwin 1961; Correll & Johnston 1970; Johnston 1988). A list of the species we used to create our model communities, along with their germination rates, is given in Table 1.

Phlox drummondii (PD) is a winter annual that reaches 10–50 cm in height (Waite & Levin 1993). It is endemic to central and south-eastern Texas and is self-incompatible. It flowers in March–May (Niehaus & Ripper 1984), and seeds germinate from October to December after periods of substantial rain. It reaches reproductive maturity in March and flowers through May and produces a few to 100 flowers per plant (Levin & Bulinska-Radomska 1988). This annual species forms discrete populations, typically composed of thousands of individuals at densities ranging from a few to 30 flowering plants per m² (Levin & Schmidt 1985). At the population level, CO₂ has a significant influence on the number of floral births, the maximum floral display, and time of commencement of flowering. There is up to a two-fold increase in both the number of floral births and the maximum floral display with increased CO₂ concentrations. PD also responds to increased CO₂ by flowering earlier (Garbutt & Bazzaz 1984).

Coreopsis tinctoria (CT) is a winter annual with taproot (Radford *et al.* 1964) that reaches 60–90 cm in height. Typically it is branched toward the top of the plant. It flowers in July–August. It forms dense stands (Freeman & Schofield 1991) which can cover large areas (e.g. acres, Kirkpatrick 1992).

Gaillardia pulchella (GP) is a winter annual that is typically erect, has a taproot, and reaches 15–20 cm in height. It typically branches from its base. It is self-incompatible. It flowers from April to June (Kirkpatrick 1992). It forms dense stands, blooms in (Freeman & Schofield 1991) and frequently is the dominant component of the late Spring flora of roadsides and pastures over much of Texas (Heywood 1986; Heywood 1993).

Linum grandiflorum (LR) is a winter annual, that is typically highly branched, has a slender stem, has numerous, small, alternately arranged leaves and a terminal branched inflorescence, and reaches heights of 20–50 cm (Munz 1974; Antonovics & Fowler 1985). It flowers from March to June (Wills & Irwin 1961). In high densities, reproductive effort is usually increased (Antonovics & Fowler 1985).

Eschsholzia mexicana (EM) is a winter annual that reaches 10–35 cm in height. Leaves are largely basal. It flowers from February to July (McDougall 1973). In springs with high rainfall, EM can cover extensive areas, representing a conspicuous floral component of the landscape (Kearney & Peebles 1951).

Eschsholzia caespitosa (EC) is an erect, tufted annual that varies from 5 to 30 cm in height (Hickman 1993). It flowers from March to June (Munz 1974).

Verbesina encilioides (VE) is a much-branched annual with a taproot that ranges in height from 30 to 120 cm. It flowers from July to October. It is a very common, hardy plant (Kirkpatrick 1992). Older plants can be highly branched (Niehaus & Ripper 1984). VE is native to tropical America and is naturalized in North America. Seeds exhibit remarkable endurance to climatic extremes and survive under extremely high temperatures (38–46 °C) and soil droughts. Germination takes place with sufficient moisture (after fall rains). It forms numerous pure stands and establishes itself in a variety of habitats. High and rapid seed germination (up to 94%), efficient seedling survival, quick vegetative and reproductive growth, extensive seed production, efficient self- and cross-pollination, and broad ecological amplitude have contributed to the biological success of this species (Kaul & Mangal 1987).

Aster tanacetifolius (AT) is an annual that reaches 10–50 cm in height. It is often profusely branched. It flowers from June to October (McDougall 1973).

Aster bigelovii (AB) is an annual with a taproot that reaches 20–100 cm in height. It is usually branched. It flowers from March to November (McDougall 1973).

Monarda citriodora (MC) is an annual that reaches 60 cm in height. It produces several stems from its base. It flowers from April to June. It often grows in large colonies and can cover several acres (Kirkpatrick 1992).

Mentzelia lindleyi (ML) is an erect annual, reaching 10–

60 cm in height. It is typically highly branched (Hickman 1993). It flowers from late March to May (Munz 1974).

Experimental communities and growth conditions

Experimental plant communities were established in tubs measuring 30 by 25 cm (width by length) by 20 cm deep. Four holes were drilled into the bottom of each tub to provide drainage. Soil within the tubs was layered as follows. The bottom 13 cm of soil was a 3:1 mixture of field mineral soil and fritted clay amended with superphosphate (1.4×10^{-2} cm⁻¹) and lime (1.9×10^{-3} cm⁻¹). On top of this layer we placed a 1–2 cm layer of fine roots for a mycorrhizal inoculum. Fine roots were collected from an unfertilized, unmown field dominated by *Agrostis*. This inoculum has proven to be successful for establishing arbuscular mycorrhizal colonization for a number of different non-native species (D. Janos, pers. comm.). Care was taken to remove all rhizomes. No *Agrostis* established in tubs, indicating that all rhizomes were removed successfully. The top 5 cm of tubs was filled with a 1:1 mineral soil, Promix layer to provide high water holding capacity and low soil density to facilitate germination and early establishment of seedlings.

We carried out a series of germination trials for seed from each of the 11 species in our experimental communities prior to starting the experiment. From these trials, we observed variations in germination rates from 14 to 70% (Table 1). Seed were sowed in the growth containers from 22 April to 24 April 1995. Using the germination rates from our experimental trials, we sowed enough seed of each species to establish communities with a density of ≈ 2500 individuals m⁻², or 175 individuals of a given species m⁻². To trigger germination, the tubs were liberally watered and exposed to a 10-day cold period 9/4 °C day/night temperature. There was a significant difference among species in the total number of successful germinants 24 days following seed sowing ($F_{(9,726)} = 157.5$, $P < 0.0001$). However, there was no effect of CO₂ treatment ($F_{(2,726)} = 0.54$, $P = 0.583$). Many species continued to germinate up until the first harvest (37 following seed sowing; Fig. 3).

In total, we established 72 experimental communities. These communities were distributed within 12 glass-sided growth chambers (internal dimensions 0.9×0.9×0.9 m), within which atmospheric temperature and CO₂ concentrations were independently controlled. We maintained atmospheric CO₂ concentrations at 375, 550, and 700 ppm using a computer controlled CO₂ sampling and injection system. In total we had four replicate chambers per CO₂ concentration. Following the cold treatment used to trigger germination, day/night temperatures were ramped up by 1 °C a day until they reached 25/26 °C. In addition to natural light which was $\approx 70\%$ full sun,

supplementary lighting over each chamber was provided with 1000 watt metal halide lamps (Philips MH1000/U). We fertilized each community with 0.5 g 20:20:20 NPK fertilizer (a total of 0.1 g N per fertilization) on 5 May, 26 May, and 16 June. All communities were watered every 1–2 days as needed.

Harvests and nitrogen uptake measurements

From the time of sowing seeds to the final harvest (10 July), the experiment lasted 86 days. During this time, we harvested eight replicate communities per CO₂ concentration (24 communities in total) three times during the experiment. These harvests took place on Day 37 (29 May), Day 58 (19 June), and Day 86 (10 July) after sowing the seeds. Four days prior to each harvest, we injected a ¹⁵N tracer into each community that was to be harvested. Once a community had received the ¹⁵N tracer, it was not watered or fertilized. The ¹⁵N tracer was applied as a 2.5 mM solution of ¹⁵NH₄¹⁵NO₃ (98% ¹⁵N). For each community, we injected 120 1 mL aliquots of tracer solution, for a total of 0.0084 g labelled N. We distributed these injections over a regular grid within each community using a specially built injection system which allowed us to inject 12 aliquots of tracer simultaneously (Berntson & Bazzaz 1997b, 1998). Each injection penetrated to a depth of ≈ 7.5 cm into the soil, and the tracer was evenly applied through this depth.

For the first harvest, we were able to separate roots and shoots for each species. For the last two harvests, the roots were so large and entangled that it was impossible to reliably separate the roots by species. Therefore, all individual species measurements were made using above-ground biomass and not below-ground biomass. As a preliminary test of this approach, we examined the allometric relationship between shoot biomass and root biomass at the first harvest (Fig. 1a). We found that this relationship did not vary between species, suggesting that above-ground biomass is a reasonably good predictor of total biomass for all species. For the second and third harvests, we recovered all fine root material but did not separate by species. Thus, community level measurements for all harvests are for whole-plant biomass.

We quantified plant nitrogen and carbon content using a CN analyser (ANCA-sl, Europa Scientific). We estimated plant nitrogen uptake by quantifying the amount ¹⁵N tracer (in excess of preinjection ¹⁵N abundance in plant tissue) in plant material with a mass spectrometer (20–20 Stable Isotope Analyser, Europa Scientific). Pre-injection ¹⁵N content of plant material was estimated by collecting leaf samples from each species prior to injection. We used the amount of ¹⁵N in excess of natural abundance to estimate N uptake rates by dividing the total amount of excess ¹⁵N in plant tissue by the duration the plants'

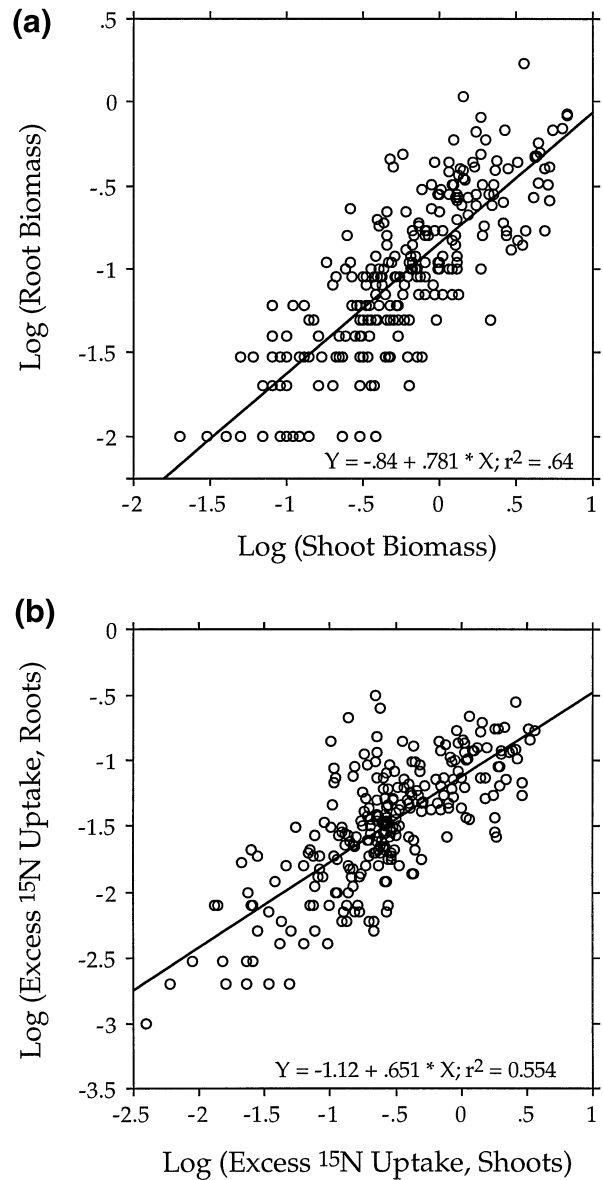


Fig. 1 Scatter-plots of above- (*x* axis) vs. below- (*y*) ground (a) plant biomass and (b) amount of ¹⁵N tracer recovered in plant tissue after 48 hour incubation. Each symbol is the total biomass or recovered tracer for a given species in a given replicate experimental community. Results are for the first harvest (out of three) only. The different species and different CO₂ treatments had nonsignificantly different slopes for both relationships (as judged from ANCOVAs with shoot biomass/¹⁵N uptake as the covariates and root biomass/¹⁵N uptake as the dependent variables).

roots were exposed to the ¹⁵N (4 days). Although this approach will underestimate actual N uptake rates during the period of incubation with the ¹⁵N tracer, as the plants are actively taking up nonlabelled N at the same time as they are taking up the tracer (Barraclough 1991), it does provide estimates of N uptake which can be used to

Table 2 Summary results of 3 way analysis-of-variance (ANOVA) for community-level measurements. Presented are degrees of freedom (d.f.) and *F*-Ratios (*F*) used to test each source within the model, as well as the percent of total model sum of squares (% Variance) explained by each term.

Dependent variable	Trans.	Source d.f.	Harvest (H)	CO ₂ (C)	H*C	Block (B)	C*B	Total
			2	2	4	3	6	54
Biomass	none	<i>F</i>	681.3***	3.6*	3.9**	1.1 NS	2.6*	
		% Variance	93.5%	0.5%	1.1%	0.2%	1.1%	
Root-to-shoot ratio	none	<i>F</i>	7.194**	1.8 NS	3.2**	3.8 NS	1.3 NS	
		% Variance	13.8%	3.5%	12.4%	10.8%	7.5%	
Total N-content	none	<i>F</i>	37.7***	4.2*	5.6**	2.5 NS	1.2 NS	
		% Variance	57.5%	3.6%	9.6%	3.2%	3.2%	
C/N (Molar)	none	<i>F</i>	473.6***	16.8***	2.8*	0.2 NS	1.3 NS	
		% Variance	89.8%	3.2%	1.1%	0.1%	0.8%	
¹⁵ N uptake (total excess)	none	<i>F</i>	30.5***	3.6*	3.0*	2.8*	1.2 NS	
		% Variance	40.8%	4.8%	7.9%	5.6%	4.8%	
Relative ¹⁵ N uptake (mg N day ⁻¹ g dry weight ⁻¹)	none	<i>F</i>	374.0***	17.6***	8.5***	08 NS	08 NS	
		% Variance	85.2%	4.0%	3.9%	0.3%	0.5%	
Relative ¹⁵ N uptake (mg N day ⁻¹ mg N ⁻¹)	none	<i>F</i>	151.5***	2.5 NS	1.4 NS	0.4 NS	0.6 NS	
		% Variance	81.3%	1.3%	1.5%	0.4%	1.0%	

****P* ≤ 0.0001; ***P* ≤ 0.001; **P* ≤ 0.05.

compare relative uptake rates. At the first harvest, we found that the total amount of ¹⁵N recovered in above-ground biomass was a good predictor of below-ground ¹⁵N content (Fig. 1b), and that this relationship did not vary by species or by CO₂ treatment. Thus, we used the amount of ¹⁵N recovered in above-ground biomass to estimate nitrogen uptake rates by each species. For entire communities, we estimated nitrogen uptake rates from the ¹⁵N recovered in both above- and below-ground biomass.

Data analysis

Community-level measurements were analysed using a three-way ANOVA, with growth CO₂ concentration, harvest, and block included as factors. Individual treatment means were compared using Scheffé posthoc means comparison, with $\alpha = 0.05$ (Velleman 1994). The relationship between the effect of elevated CO₂ on resource acquisition and net biomass production were analysed using linear regression with the mean values of each species by harvest combination as an independent observation. When necessary, compliance with assumptions of homoscedasticity and normality of the distribution of data was improved by applying a log transformation prior to analysis.

Results

Community-level responses

Elevated CO₂ led to an increase in biomass production (Table 2). However, this effect was variable through time

(significant CO₂ by harvest time interaction), with the greatest enhancement in biomass production at the middle harvest date. There was a trend toward reduced biomass production in elevated CO₂ environments at the final harvest (Fig. 2a). Changes in relative allocation between above- and below-ground biomass showed low variation through time relative to total biomass (Fig. 2B). The observed range of root-to-shoot ratios for the entire experimental communities was within the range typically observed for winter-spring annuals of south-western U.S.A. (Gutierrez & Whitford 1987).

Elevated CO₂ led to an overall reduction in total plant nitrogen content. Similar to biomass, this pattern was variable through time (Table 2, Fig. 2c). At the first harvest there was minimal variation in total plant nitrogen content between the different CO₂ levels. For the second and third harvests, elevated CO₂ led to a decrease in whole-plant community nitrogen content. This effect was most pronounced at the middle harvest.

Elevated CO₂ led to an increase in plant molar carbon-to-nitrogen (C/N) ratios (Table 2, Fig. 2d). Similar to biomass production and plant nitrogen content, this effect was most pronounced at the intermediate harvest. However, the overall trend of increased C/N was apparent at every harvest.

Rates of ¹⁵N-nitrogen uptake by the plant communities showed variable responses to increased CO₂ levels through time (Table 2, Fig. 2e). For the first harvest, there was no significant effect of elevated CO₂. For the intermediate harvest, and to a lesser extent at the final harvest, uptake rates were significantly reduced with increasing CO₂ levels.

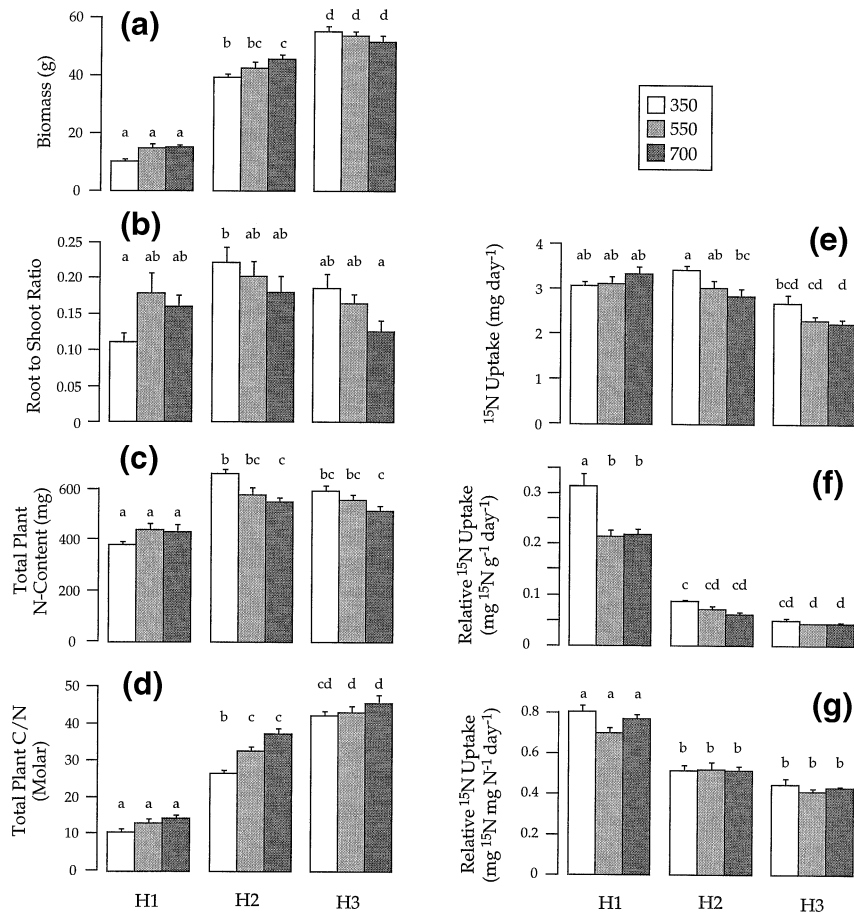


Fig. 2 Summary of community-level responses to three atmospheric CO₂ treatments (375 ppm, 550 ppm and 700 ppm; Shown with different shaded bars) at the three harvests. (a) biomass, (b) ratio of root biomass to shoot biomass, (c) plant nitrogen content, (d) whole-plant molar carbon-to-nitrogen ratios, (e) plant ¹⁵N-nitrogen uptake rates, (f) plant ¹⁵N-nitrogen uptake rate relative to plant biomass, (g) plant ¹⁵N-nitrogen uptake rate relative to plant nitrogen content. Each of these variables represents total above- and below-ground plant biomass and/or nitrogen content pooled together for all 11 species within each replicate experimental community. Error bars are a single standard error of the mean. Different letters above each CO₂ by harvest combination represent significant differences ($P < 0.05$) from a Scheffé posthoc means comparison, with $\alpha = 0.05$ (Velleman 1994). See Table 2 for the full ANOVA summary.

Plant ¹⁵N-nitrogen uptake rates relative to whole plant biomass showed a decreasing trend with increasing CO₂ concentrations (Fig. 2f). However, this pattern was more pronounced at the first harvest than the last two. At the first harvest, this effect was entirely due to the difference between the ambient CO₂ concentration and 550 ppm. There was no difference between the 550 ppm and 700 ppm treatments.

Plant ¹⁵N-nitrogen uptake rates relative to whole plant nitrogen content was not affected by changes in atmospheric CO₂ concentrations (Fig. 2g). Overall, this parameter showed a decline from the first to second harvest, but showed no decline from the second to third harvests.

Species-level responses

For every parameter we measured, we observed highly significant species by CO₂ interactions, even when the overall CO₂ effect was not significant (Table 3).

Elevated CO₂ did not have a significant effect on the number of plants when all species were pooled together (Table 3, Fig. 3). There were no effects of CO₂ on germination rates and therefore no effects of CO₂ on the number of plants for any of the species before or at the first harvest. However, there were a few species (GP, EC, MC, and EM) that showed a significant effect of CO₂ on the number of individuals surviving to the second and third harvests. In every case, elevated CO₂ led to increases in

Table 3 Summary results of 4-way analysis-of-variance (ANOVA) for species-level measurements. Presented are degrees of freedom (d.f.) and *F*-Ratios (*F*) used to test each source within the model, as well as the percent of total model sum squares (% variance) explained by each term.

Dependent Variable	Trans.	Source										Total
		Species (S)	Harvest (H)	S*H	CO ₂ (C)	S*C	H*C	S*H*C	Block (B)	C*B	Total	
	d.f.	10	2	20	2	20	4	40	3	6	776	
Plant density	Log	127.7***	78.5***	20.3***	26.5***	3.9***	4.4**	1.2 NS	6.3**	2.3*		
	% Variance	49.1%	6.0%	15.6%	2.0%	3.0%	0.7%	1.9%	0.7%	0.5%		
Biomass	Log	496.1***	476.4***	34.7***	46.6***	4.8***	2.0 NS	1.5*	7.2***	0.8 NS		
	% Variance	65.6%	12.6%	9.2%	1.2%	1.3%	0.1%	0.8%	0.3%	0.1%		
Total N-content	Log	404.0***	26.6***	23.9***	2.6†	5.2***	1.8 NS	1.3 NS	7.7***	0.3 NS		
	% Variance	75.1%	1.0%	8.9%	0.1%	1.9%	0.1%	1.0%	0.4%	0.0%		
C/N (molar)	Log	159.9***	2319.4***	17.3***	153.3***	2.6***	4.7***	0.9 NS	5.4**	4.6***		
	% Variance	20.1%	58.4%	4.4%	3.9%	0.7%	0.2%	0.5%	0.2%	0.3%		
¹⁵ N uptake (total excess)	Log	386.0***	299.1***	47.3***	11.3***	5.6***	4.6**	1.5*	8.8***	1.7 NS		
	% Variance	62.9%	9.8%	15.4%	0.4%	1.8%	0.3%	1.0%	0.4%	0.2%		
Relative ¹⁵ N uptake (mg N day ⁻¹ dry weight ⁻¹)	Log	206.8***	1772.6***	21.9***	4.8**	2.7***	4.9**	1.0 NS	3.0*	1.6 NS		
	% Variance	30.5%	52.4%	6.5%	0.1%	0.8%	0.3%	0.6%	0.1%	0.2%		
Relative ¹⁵ N uptake (mg N day ⁻¹ mg N ⁻¹)	Log	281.3***	703.3***	30.3***	9.3***	2.5**	4.3**	1.0 NS	3.4*	1.6 NS		
	% Variance	51.3%	25.6%	11.0%	0.3%	0.8%	0.3%	0.8%	0.2%	0.2%		

****P* ≤ 0.0001; ***P* ≤ 0.001; **P* ≤ 0.05

the number of individuals surviving. Three of the 11 species showed clear signs of senescence and loss of individuals by the final harvest (EC, EM, and ML).

In every case where we observed a trend in the fraction of experimental communities which had individuals flowering, elevated CO₂ led to an increase in flowering (Fig. 4). Five of the 11 species had peaked in flowering and were declining by the third harvest (GP, EC, EM, LR, and ML). All of the species which showed declining

numbers by the end of the experiment had declining flowering. In contrast, four species showed either little to no flowering through the majority of the experiment, exhibiting the highest flowering rates at the end of the experiment (MC, AB, AT, and VE).

For above-ground biomass, we observed that only 4 of the 11 species showed significant responses to elevated CO₂. There was no relationship between the overall size of the species (as estimated by contribution to whole community biomass) and how responsive it was to elevated CO₂ as the CO₂-responsive species biomass ranged from the second smallest to the largest fraction of whole-community biomass (Fig. 5, Table 4). Three of the four responsive species showed increases in biomass (EC, EM, and ML). One species (VE) showed a significant decrease in biomass, particularly at the final harvest. The species that showed large and significant decreased biomass production in elevated CO₂ at the final harvest represented the single largest contribution to total biomass in the communities.

For most species, elevated CO₂ had a minimal effect on above-ground plant nitrogen content (Tables 3 and 4, Fig. 6). The two exceptions to this pattern are EC and VE. EC, one of the smallest species, showed a consistent significant trend of increased nitrogen content in elevated CO₂. In contrast, VE, the largest species, showed a highly significant reduction in plant nitrogen content in both the second and third harvests.

Above-ground plant C/N ratios were largely a function of time of harvest, where every species at each CO₂ level showed increased C/N ratios with each harvest (Table 3 and Fig. 7). In contrast to biomass and total nitrogen content, every species showed a significant increase in C/N ratios with elevated CO₂ (Table 4).

Rates of ¹⁵N-nitrogen uptake were less sensitive to changes in CO₂ than were C/N ratios (Table 3). For most

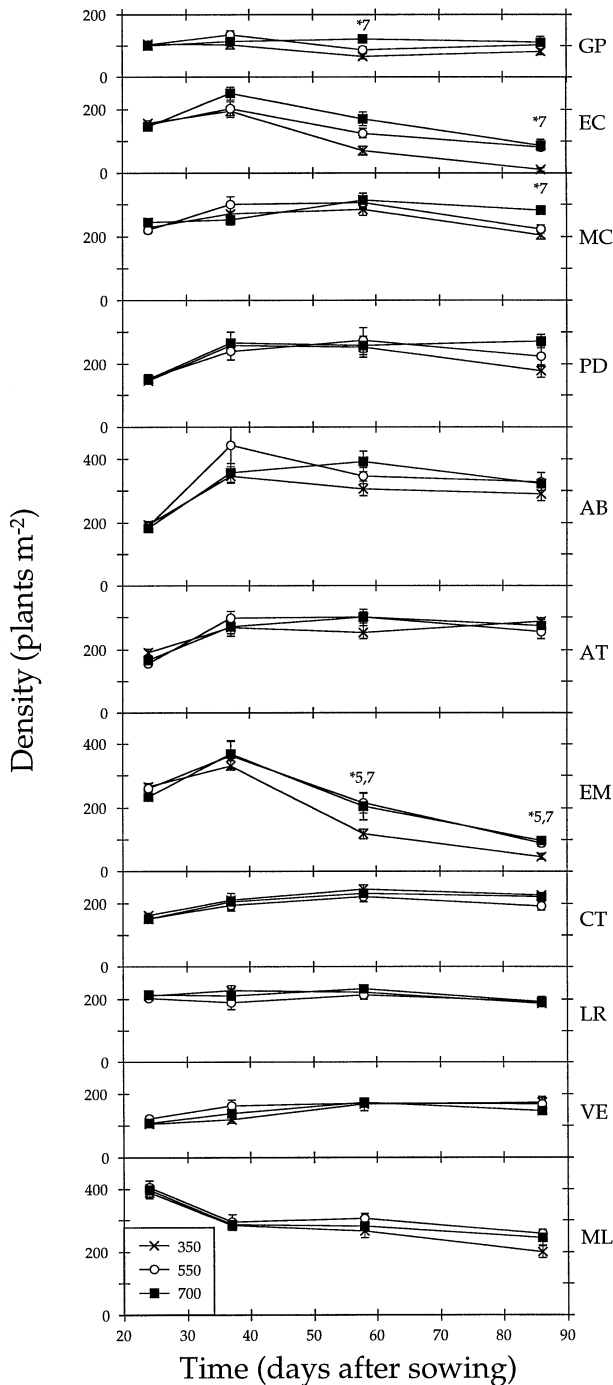


Fig. 3 Summary of species-level changes in the density of individuals present in each community in responses to the atmospheric CO₂ treatments (ambient, 550 ppm and 700 ppm) 24 days following the sowing of the seed and at the three harvests. Species are sorted in order of increasing relative biomass at the first harvest. Species codes listed along the right-hand axis are defined in Table 1. For each species, if the CO₂ treatments were significantly different from one another at a given time ($P < 0.05$ from a Scheffé posthoc means comparison, with $\alpha = 0.05$, Velleman 1994), then an asterisk is present. Note: the *post-hoc* comparisons were performed separately from a three-way analysis of variance for each species. Adjacent to each asterisk is a numeric code of '7' and/or '5'. '7' indicates that the 700 ppm treatment was significantly different from the ambient CO₂ treatment. '5' indicates that the 550 ppm treatment was significantly different from the ambient treatment. See Table 3 for the full ANOVA summary. Error bars are a single standard error of the mean.

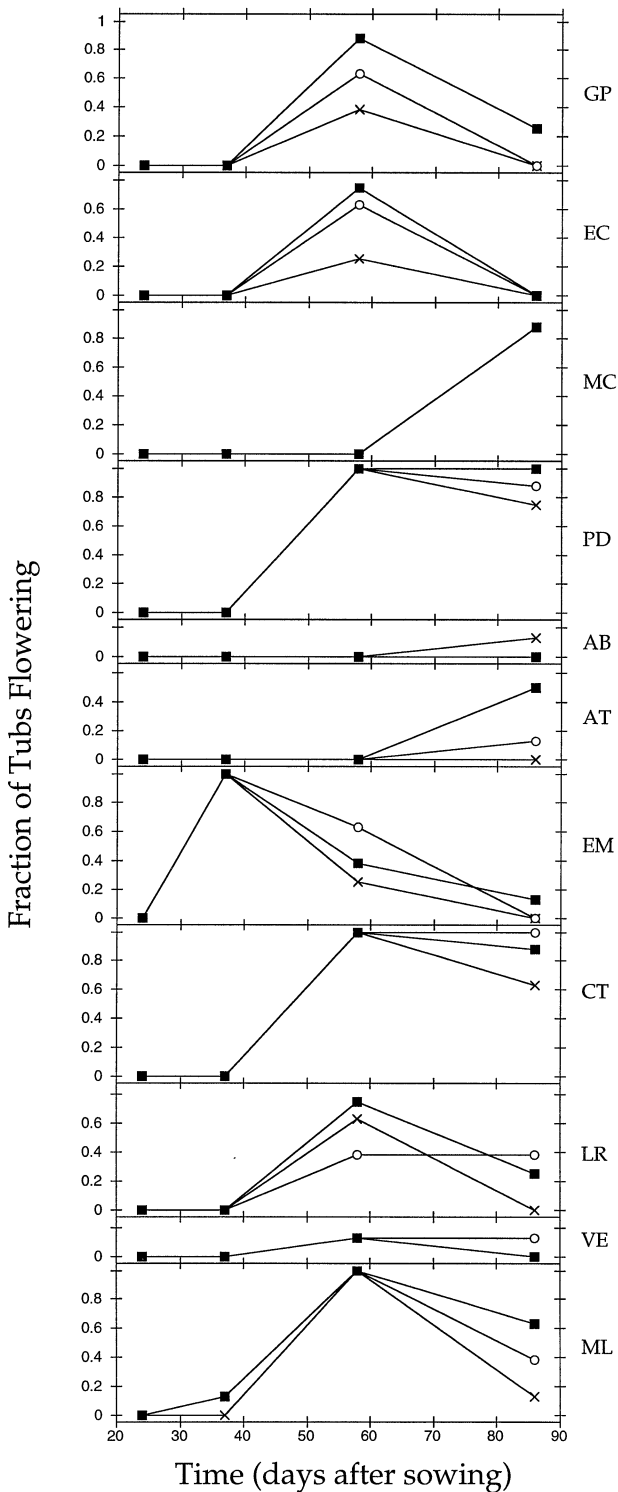


Fig. 4 Summary of species-level changes in the fraction of experimental communities which had at least one individual of a given species which was flowering. The layout of the figure is identical to Fig. 3, including symbol types, except that there are no estimates of error for each point.

species, rates were not significantly affected (Table 4, Fig. 8). A few species (MC, CT, and ML) showed trends (nonsignificant) of increased uptake either at the first or the last harvest. The single species that showed highly significant effects of elevated CO₂ on rates of nitrogen uptake was VE. Similar to patterns of whole plant nitrogen content, VE showed highly significant decreases in nitrogen uptake rates with elevated CO₂ at the second and third harvests.

Rates of ¹⁵N-nitrogen uptake relative to biomass were reduced in elevated CO₂ for most species (Table 3, Fig. 9). In general, these reductions were significant mostly for the larger species at the first harvest (Table 4). Rates of ¹⁵N-nitrogen uptake relative to plant nitrogen content were for most species less affected by CO₂ concentrations (Table 4, Fig. 10). None of those species that showed significant decreases in nitrogen uptake rates relative to biomass in elevated CO₂ showed reduced nitrogen uptake rates relative to plant nitrogen content. The only species that showed a significant change in nitrogen uptake rates relative to plant nitrogen content in elevated CO₂ was EC. EC showed an increase at the final harvest. Several other species showed trends, albeit nonsignificant, of increased nitrogen uptake rates relative to nitrogen content in elevated CO₂ (GP, MC, PD, CT), though VE showed a trend toward decreased relative uptake rates.

Discussion

In this study we measured the biomass production and nitrogen uptake within experimental communities of annual plants in a range of atmospheric CO₂ levels from ambient to double-ambient. This experiment is unique in combining the following:

- experimental communities consisted of 11 naturally co-occurring species.
- by carrying out a series of destructive harvests coupled with *in situ* ¹⁵N tracer applications, we documented how the structure and function of these experimental communities are affected by elevated CO₂ through ontogeny;
- the experimental communities were maintained at two concentrations of CO₂ in addition to ambient levels;
- by applying a ¹⁵N tracer directly to the intact communities, we measured *in situ* patterns of nitrogen uptake which integrate a number of individual architectural, physiological, symbiotic and environmental factors.
- in each experimental community, we studied both community-level and species-level biomass, nitrogen content, and nitrogen uptake through time.

Community- vs. species-level responses

We observed highly significant species by CO₂ interactions for every parameter that we measured — from

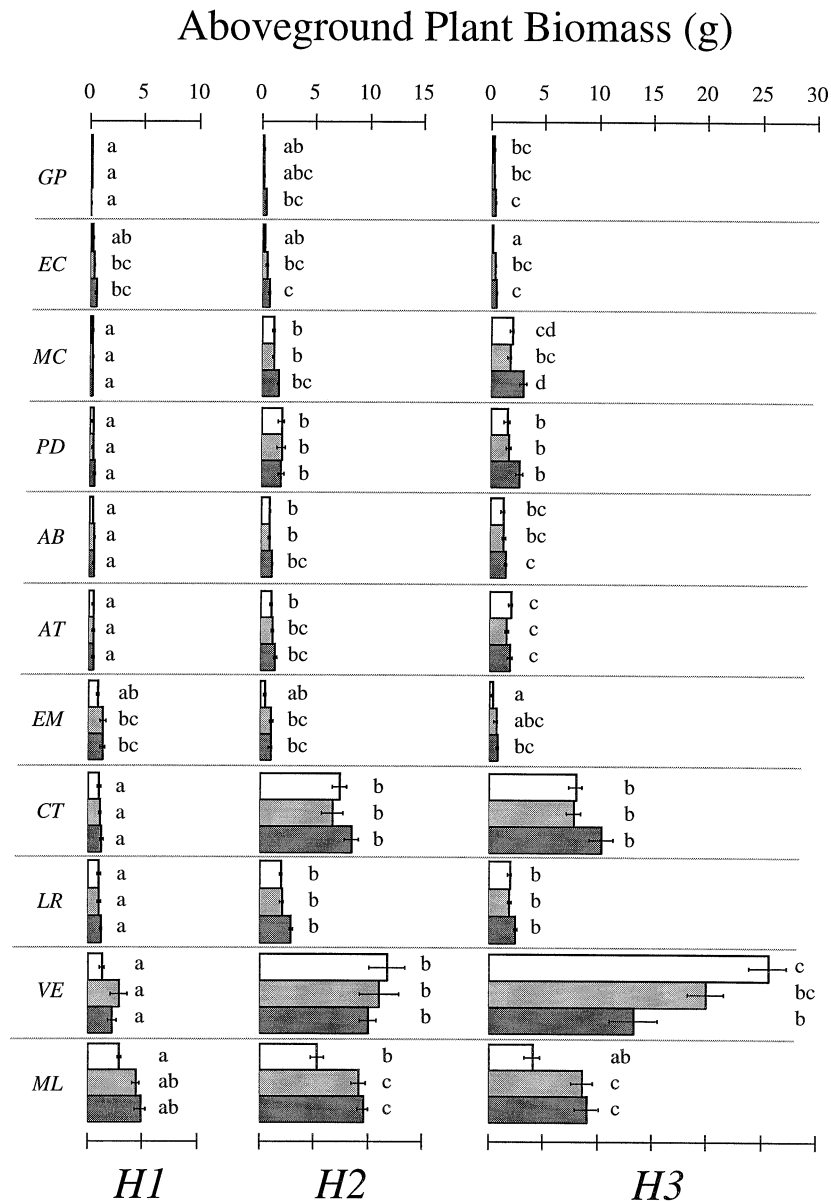


Fig. 5 Summary of species-level above-ground biomass responses in the atmospheric CO₂ treatments (ambient, 550 ppm and 700 ppm) at the three harvests (H1, H2, and H3). Species are sorted in order of increasing relative biomass at the first harvest. Error bars are a single standard error of the mean. Species codes listed along the left-hand axis are defined in Table 1. For each species, different letters next to each CO₂ by harvest combination represent significant differences ($P < 0.05$) from a Scheffé posthoc means comparison, with $\alpha = 0.05$ (Velleman 1994). Note: the *post-hoc* comparisons were performed separately from a three-way analysis of variance for each species. See Table 3 for the full ANOVA summary. Shading of different bars to denote CO₂ treatments is the same as in Fig. 2.

net biomass production and number of individuals surviving to a given time to relative nitrogen uptake rates. This implies that the community-level responses summarized in Fig. 2 were the result of an amalgam of different species-level responses (e.g. Zangerl & Bazzaz 1984). The only exception to this pattern is that every species showed qualitatively similar patterns of increases C/N ratios with increased CO₂. One limitation to the data we present on species-level responses is that all species-level data that

we present are derived from above-ground plant biomass. If above- and below-ground biomass had equivalent scaling relationships for each species at a given time, then we could safely use above-ground biomass as a measure of relative differences between species. For the first harvest we know this is the case (Fig. 1), but we are unable to state that this is the case for the last two harvests. The low variation in whole-community root to shoot biomass ratios through time relative to such factors

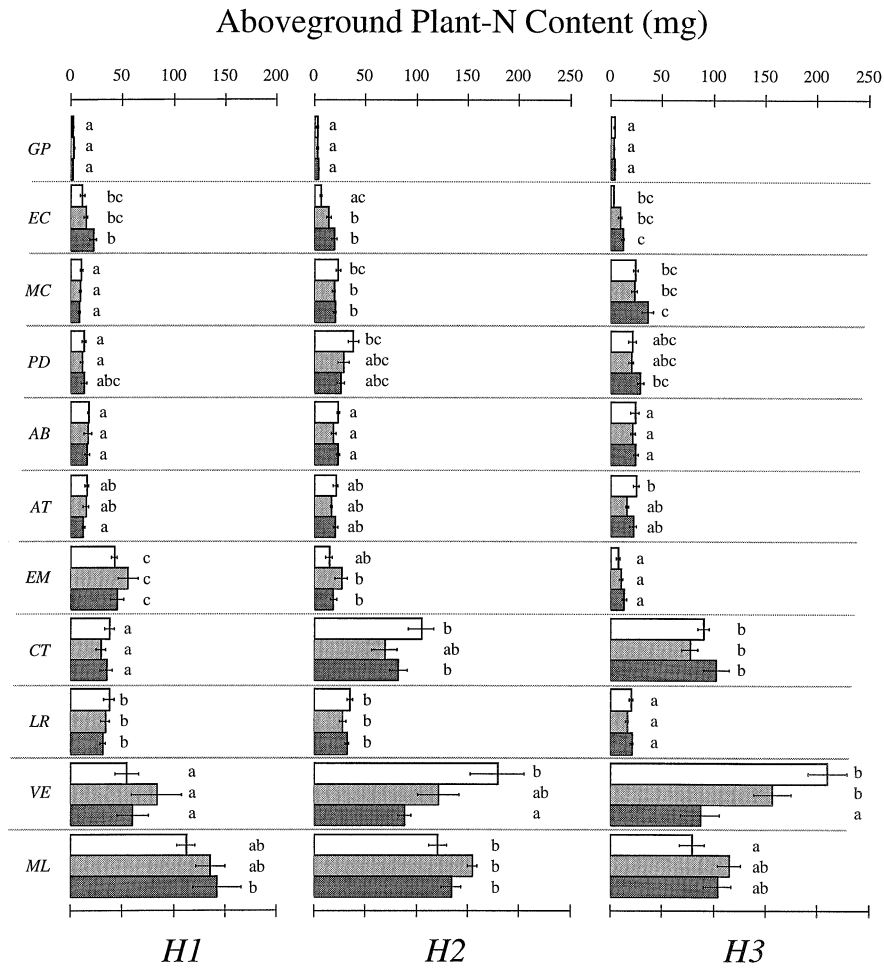


Fig. 6 Summary of species-level above-ground plant nitrogen content. Layout and shading of bars identical to Fig. 5.

Table 4. Overview of the effects of CO₂ on biomass production, nitrogen content, and nitrogen uptake in all 11 species within the experimental communities. Species are listed in order of increasing relative biomass at the first harvest (same as Figs 3–8). The symbols presented are a summary of ANOVAs carried out for each individual species. ● = no CO₂ effect, ↑ = increased CO₂ leads to increased response, ↓ = increased CO₂ leads to decreased response. When multiple symbols are present and separated by a slash, then there was a significant CO₂ by harvest interaction and the different symbols present a qualitative summary of the changing effects of elevated CO₂ with time. See Figs 3–8 for a detailed view of these patterns.

Species	Density	Biomass	N-content	C/N	¹⁵ N uptake	¹⁵ N uptake (Biomass ⁻¹)	¹⁵ N uptake (N-content ⁻¹)
GP	●/↑/●	●	●	↑	●	●	●
EC	●/↑	↑	↑	↑	●	●	↑/●
MC	●/↑	●	●	↑/●	●	●	●
PD	●	●	●	↑	●	●	●
AB	●	●	●	↑	●	↓/●	●
AT	●	●	●	↑	●	↓/●	●
EM	●/↑	↑	●	↑	●	↓/●	●
CT	●	●	●	↑	●	↓/●	●
LR	●	●	●	↑	●	↓/●	●
VE	●	●/↓	●/↓	↑	↓	↓	●
ML	●	↑	●	↑	●	↓/●	●

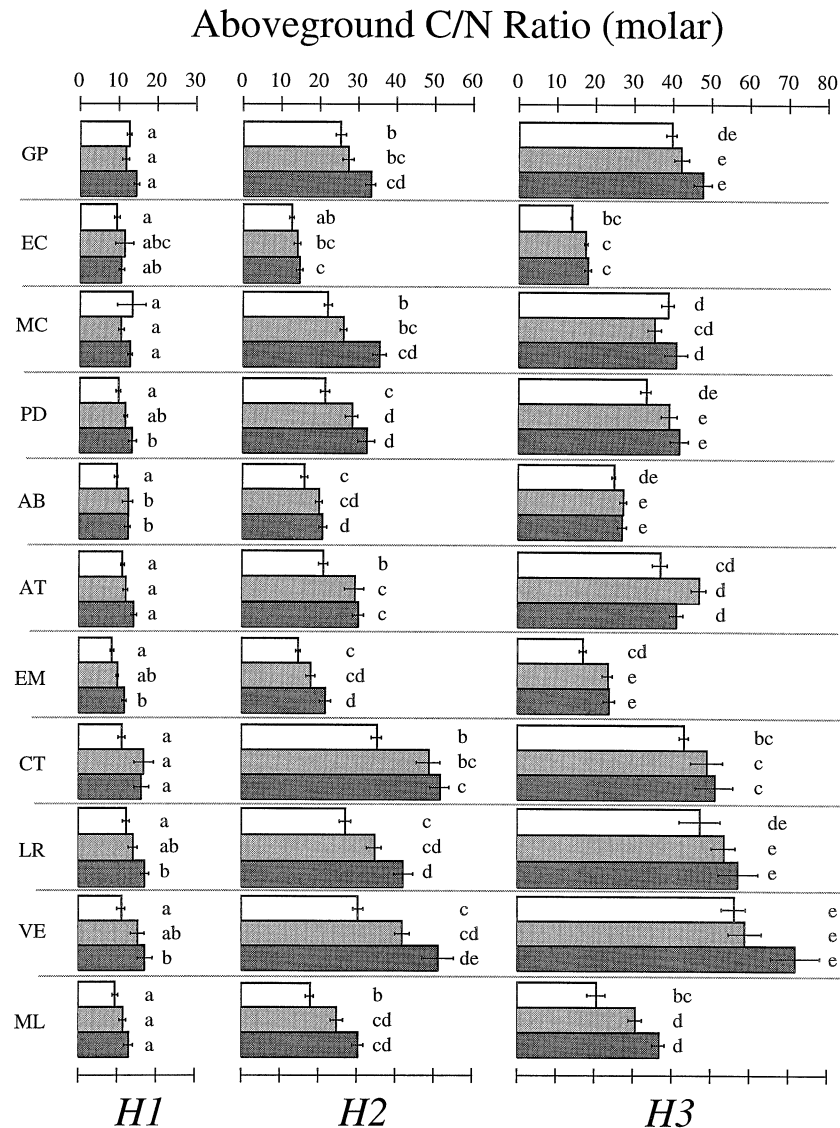


Fig. 7 Summary of species-level above-ground plant molar carbon-to-nitrogen ratios. Layout and shading of bars identical to Fig. 5.

as total biomass, C/N ratios, and relative N uptake rates (Table 2, Fig. 2) suggests that changes in allocation may not have been as important as changes in other factors.

Many of the community-level measurements were influenced to a large degree by one or a few species that comprise the majority of each community's above-ground biomass. For example, the community-level trend toward decreased biomass in elevated CO_2 at the last harvest was the result of one species' response to elevated CO_2 . Most species (7 out of 11) showed no change in biomass with elevated CO_2 , three showed significant increases, and one showed a significant decrease (Table 4, Fig. 5). The single species (VE) whose biomass decreased significantly in elevated CO_2 comprised the single largest

fraction of whole community above-ground biomass. The reduced biomass production of this species in elevated CO_2 was not due to accelerated senescence (e.g., St. Omer & Horvath 1983), as this species showed no increase in mortality (Fig. 3) and no clear trend to accelerated or declining flowering (Fig. 4). This species grows to a larger maximum size than any of the other species in our communities (120 cm tall), and is a successful weed pest in North America and India (Kaul & Mangal 1987). Thus it is not surprising that this species dominated the biomass production in our communities. The significant reduction in biomass for this species, however, resulted in a reduction in community-level biomass even though most species showed either no increase or a significant increase

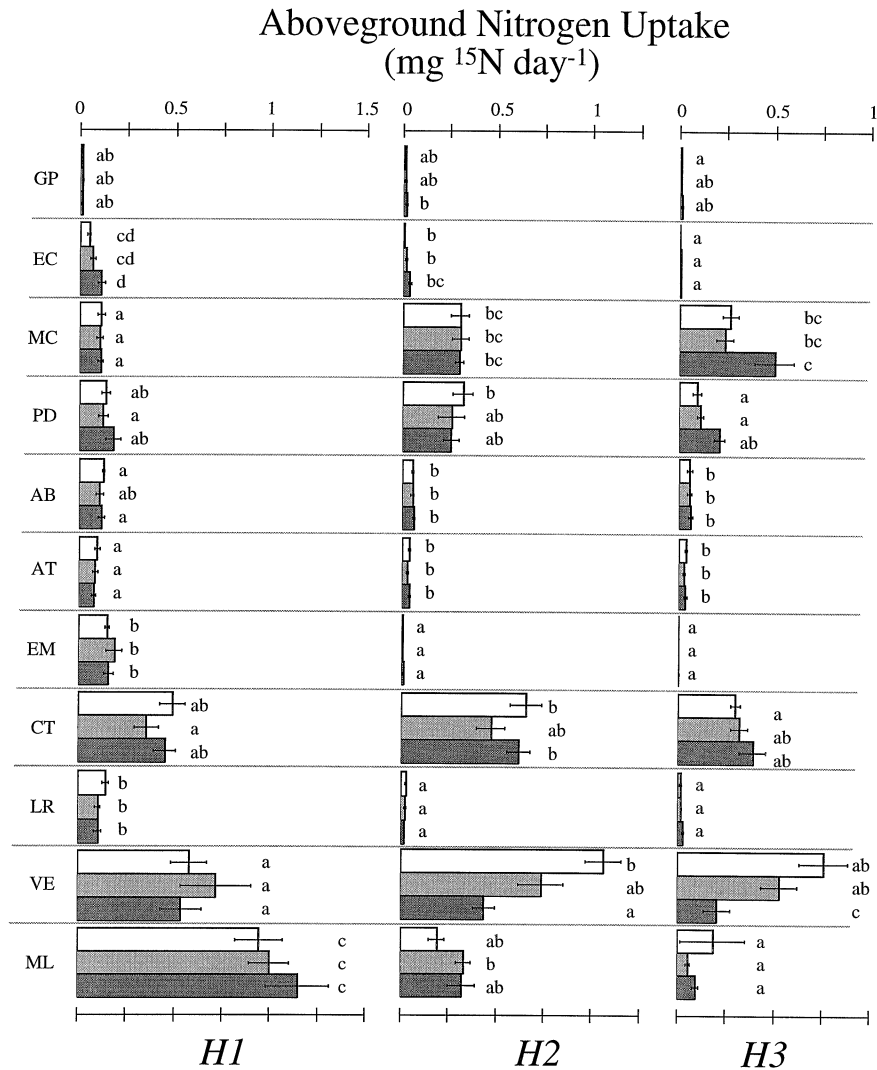


Fig. 8 Summary of species-level above-ground plant ¹⁵N-nitrogen uptake rates. Layout and shading of bars identical to Fig. 5.

in biomass. The same general pattern was true for community- vs. species-level nitrogen content as well.

Nitrogen uptake rates within an intact, multi-species community

The measurements of nitrogen uptake we present in this paper have certain advantages and disadvantages in relation to how other studies have studied plant nitrogen uptake in elevated CO₂ atmospheres (BassiriRad *et al.* 1996a,b; Jackson & Reynolds 1996). The advantage of the approach taken here is that our measurements of nitrogen uptake integrate a number of plant architectural, physiological, and symbiotic factors along with environmental constraints because we measure *in situ* uptake rates. The disadvantage of this approach is that because we have

not measured how elevated CO₂ affects each of the different processes that regulate uptake that we cannot assess the relative importance of these factors.

Recent studies have focused on nitrogen uptake (separately for NH₄⁺ and NO₃⁻) on excised root fragments (BassiriRad *et al.* 1996a,b; Jackson & Reynolds 1996). Together, these studies have documented the range of potential responses, from increases to decreases, in physiological capacity for nitrogen uptake. We found that, at the community-level, elevated CO₂ appears to reduce whole-plant nitrogen uptake rates for our dual-labelled ¹⁵NH₄⁺¹⁵NO₃⁻, but these reductions appear only after the communities had been developing for a while. At the first harvest, uptake rates were not significantly affected by CO₂.

It is possible that these reductions were the result of

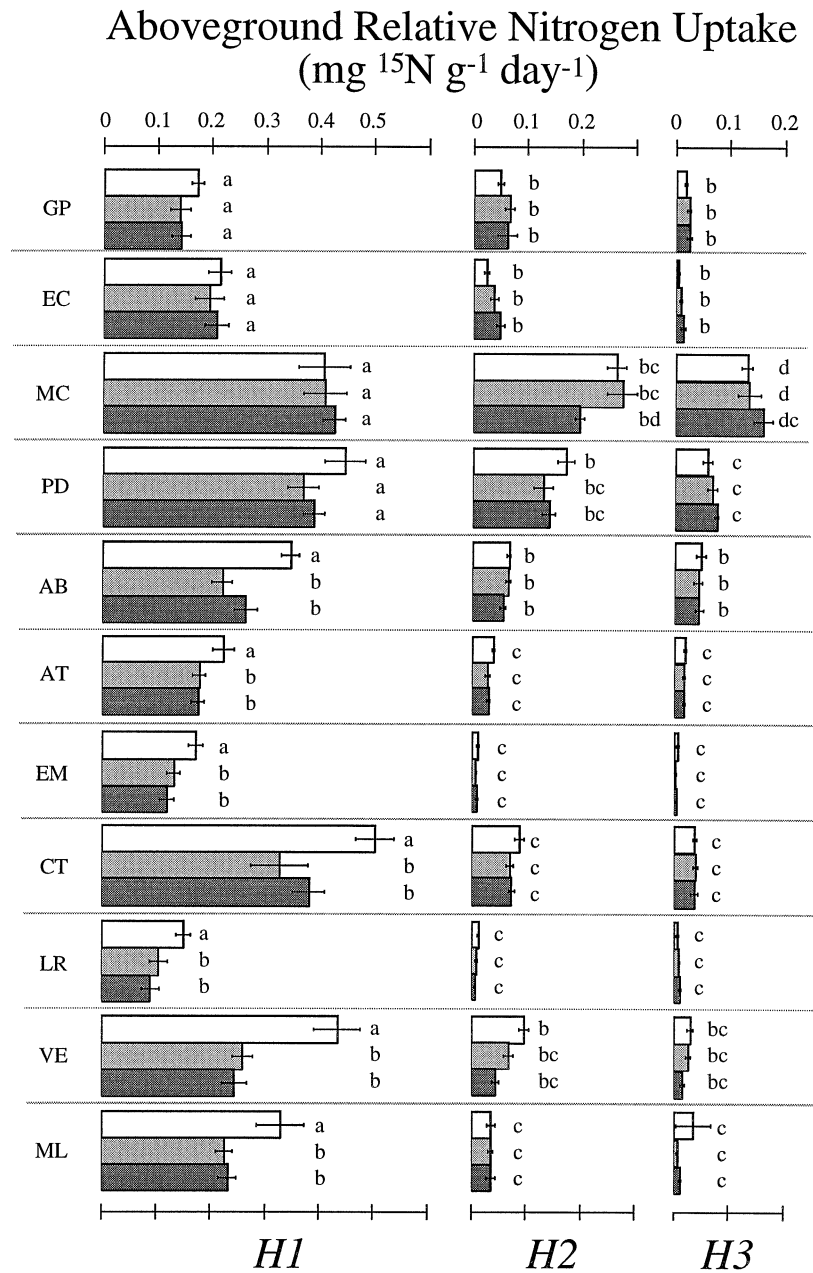


Fig. 9 Summary of species-level above-ground plant ¹⁵N-nitrogen uptake rate relative to plant biomass. Layout and shading of bars identical to Fig. 5.

reduced physiological capacity for uptake. However, a number of studies have documented that plant acquisition of nitrogen in elevated CO₂ environments can be limited by other environmental factors which reduce the amount of nutrients available to plants, including immobilization by soil microflora (Díaz *et al.* 1993), and reduced rates of mineralization (Berntson & Bazzaz 1997b, 1998). While we did not directly measure immobilization of the applied ¹⁵N tracer by soil microbes, we did recover

less of the applied tracer in elevated CO₂ environments toward the end of the experiment, which is consistent with its immobilization elsewhere in our experimental communities.

The rate of nitrogen uptake relative to plant biomass in our study was not affected at the community-level in the last two harvests. This relative rate was, however, significantly greater for ambient CO₂ concentrations at the first harvest. The decrease in nitrogen uptake rates

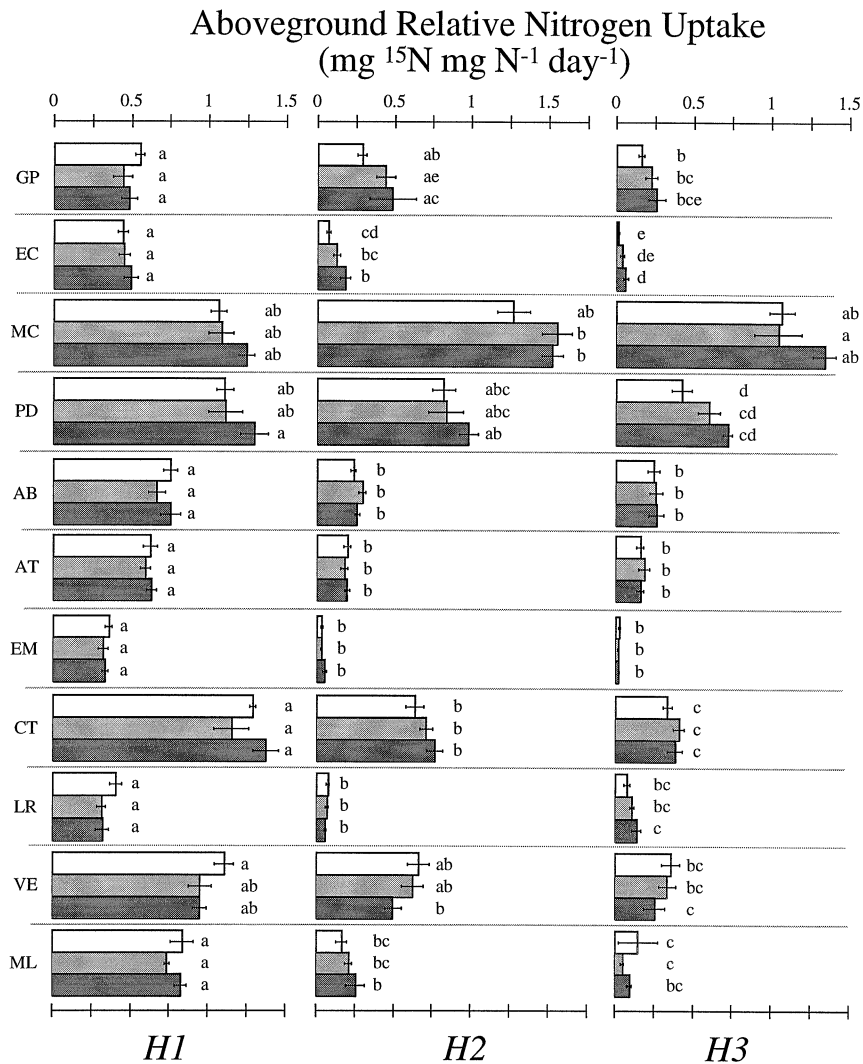


Fig. 10 Summary of species-level above-ground plant ¹⁵N-nitrogen uptake rate relative to plant nitrogen content. Layout and shading of bars identical to Fig. 5.

in relation to biomass for the first harvest is likely to be a size-effect. Through the course of the study, plant biomass increased to a much greater degree than did nitrogen uptake rates. Thus, nitrogen uptake rates relative to biomass showed a precipitous decline through ontogeny. The relative effect of elevated CO₂ on plant biomass was greater at the first harvest than at the last two (Fig. 3a), possibly explaining the reduction in relative uptake rates of nitrogen uptake in elevated CO₂.

Total plant nitrogen content changed to a much lesser degree through ontogeny than did plant biomass (compare Fig. 3a and b), leading to large increases in plant C/N ratios through ontogeny. If changes in the acquisition of nitrogen from the soil in an elevated CO₂ environment is regulated more by physiological capacity (Bassirirad *et al.* 1996a,b; Jackson & Reynolds 1996) than plant size or root system architecture (Berntson & Woodward 1992;

Rogers *et al.* 1992; Berntson 1994), then we would expect that observed variations in nitrogen uptake rates could be explained by variations in plant protein and thus nitrogen content. Our results support this hypothesis. Plant nitrogen uptake rates relative to nitrogen content were not affected by CO₂ concentration at the community-level (Table 2, Fig. 2). Furthermore, at the species level, plant nitrogen uptake rates relative to nitrogen content removed the effect of CO₂ for all those species for which we observed significant reductions in uptake rates relative to plant biomass (Table 4).

The relationship between nitrogen uptake and net growth responses to elevated CO₂

The observations we have presented thus far represent an overview of how elevated CO₂ affected patterns of

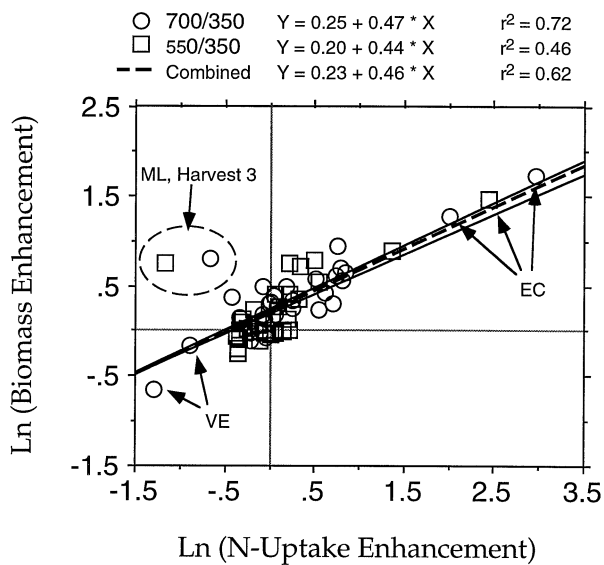


Fig. 11 Relationship between enhancement in nitrogen uptake (¹⁵N-nitrogen, x-axis) and enhancement in biomass production (above-ground, y-axis) for 550 and 700 ppm CO₂ relative to ambient. Each point within the graph represents the ratio of elevated over ambient treatment means for each species by harvest combination ($n = 33$). The thick, dashed line represents the best fit regression line when enhancement ratios for 550 and 700 ppm CO₂ were pooled together. Regressions were statistically significant for 550 and 700 ppm CO₂ ($F = 80.5_{(1,32)}$, $P < 0.0001$ and $F = 26.83_{(1,32)}$, $P < 0.0001$) as well as the combined regression ($F = 101.54_{(1,64)}$, $P < 0.0001$). A single significant outlier for both sets of CO₂ enhancement ratios is indicated within the figure by a dashed circle. This point corresponds to species ML at the final harvest which had anomalously high ¹⁵N content in plant material at ambient CO₂ levels, leading to low (< 1.0) nitrogen uptake enhancement ratios. Removal of this outlier improved the r^2 of regressions to 0.85, 0.75, and 0.80 for 550 ppm, 700 ppm CO₂ and the combined regression, respectively.

growth and nitrogen uptake at the level of individual species and entire communities. To understand how changes in the ability to acquire soil nutrients influences biomass enhancement for individual species in elevated CO₂, we need to examine the relationship between the effects of elevated CO₂ on nutrient acquisition and biomass production. This relationship (Fig. 9; here presented on a log-log scale to improve the distribution of residuals) between the enhancement of nitrogen uptake and biomass production is highly statistically significant for both elevated CO₂ levels relative to ambient. Interestingly, the relationship between enhancement ratios for both elevated CO₂ levels did not statistically differ from one another (overlapping 99% confidence limits for both slopes and intercepts). Thus, it appears that there is a single relationship we can use to describe the relationship between relative enhancement of nitrogen uptake and biomass production on a species-by-species basis. An important feature

of this relationship is that we find a greater range of variation in nitrogen uptake than we do in biomass production, so that on average a unit relative increase in biomass production requires a greater relative increase in nitrogen uptake.

This relationship may appear contrary to the general pattern of decreased plant nitrogen content with elevated CO₂ observed in this study. Figure 9 presents the relationship between enhancement in nitrogen uptake and biomass production in elevated CO₂ for each species. Those species that were able to increase nitrogen uptake were the ones that had the greatest enhancement in biomass, even though all species showed reduced relative total nitrogen content. These patterns were not positively related to the dominance of the species within the communities. The single largest species in the communities (VE) showed reductions in nitrogen uptake and biomass production (enhancement ratios < 1.0), and the species that showed the largest enhancement for both these parameters was one of the smallest in terms of total biomass contribution to the communities (EC, Fig. 3).

Conclusions

In this study we found that community-level responses to elevated CO₂ were generally consistent with the majority of other studies of individual- and multispecies assemblages, where elevated CO₂ leads to enhanced biomass production early on, but this enhancement declines through time. In contrast, the responses of the individual species within our experimental communities was highly variable, showing the full range of responses from positive to negative. Due to the large variation in size between the different species, community-level responses were generally determined by the responses of only one or a few species. Thus, while several of the smaller species showed trends of increased biomass and nitrogen uptake in elevated CO₂ at the end of the experiment, community-level patterns showed a decrease in these parameters due to the significant reduction in biomass and nitrogen content in the single largest species.

The relationship between enhancement of nitrogen uptake and biomass production in elevated CO₂ was highly significant for both 550 ppm and 700 ppm CO₂ relative to ambient CO₂ levels (Fig. 11). This relationship suggests that the ability of plants to increase nitrogen uptake (through changes in physiology, morphology, architecture, and/or mycorrhizal symbionts) may be an important determinant of which species in a community will be able to respond to increased CO₂ levels with increased biomass production. The fact that the most dominant species within the community showed

reduced enhancement and the smaller species showed increased enhancement suggest that through time, elevated CO₂ may lead to significant changes in community composition.

At the community level, nitrogen uptake rates relative to plant nitrogen content were invariable between the three different CO₂ levels at each harvest. This was in contrast to significant reductions in total plant nitrogen uptake and nitrogen uptake relative to total plant biomass. This patterns supports the hypothesis that plant nitrogen uptake may be to a large extent regulated by physiological activity, assuming that physiological activity is controlled by protein and thus nitrogen content.

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