

Differential phosphorus and nitrogen effects drive species and community responses to elevated CO₂ in semi-arid grassland

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Summary

1. Productivity of dryland communities is often co-limited by water and nutrients. Since atmospheric CO₂ enrichment induces water savings by plants, elevated CO₂ and nutrients could interact to reduce growth limitation, irrespective of the direct influence of CO₂ on photosynthesis. We studied CO₂ effects in model communities from the semi-arid Negev of Israel with 17 mostly annual C₃ species at three CO₂ concentrations and three nutrient treatments.

2. Community biomass increased at elevated (440 and 600 µL L⁻¹) compared to pre-industrial CO₂ (280 µL L⁻¹) by 34% on average in the low-nutrient control, by 45% in the high P and by 50% in the high NPK treatment. Less evapotranspiration at elevated CO₂ increased soil water content by 30–40% on average. Significant CO₂–fertilization interactions indicated that plant responses to CO₂ enrichment were constrained by nutrient availability.

3. Responses of biomass and water-use efficiency (dry-matter accumulation per cumulative evapotranspiration) to CO₂ enrichment were non-linear and were saturated at 440 µL L⁻¹ at low nutrient and high P supply. CO₂ effects were further increased up to 600 µL L⁻¹ only under full NPK fertilization.

4. The overall CO₂ effect on biomass depended on the differential response of plant functional groups, with the P-dependent legume response dominating at low nutrient and high P supply, and the N-dependent grass response dominating at high NPK. With the exception of grasses, species responded differently to combinations of CO₂ enrichment and nutrient addition, even within functional groups.

5. Biomass production was co-limited by CO₂ and nutrients in this semi-arid seasonal community, with both effects possibly mediated by water availability. Nutrient losses associated with desertification will thus diminish potential gains in biomass due to elevated CO₂. Growth stimulation by CO₂ enrichment beyond close-to-current concentrations will only be seen under nutrient-rich conditions in semi-arid and possibly other drought-stressed grasslands.

Key-words: Biodiversity, elevated CO₂, non-linear CO₂ response, nitrogen, phosphorus

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Introduction

Plant growth and survival in semi-arid ecosystems is strongly limited by water supply. The already scarce availability of water might further decrease in many areas as a consequence of desertification, which is particularly grave in semi-arid regions (Schlesinger *et al.* 1990; Warren, Sud & Rozanov 1996). Any factor

increasing water availability is likely to increase plant production in these regions. Atmospheric CO₂ enrichment often improves plant water status besides its direct impact on photosynthesis (Körner 2000). The mechanism for this effect in both C₃ and C₄ plants is a reduction in leaf diffusive conductance caused by increased substomatal CO₂ concentration (Drake, González-Meler & Long 1997; Ghannoum *et al.* 2000; Wand *et al.* 1999), resulting in decreased transpiration and increased soil water content (Bremer, Ham & Owensby 1996; Field *et al.* 1997; Grünzweig & Körner 2001b; Niklaus, Spinnler & Körner 1998b). Volk *et al.* (2000) suggested that most CO₂ effects on grassland

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productivity are caused by water savings and increased soil water content rather than by photosynthetic stimulation. Under dry conditions, water-saving effects should be particularly important for plant growth and ecosystem productivity. Therefore, dryland ecosystems are expected to respond more strongly to elevated CO₂ than more humid ones (Lockwood 1999; Mooney *et al.* 1991; Smith *et al.* 2000). However, productivity in subhumid (Navas *et al.* 1995; Shaw *et al.* 2002) and semi-arid grasslands (Grünzweig & Körner 2001b) increased by 5–20% at elevated CO₂, similar to that found in mesic communities (Mooney *et al.* 1999).

The lack of more pronounced growth stimulation in dryland communities might be related to co-limitation by CO₂ and nutrients. Productivity in dry regions (at ambient CO₂) is often co-limited by water and N (Hooper & Johnson 1999), contrary to the common belief of limitation by water only. This might have consequences for biomass production under desertification, which causes the nutrient status of ecosystems to deteriorate (Schlesinger *et al.* 1990). Water shortage does not only limit growth directly, it also restricts soil nutrient availability (Chapin 1991). This effect could potentially be overcome by addition of readily available nutrients. Fertilization increased CO₂ responses of above-ground and/or total biomass in a number of grassland communities (Daepf *et al.* 2000; Joel *et al.* 2001; Owensby, Auen & Coyne 1994; Stöcklin, Schweizer & Körner 1998), whereas other studies with grasslands from various climatic zones (temperate, alpine, subhumid Mediterranean) showed no or a negative change in biomass response to CO₂ enrichment (Hebeisen *et al.* 1997; Loiseau & Soussana 2000; Reich *et al.* 2001; Schächli & Körner 1996; Shaw *et al.* 2002). Because of the water-saving effect of elevated CO₂ we hypothesize that biomass accumulation in dryland communities is co-limited by CO₂ and N (or nutrients in general). This would also mean that CO₂ responses are nutrient-limited.

Biomass responses to CO₂ enrichment were often non-linear, e.g. biomass accumulation was increased over a lower range of elevated CO₂ concentrations, but was negligible over a higher range of concentrations (Zangerl & Bazzaz 1984). This was particularly obvious when below-ambient CO₂ concentrations were included (Hättenschwiler & Körner 1996; Grünzweig & Körner 2001b), and was potentially caused by non-linearity or thresholds of photosynthesis and other physiological processes and by acclimation (Gill *et al.* 2002; Körner 2000). However, linearity of CO₂ effects might depend on resource supply (Körner 2000). We expected non-linear CO₂ effects and dependence of this pattern on nutrient availability.

Most, but not all legume species were often more responsive to CO₂ enrichment than other functional groups (Grünzweig & Körner 2001a; Hebeisen *et al.* 1997; Joel *et al.* 2001; Stöcklin & Körner 1999). Legume growth depends heavily on N₂ fixation (Lüscher *et al.* 2000; Niklaus *et al.* 1998a), which is controlled by water and P availability and carbohydrate supply to

N₂-fixing bacteria (Bordeleau & Prévost 1994; Schulze, Adgo & Schilling 1994). Legumes might profit from elevated CO₂ by increased carbohydrate supply to symbiotic associations, as N₂ fixation is a process with high respiratory costs for plants (Ainsworth *et al.* 2002; Lloyd & Farquhar 1996; Niklaus *et al.* 1998a). In some cases, carbohydrate supply appeared to be sufficient at ambient CO₂ (Almeida *et al.* 2000; Serraj, Sinclair & Allen 1998), and the CO₂ effect on legumes was explained by increased growth rate at elevated CO₂ subsequently leading to greater N demand, which could be met by increased N₂ fixation (Zanetti *et al.* 1996). Grasses and other non-N₂-fixing plant species might not be able to meet their N demand at elevated CO₂, and therefore greater responsiveness of legumes was expected.

Besides a generally low nutrient status, dry regions are typically low in P (Schlesinger *et al.* 1990), which commonly limits legume growth. Moreover, P limitation could have caused negative CO₂ effects in a subhumid grassland under different global change scenarios (Shaw *et al.* 2002). Grass and community biomass production at ambient CO₂ is often nutrient (NPK) limited, while legume growth can be depressed by N fertilization (Elisseou, Veresoglou & Mamolos 1995; Kirkham, Mountford & Wilkins 1996; Mitchley, Buckley & Helliwell 1996; Niklaus *et al.* 1998a). We hypothesized that the CO₂ response of grasses and non-leguminous forbs depended on general soil fertility (mainly N), whereas legumes should reach full responsiveness to CO₂ by P fertilization alone. In addition, we predicted that species will respond differently even within functional groups because of species-specific response potential and interspecific competition.

We tested the interactive effect of CO₂ and P or NPK in species-rich grassland assemblages from the northern Negev under a typical dry-year scenario (40% below mean annual precipitation), and analysed biomass- and water-related responses at the species, functional-group and community level. The use of three CO₂ concentrations (280, 440 and 600 µL L⁻¹), including pre-industrial, allowed us to assess the linearity of CO₂ responses.

Materials and methods

The grassland that served as the source for plants and soil is located in the hills of the northern Negev, Israel (400 m, 31°21' N, 34°51' E). The site is part of the Lehavim Long-Term Ecological Research area and of the Bedouin Demonstration Farm (Agricultural Research Organization), and has a long history of grazing by domestic animals. The region is part of the Irano-Turanian phytogeographical region, but the vegetation includes many species of Mediterranean and Saharo-Arabian origin (Feinbrun-Dothan & Danin 1991), and is dominated by scattered evergreen shrubs and ephemeral grassland species. Seeds (bulbils in the case of *Poa bulbosa* L. var. *bulbosa*) of the following 16 mostly annual species representing the ephemeral grassland patches were collected in late

spring of 1996 and 1997 (Feinbrun-Dothan & Danin 1991): *Aegilops kotschyi* Boiss., *Brachypodium distachyon* (L.) Beauvois, *Bromus fasciculatus* C. Presl, *Crithopsis delileana* (Schultes et Schultes fil.) Roshev., *Stipa capensis* Thunb. (annual grasses); *Poa bulbosa* (perennial grass); *Hymenocarpus circinnatus* (L.) Savi, *Medicago minima* (L.) Barta, *M. truncatula* Gaertner, *Trifolium campestre* Schreber (annual legumes); *Carrichtera annua* (L.) DC., *Daucus subsessilis* Boiss., *Hedypnois cretica* (L.) Dum.-Courset, *Plantago coronopus* L. ssp. *commutata* (Guss.) Pilger, *P. cretica* L., *Scabiosa porphyronera* Blakelock (annual non-leguminous forbs). In summer 1997, soil was collected to a depth of 30 cm. Soil was light lithosol on calcareous bedrock with pH 7.9, mineral N 7.5 $\mu\text{g g}^{-1}$, available P 3.0 $\mu\text{g g}^{-1}$ (extracted with CO_2 -saturated water; Eidg. Forschungsanstalten 1996) and water-extractable K 4.7 $\mu\text{g g}^{-1}$. Plant material and soil were shipped to the University of Basel, Switzerland. *Ranunculus asiaticus* L. (geophyte) emerged spontaneously from the diaspore bank in the soil during the experiment at similar rates in all containers and was left as part of the plant communities, increasing the number of species in model communities to 17. All species were of the C_3 photosynthetic type.

Plants were grown on nutrient-poor 1 : 9 mixtures of Negev soil and calcareous marl (particle diameter 0–8 mm) amended with a base supply of inorganic slow-release full fertilizer (3, 1.8 and 2.3 g m^{-2} N, P and K, respectively, plus other macro- and microelements; Optima, Münchenstein, Switzerland). Forty-five containers (0.265 m \times 0.165 m, 0.21 m deep) were each filled with 13 kg of this substrate, which permitted only slow growth, comparable with that at the site in the northern Negev (Osem *et al.* 2002). On top of this base supply, fertilizer treatments included the addition of liquid plant-available nutrient solutions corresponding to either 6 g m^{-2} P only, or 6 g m^{-2} N, P and K each (as NH_4NO_3 , triple-superphosphate and KCl, Landor, Birsfelden, Switzerland). Each fertilizer was added in two applications of 3 g m^{-2} at the end of week 3 and week 8 after sowing. Control containers (labelled 'low-nutrient control') received only the base supply of nutrients. Each of the two fertilization treatments and the control consisted of 15 containers, five for each of the three CO_2 treatments (45 containers in total). The substrate was subsampled for measurement of water content to determine soil dry-weight in containers.

The plant communities were grown in closed growth cabinets (1.2 m^2 area; one cabinet for each of the three CO_2 treatments), which were equipped with a transparent top cover to allow penetration of natural daylight. The elevated CO_2 concentrations (440 and 600 $\mu\text{L L}^{-1}$) were regulated by an infrared gas analyser (WMA-2, PP Systems, Stotfold, Hitchin, Herts, UK) and a computerized injection system. Pre-industrial CO_2 concentration (280 $\mu\text{L L}^{-1}$) was produced by a small computer-controlled bypass duct passing air in the growth cabinet through a soda-lime scrubber. CO_2 treatments were maintained during day and night. The

following temperature regime represented early, mid and late Negev winter, respectively: During the first 40 days, 15/10 $^\circ\text{C}$ (day/night) were applied, followed by 30 days of 12/8 $^\circ\text{C}$ and 30 days of 14/10 $^\circ\text{C}$. In order to compensate for greater cloudiness in Basel as compared to the Negev, natural daylight was supplemented for 10 h per day with three daylight lamps (Power Star HQI-T 1000/D, Osram, Munich, Germany) per chamber, thus achieving a photosynthetically active photon flux density of 500–900 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at canopy level. Deionised water was provided at a total of 167 mm, which represented a dry year, 40% below the long-term average rainfall in this region of the Negev (Baram 1996). Water was applied at 2–11 mm every 1–10 days, representing the lower half of the range of rain frequency and rain amount per event during a dry year, thus preventing drainage from containers through excessive watering. Some drought-induced wilting was observed particularly in the larger high NPK-treated communities several times during the experiment, with plants at 280 $\mu\text{L L}^{-1}$ appearing more affected than plants at elevated CO_2 .

Prior to sowing, legume seed coats were slightly scarified with sand paper to break dormancy (seed-coat impermeability) of these species. In addition, all seeds were stratified in moist soil at 10 $^\circ\text{C}$ in the dark for 3 days immediately after sowing to improve germination. Seeds were dispersed uniformly over the container according to the mean natural species densities in ungrazed hillside plots (Y. Osem, M. Sternberg and J. Kigel, personal communication). At harvest, plant density averaged 2400 ± 30 individuals m^{-2} among the 45 containers. Plant density was similar among all treatment combinations, except for a 17% higher density in high NPK-treated systems at 440 and 600 $\mu\text{L L}^{-1}$ compared to those at 280 $\mu\text{L L}^{-1}$ CO_2 ($P = 0.043$ and 0.015, respectively; Tukey-Kramer HSD test), possibly caused by lower seedling mortality.

Fifteen containers (five for each of the three fertilization treatments) were located in each of the three growth chambers. In order to minimize possible chamber effects, we randomised CO_2 treatments among growth chambers and positions of the containers within growth chambers every week throughout the 14-week growing period (sowing to peak of vegetative biomass).

The ecosystem water balance was monitored periodically by weighing containers. Soil water content was determined by subtracting the weights of dry soil and empty container from the total weight. Total cumulative evapotranspiration during the entire experiment was determined by subtracting soil water content at harvest from the sum of the total amount of water applied during the experiment and the soil water content at the beginning of the experiment (no water drainage from the containers was noted). The fraction of flowering plants (individuals with at least one flower at anthesis per total number of individuals) was determined weekly for all legumes, the early flowering forb *Carrichtera annua* and the grass *Bromus fasciculatus*.

The experiment ended with a harvest at the time of early anthesis of grasses (the last functional group to flower) and close to maximum biomass (100 days after sowing). Above-ground plant parts were harvested separately for each species, and were divided into stems, dead leaves, green leaves and reproductive parts. Roots could not be separated into species, and were harvested in bulk. Before drying, leaf area was measured by a photoplanimeter (Li-3100, Licor, Lincoln, Nebraska, USA), and plant material was dried at 80 °C. The term 'biomass' (amount of living matter) was used here instead of the term 'phytomass,' although a small amount of dead matter was present in the communities.

Results were analysed at the community, functional group and species levels (functional groups were 'grasses', 'legumes' and 'forbs'; grasses included five annuals and one perennial). At the community level, variables were analysed with three-way factorial ANOVA (with the factors CO₂, fertilization and functional group) for above-ground biomass and its components, and with two-way ANOVA (CO₂ and fertilization) for other variables. At the functional-group level, three-way ANOVA (CO₂, fertilization and species) was conducted. Where needed, data were log-transformed prior to analysis, in order to homogenise variance. Angular transformation was performed for fractions. Multiple comparisons were performed with the Tukey-Kramer honestly significant difference (HSD) *post hoc* test.

Results

BIOMASS AND LEAF AREA AT THE COMMUNITY LEVEL

Phosphorus and NPK fertilization significantly increased above-ground, below-ground and community biomass per unit ground area irrespective of CO₂ treatment (Fig. 1). Compared with the low-nutrient control, total community biomass was 40% larger following high P

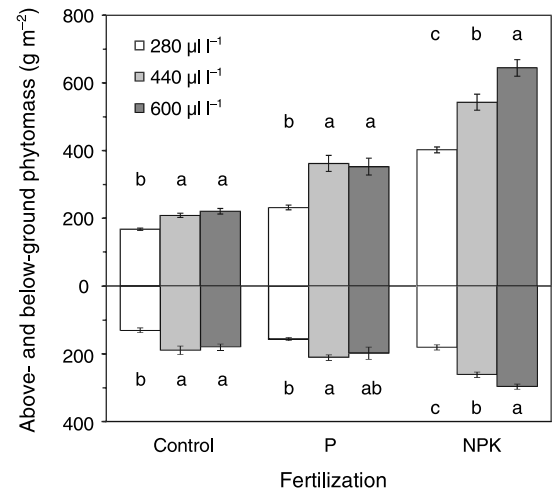


Fig. 1. Above-ground (upper part of chart) and below-ground (lower part of chart) biomass at three CO₂ concentrations (280, 440, 600 μL L⁻¹) and three fertilization treatments (low-nutrient control, high P, high NPK). Mean ± SE, *n* = 5 experimental communities. Means of CO₂ treatments within the same fertilization treatment that were not indicated by a common letter were significantly different at *P* ≤ 0.05. Fertilization effect: at each CO₂ concentration, control, P and NPK are significantly different from each other, except for below-ground biomass at 440 and 600 μL L⁻¹, where no difference was observed between control and P.

addition alone and 110% larger following high NPK addition.

The CO₂ effect on biomass depended on the CO₂ range considered and on nutrient availability (significant interaction of CO₂ and fertilization; Table 1). Tested separately for each nutrient treatment, biomass was significantly greater at 440 than at 280 μL L⁻¹ CO₂ in all nutrient treatments (increase in total biomass by 34, 48 and 38% in the control, high P and high NPK treatments). This response was saturated at 440 μL L⁻¹ in the control and the high P treatment (no further increase from 440 to 600 μL L⁻¹; Fig. 1). NPK

Table 1. *F*-values and probability levels from three-way ANOVA (above-ground biomass and its components) and two-way ANOVA for variables at the community level. Main factors were CO₂, fertilization and functional group. Only above-ground dry mass could be separated into functional groups

Variable	CO ₂ (d.f. = 2)	Fert. (d.f. = 2)	Group (d.f. = 2)	CO ₂ × Fert. (d.f. = 4)	CO ₂ × Group (d.f. = 4)	Fert. × Group (d.f. = 4)	CO ₂ × Fert. × Group (d.f. = 8)
Above-ground biomass	60***	139***	973***	3.6**	20***	122***	1.5
Stem fraction	3.9*	1.4	672***	0.5	0.7	2.4	1.3
Dead-leaf fraction	1.7	23***	572***	1.7	6***	46***	1.4
Green-leaf fraction	1.7	2.3	452***	0.7	3.3*	5.3***	0.5
Reproductive fraction	0.5	11***	170***	1.8	0.7	3.9**	0.5
Dead/total leaf ratio	2.2	17***	1026***	1.2	9.5***	39***	1.7
Below-ground biomass	48***	55***		4.6**			
Total production	83***	297***		11***			
Leaf area index	13***	140***		4.2***			
Cumul. evapotranspiration	4.6*	51***		1.3			
Water use efficiency	146***	383***		14***			
Soil water content	36***	128***		1.5			
Below-/above-ground ratio	0.9	63***		1.8			
Below-ground bulbil mass	4.7*	0.2		0.8			

P* ≤ 0.05, *P* ≤ 0.01, ****P* ≤ 0.001.

Table 2. Biomass, leaf-area and water-relation responses to CO₂ enrichment at the end of the experiment (100 d after sowing). Soil water content is an average over the second 50 d of the experiment. Mean \pm SE, $n = 5$ experimental communities. Means of CO₂ treatments within the same fertilization treatment that were not indicated by a common letter were significantly different at $P \leq 0.05$ (multiple comparisons were conducted, if CO₂ \times fertilization was statistically significant, see Table 1)

Variable	Fertilization	CO ₂ concentration ($\mu\text{L L}^{-1}$)		
		280	440	600
Leaf area index ($\text{m}^2 \text{m}^{-2}$)	Control	0.53 \pm 0.02 ^b	0.63 \pm 0.05 ^{ab}	0.68 \pm 0.02 ^a
	P	0.88 \pm 0.06 ^b	1.58 \pm 0.17 ^a	1.60 \pm 0.23 ^a
	NPK	1.66 \pm 0.12 ^a	1.72 \pm 0.04 ^a	1.74 \pm 0.06 ^a
Cumulative evapotranspiration (ET) ($\text{kg H}_2\text{O m}^{-2}$)	Control	155 \pm 1	148 \pm 1	149 \pm 2
	P	160 \pm 1	159 \pm 2	157 \pm 2
	NPK	165 \pm 1	163 \pm 2	165 \pm 2
Water use efficiency ($\text{g total dry wt. kg}^{-1} \text{H}_2\text{O ET}$)	Control	1.9 \pm 0.1 ^b	2.7 \pm 0.1 ^a	2.7 \pm 0.1 ^a
	P	2.4 \pm 0.04 ^b	3.6 \pm 0.1 ^a	3.5 \pm 0.1 ^a
	NPK	3.5 \pm 0.1 ^c	4.9 \pm 0.1 ^b	5.7 \pm 0.1 ^a
Soil water content ($\text{kg H}_2\text{O m}^{-2}$)	Control	12.8 \pm 0.3	16.3 \pm 0.6	17.3 \pm 0.5
	P	9.3 \pm 0.4	11.7 \pm 0.7	13.8 \pm 0.8
	NPK	6.4 \pm 0.8	8.7 \pm 0.3	8.8 \pm 0.4
Below-ground/above-ground ratio	Control	0.77 \pm 0.04	0.91 \pm 0.06	0.81 \pm 0.05
	P	0.67 \pm 0.03	0.59 \pm 0.02	0.58 \pm 0.07
	NPK	0.45 \pm 0.02	0.48 \pm 0.02	0.46 \pm 0.01
Below-ground bulbil dry wt. (g m^{-2})	Control	33 \pm 6	39 \pm 5	42 \pm 8
	P	32 \pm 5	38 \pm 7	41 \pm 8
	NPK	20 \pm 3	44 \pm 7	41 \pm 2

fertilization, however, led to a significant increase in biomass by 17% at 600 compared to 440 $\mu\text{L L}^{-1}$ (+61% compared to 280 $\mu\text{L L}^{-1}$).

All combinations of elevated CO₂ and fertilization yielded more than would correspond to the sum of the separate responses to each factor. This was most obvious for the combination of 600 $\mu\text{L L}^{-1}$ CO₂ and high NPK, which resulted in a 220% increase over the combination of 280 $\mu\text{L L}^{-1}$ CO₂ and low-nutrient control. The separate responses summed up to an increase of only 160% (+35% from 600 vs. 280 $\mu\text{L L}^{-1}$ CO₂ in the low-nutrient control, +100% from NPK fertilization vs. control at 280 $\mu\text{L L}^{-1}$ CO₂).

Leaf area index (LAI) remained below 0.7 without additional fertilization, and increased by 70–210% when high P and high NPK were applied (Table 2). Leaf-area responses to elevated CO₂ were nutrient-dependent (CO₂-fertilization interaction; Table 1), and they were smaller than growth responses (17% at 440 $\mu\text{L L}^{-1}$ and 27% at 600 $\mu\text{L L}^{-1}$ compared to 280 $\mu\text{L L}^{-1}$ CO₂) in the low-nutrient control, but greater than the growth response in the high P-only treatment (80% at both 440 and 600 $\mu\text{L L}^{-1}$). Surprisingly, CO₂ enrichment had no effect on LAI when combined with high NPK. This means that CO₂ enrichment increased LAI primarily when nutrition stimulated an exclusive legume response (high P-only treatment; see below).

WATER USE AT COMMUNITY LEVEL

Cumulative evapotranspiration (ET) over the 100-days growing period increased up to 10% following high P

and NPK additions as a consequence of larger biomass (leaf area in the case of P; Table 2). Elevated CO₂ reduced cumulative ET by 0.5–4.5%, independently of nutrient treatment (no interaction; Table 1). This relatively small decrease in ET needs to be seen in light of the larger LAI present at elevated CO₂.

Increased ET in the high P and high NPK treatments resulted in 25 and 50% reduced mean soil water content as compared to that in the low-nutrient control (Table 2). On the other hand, reduction in ET at elevated CO₂ resulted in a less depleted soil water content, to a similar extent at all nutrient treatments (Table 1). Soil was on average 30% wetter at 440 than at 280 $\mu\text{L L}^{-1}$ and 40% wetter at 600 than at 280 $\mu\text{L L}^{-1}$. At harvest, soil water content correlated negatively with cumulative ET across all CO₂ and nutrient treatments ($r^2 = 0.98$).

Water-use efficiency (WUE) expressed as the ratio of total net biomass accumulation to cumulative ET increased in the high P and NPK treatments, because production was greater than the increment of ET (Table 2). WUE increased by 40–60% at elevated CO₂, depending on nutrient additions (Tables 1 and 2).

BIOMASS AT THE FUNCTIONAL-GROUP AND SPECIES LEVEL

Functional groups responded differently to fertilization (significant interactions at the community level; Table 1), as fertilizing grasses and legumes with high P alone resulted in a 10 and 280% increase, respectively, in above-ground dry matter, and fertilizing with NPK

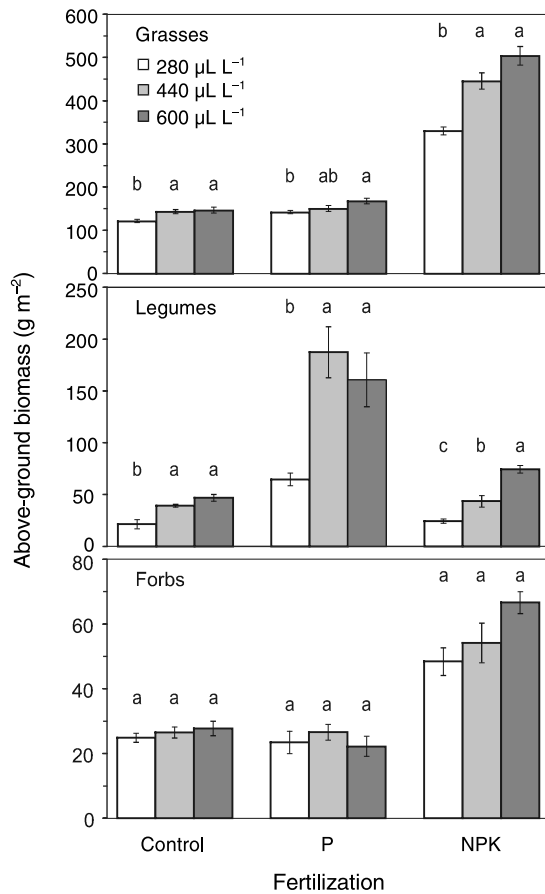


Fig. 2. Above-ground biomass in grasses, legumes and forbs at three CO₂ concentrations (280, 440, 600 μL L⁻¹) and three fertilization treatments (low-nutrient control, high P, high NPK). Mean ± SE, $n = 5$ experimental communities. Note different scales on the y-axis.

improved growth by 210 and 30% compared to the low-nutrient control (Fig. 2). Grasses appeared to suppress legumes once well supplied with nutrients (mainly N) other than P. Non-leguminous forbs were not affected by fertilization of P alone, but responded strongly to high NPK addition (+110%). All grass species were similarly affected by fertilization (no species–fertilization interaction), whereas legume species responded to fertilization in the same direction, but to different extents (species–fertilization interaction; Table 3, Fig. 3). Forb responses differed greatly among species, with some forbs, such as *Carrichtera annua*, gaining substantially from high NPK addition, while others, such as *Scabiosa porphyro-neura*, were unresponsive.

The CO₂ response of grasses was nutrient-dependent, with a 5–20% increase at elevated CO₂ in the low-nutrient control and the high P treatment, and 35–50% increase in the high NPK treatment (Table 3, Fig. 2). Legumes were more responsive to CO₂ enrichment than the other functional groups, and the extent of this response was again nutrient-dependent (increase in above-ground biomass by 80–120% in the low-nutrient control and the high NPK treatment, and by 150–190% following high P addition alone). The CO₂ response of forbs was not affected by nutrient treatments.

All grass species were stimulated similarly by elevated CO₂ (Table 3, Fig. 3). CO₂ enrichment significantly stimulated the growth of all legume species, yet the extent of the increase in biomass above 280 μL L⁻¹ CO₂ varied greatly among them (Table 3, Fig. 3). For example, the relative biomass increase at 600 vs. 280 μL L⁻¹ CO₂ in the low-nutrient control amounted to 40% in *Hymenocarpus circinnatus* and more than

Table 3. *F*-values and probability levels from three-way ANOVA of above-ground variables at the functional-group level. Main factors were CO₂, fertilization and species

Variable	CO ₂	Fert.	Species	CO ₂ × Fert.	CO ₂ × Species	Fert. × Species	CO ₂ × Fert. × Species
Grasses	(d.f. = 2)	(d.f. = 2)	(d.f. = 4)	(d.f. = 4)	(d.f. = 8)	(d.f. = 8)	(d.f. = 16)
Above-ground biomass	30***	841***	308***	4.8***	0.8	2.5	0.9
Stem fraction	17***	3.6*	87***	3.6**	2.3*	6.3***	1.2
Dead-leaf fraction	15***	181***	303***	1.3	3.3**	0.7	0.4
Green-leaf fraction	121***	23***	207***	8.6***	6.7***	8.1***	1.0
Reproductive fraction	1.2	25***	605***	0.8	0.9	4.8***	1.6
Dead/total leaf ratio	57***	132***	248***	1.2	3.3**	2.2*	0.8
Legumes	(d.f. = 2)	(d.f. = 2)	(d.f. = 3)	(d.f. = 4)	(d.f. = 6)	(d.f. = 6)	(d.f. = 12)
Above-ground biomass	105***	181***	417***	3.6**	2.4*	4.8***	1.3
Stem fraction	0.4	2.2	96***	1.2	3.0**	1.0	1.6
Dead-leaf fraction	3.6*	27***	110***	0.3	3.0**	12.0***	0.7
Green-leaf fraction	2.1	0.8	71***	1.3	3.0**	5.7***	1.1
Reproductive fraction	13***	12***	112***	3.1*	8.2***	2.3*	2.0*
Dead/total leaf ratio	3.6*	27***	112***	0.3	3.2**	12***	0.6
Non-leguminous forbs	(d.f. = 2)	(d.f. = 2)	(d.f. = 5)	(d.f. = 4)	(d.f. = 10)	(d.f. = 10)	(d.f. = 20)
Above-ground biomass	3.3*	33.0***	103***	1.6	2.4*	2.7**	0.9
Stem fraction	4.2*	4.3*	1137***	0.4	1.0	3.4***	1.1
Dead-leaf fraction	0.5	1.7	192***	0.9	1.9	1.9*	0.7
Green-leaf fraction	4.2*	0.1	750***	0.1	3.4***	2.4**	1.0
Reproductive fraction	1.1	2.1	368***	0.4	1.6	1.1	1.0
Dead/total leaf ratio	0.1	2.2	130***	0.7	1.7	1.9*	1.3

$P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

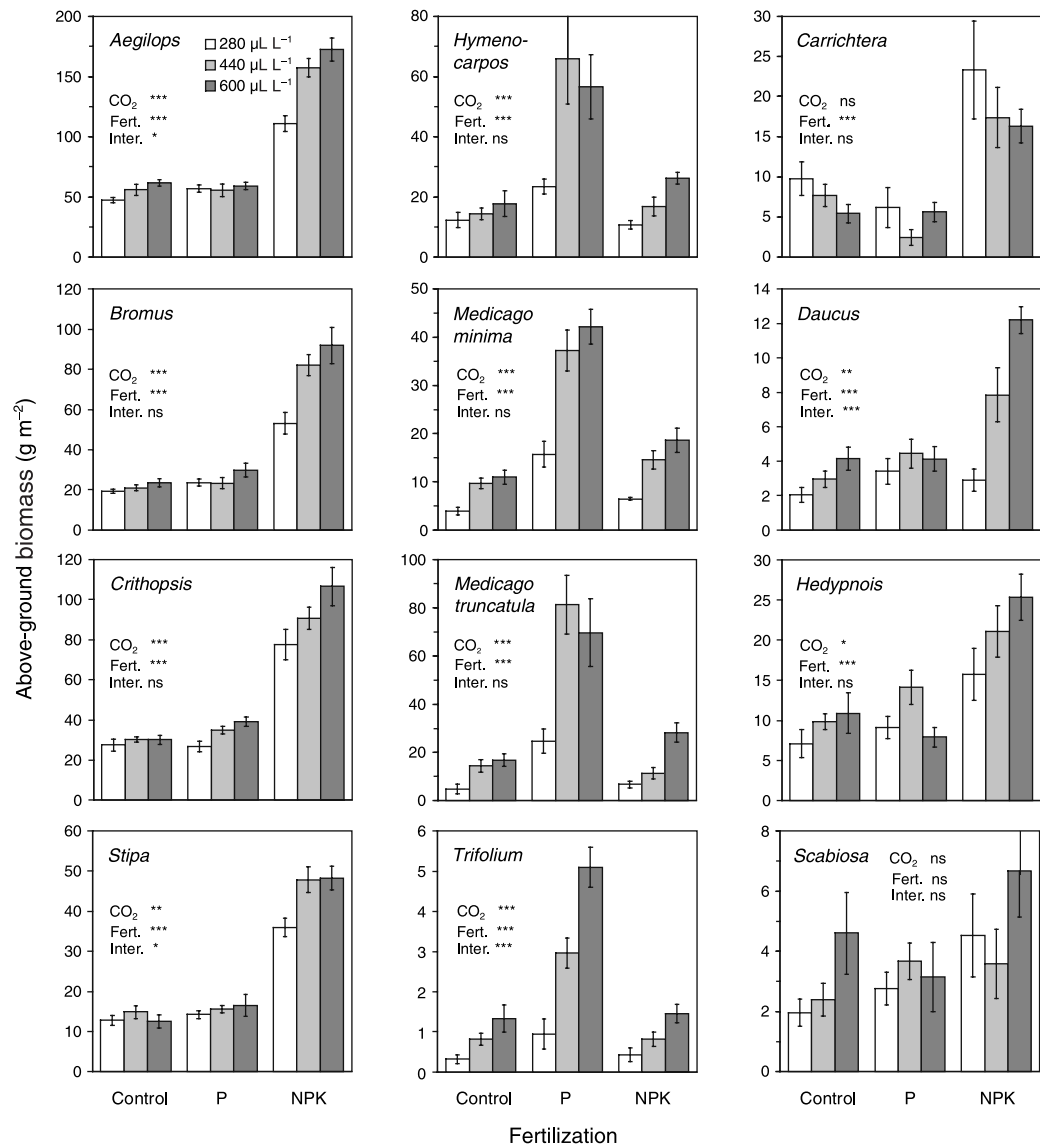


Fig. 3. Species above-ground biomass at three CO₂ concentrations (280, 440, 600 $\mu\text{L L}^{-1}$) and three fertilization treatments (low-nutrient control, high P, high NPK). The four species on the left are grasses, the ones in the middle are legumes, and the ones on the right are non-leguminous forbs. Mean \pm SE, $n = 5$ experimental communities. Probabilities of two-way ANOVAs are indicated for the two factors CO₂ and fertilization and for the interaction between them (* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; ns, non-significant). Note different scales on the y-axis.

300% in *Trifolium campestre*. Forb species responded to different extents and sometimes in different directions (Table 3, Fig. 3). Significant production gains at elevated CO₂ were found only for *Hedypnois cretica* and *Daucus subsessilis* (for the latter species only in the high NPK treatment), while *Carrichtera annua* tended to lose about 30% above-ground biomass in all nutrient treatments. Many of the differences between 440 and 600 $\mu\text{L L}^{-1}$ CO₂ were non-significant, hence significant CO₂ effects resulted largely from the pre-industrial reference CO₂ concentration.

PARTITIONING

NPK fertilization considerably decreased dead-leaf fraction in grasses on the functional-group level (Table 3, Fig. 4) and in all species (data not shown).

CO₂ enrichment resulted in a general but relatively small increase in stem fraction at the community level (Table 1, Fig. 4). More significantly, CO₂ enrichment reduced green-leaf fraction in grasses by 15–40% and increased dead-leaf fraction by 7–15% in all nutrient treatments (Table 3, Fig. 4). Consequently, the ratio of dead to total leaf mass increased, suggesting earlier leaf senescence. It appears that the lack of a CO₂ effect on community LAI in the high NPK treatment (despite a large biomass response; Table 2, Fig. 1) reflected leaf senescence in grasses, as these communities had 80% grass dominance. Reproductive fraction was less than 20% in most grasses, but was increased at elevated CO₂ in *Bromus fasciculatus*, the first grass species to flower (Table 4, Fig. 4). Changes in partitioning in legumes and forbs were small and affected only a few species. Variation in partitioning was not a simple

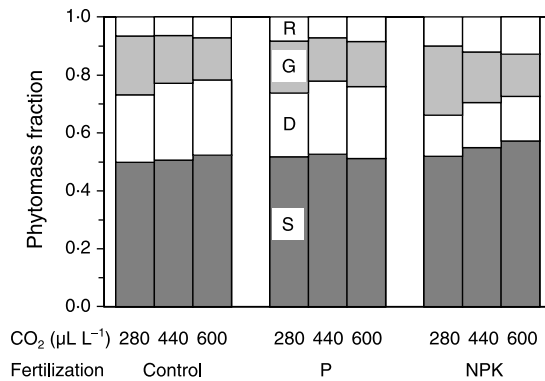


Fig. 4. Partitioning of above-ground phytomass in grasses at three CO₂ concentrations (280, 440, 600 μL L⁻¹) and three fertilization treatments (low-nutrient control, high P, high NPK). Fractions of stem (S), dead leaves (D), green leaves (G) and reproductive structures (R) are the proportion of the plant-part mass to above-ground phytomass. Mean, *n* = 5 experimental communities.

correlate of plant size (data not shown), and thus might be a true response to CO₂ enrichment, although some contribution by ontogenetic drift cannot be ruled out (McConaughay & Coleman 1999).

The ratio of below-ground (mainly roots) to above-ground biomass was decreased by high P-only addition, and was further reduced by high NPK addition (Tables 1 and 2). In contrast, CO₂ enrichment had no significant effect on below-ground/above-ground ratio irrespective of nutrient availability. The size of special below-ground storage organs (bulbils) was not affected

Table 4. Above-ground partitioning of biomass of the main species. A positive, statistically significant CO₂ effect (two-way ANOVA) was indicated by +, a negative effect by -, with one, two and three + or - signs indicating probability levels of ≤ 0.05, ≤ 0.01 and ≤ 0.001. x stands for a significant CO₂-fertilization interaction, x(+) and x(-) for an interaction with a significant positive and negative CO₂ effect, respectively, at least in one fertilization treatment

Species	Stems	Dead leaves	Green leaves	Reprod. parts	Dead/total leaf ratio
Grasses					
<i>Aegilops kotschy</i>	x		x(-)		++
<i>Brachypodium distachyon</i>	x(+)	+	---		+++
<i>Bromus fasciculatus</i>	+		x(-)	+++	+++
<i>Crithopsis delileana</i>			x(-)		
<i>Stipa capensis</i>	x(+)	+++	---		+++
Legumes					
<i>Hymenocarpus circinnatus</i>	+	--			---
<i>Medicago minima</i>				+	
<i>Medicago truncatula</i>	x(+)				
<i>Trifolium campestre</i>	-	n.a.	-	x(+)	n.a.
Forbs					
<i>Carrichtera annua</i>			---		x
<i>Daucus subsessilis</i>	+++		---		
<i>Hedypnois cretica</i>	+				
<i>Plantago cretica</i>					
<i>Scabiosa porphyreoneura</i>		-			

n.a., not applicable; all leaves in *Trifolium* were green.

by fertilization, but was increased by CO₂ enrichment (Tables 1 and 2). Bulbil dry-matter was more than doubled when CO₂ enrichment was combined with high NPK addition. This stimulation was mainly caused by *Poa bulbosa*, which contributed 60% to the total mass of such storage organs (data not shown).

FLOWERING

Time of anthesis was similar among CO₂ treatments in all species, except for *Bromus*, which showed earlier flowering by one week at elevated compared to pre-industrial CO₂ (data not shown; *Bromus* production was 16% of that of all grass species combined). The fraction of flowering individuals averaged over the whole flowering period was increased at elevated CO₂ for the legume species, which reflected their overall growth (size) response ($P = 0.014$, < 0.001 , < 0.001 and < 0.001 for CO₂ in two-way ANOVA for *Hymenocarpus circinnatus*, *Medicago minima*, *M. truncatula* and *Trifolium campestre*). No CO₂-fertilization interaction on flowering was found in these species.

Discussion

NON-LINEARITY OF CO₂ EFFECTS

This study of semi-arid model communities revealed a notable non-linearity of CO₂-effects, with large differences in plant responses between pre-industrial (280 μL L⁻¹) and close-to-current CO₂ concentration (440 μL L⁻¹), and small differences between 440 μL L⁻¹ and distant future CO₂ concentration (600 μL L⁻¹). However, CO₂ effects were not saturated at 440 μL L⁻¹ at high nutrient (NPK) availability. Similar CO₂ saturation of biomass production and soil carbon storage at 400–450 μL L⁻¹ and natural nutrient supply was reported from studies with different annual and perennial communities (Gill *et al.* 2002; Grünzweig & Körner 2001b; Hättenschwiler & Körner 1996; Zangerl & Bazzaz 1984). Hence, the results of the current study reflected largely past and perhaps current responses in the real world. This is a significant aspect in the interpretation of our data because most other studies used 350–370 μL L⁻¹ as low CO₂ and 560–720 μL L⁻¹ as high CO₂ (at ambient nutrient availability), a range of CO₂ concentrations which is above the most responsive range covered in our study.

CO-LIMITATION OF CO₂ AND NUTRIENTS AT THE COMMUNITY LEVEL

Atmospheric CO₂ enrichment reduced water consumption and increased community biomass, WUE and soil water content in these semi-arid communities. These results were expected as water savings under CO₂ enrichment should ease drought. Part of this effect might reflect improved nutrient availability because nutrient mobility and diffusion to root surfaces

increase in wetter soil (Chapin 1991). In Californian grassland, N uptake by plants increased at elevated CO₂ due to this effect (Hu *et al.* 2001; Hungate *et al.* 1997). Nevertheless, nutrient addition increased growth responses to elevated CO₂, suggesting a limitation of CO₂ effects by low nutrient availability. Increased nutrient supply appears to alleviate constraint on use of higher CO₂ concentrations by plants under dry conditions. In addition to the effect of nutrient supply itself, water-mediated CO₂ effects could be increased as a consequence of the drier soil in fertilized as compared to control communities. Irrigation experiments (at natural nutrient supply) showed greater relative biomass responses to elevated CO₂ at low than at high water availability (Körner 2000; Poorter & Pérez-Soba 2001). Consequently, low soil water content following fertilization could have increased CO₂ effects *per se*, i.e. irrespective of increased availability of nutrients. Interestingly, interactive effects of CO₂ and fertilization were observed only in the primarily growth-related variables (biomass, LAI, WUE), but not in the water-related variables (ET, soil water content). Co-limitation by CO₂ and nutrients was observed in tall-grass prairie during a dry year (Owensby *et al.* 1994), and in individually grown plants in low water treatments (Arp *et al.* 1998). These interactive responses of growth to CO₂ enrichment and nutrient availability confirm our hypothesis of co-limitation of biomass accumulation by these factors in droughted communities, and reflect the known water-nutrient co-limitation in drylands (Hooper & Johnson 1999). Hence, the CO₂ effect observed here was largely a water effect, similar to that shown for temperate grassland (Volk *et al.* 2000).

FUNCTIONAL GROUPS DRIVE DIFFERENTIAL COMMUNITY RESPONSES TO P AND N

The overall community response to CO₂ enrichment and fertilization depended on the differential responses of grasses and legumes, and on the nutrient in question, i.e. P or N [addition of N with K is likely to be a N effect because K limitation is rare (Aerts & Chapin 2000)]. At low nutrient supply (control) and in the high P treatment, increased community biomass at elevated CO₂ was caused mainly by the strong response of legumes. It seems that even the low-nutrient control supplied enough P to facilitate a relatively strong legume response to CO₂ enrichment, a response which was enhanced at high P fertilization. Legume species at elevated CO₂ most likely profited from increased N₂-fixation rates even under dry conditions, as CO₂ enrichment increases drought tolerance of legumes (Serraj *et al.* 1998). It was hypothesized that P availability to plants could also be increased at elevated CO₂ (Gifford, Lutze & Barrett 1996), thus further stimulating legume growth, but there is little evidence for enhanced P uptake by legumes at natural P supply (Gavito, Schweiger & Jakobsen 2003; Staddon, Graves & Fitter 1999). Phosphorus fertilization increased

community production response to elevated CO₂ in calcareous grassland (Stöcklin *et al.* 1998), an effect that depended on the presence of legumes (Stöcklin & Körner 1999). Similar to these findings, P fertilization (in combination with K) had no effect on legume production at ambient CO₂, but doubled production at elevated CO₂ in Californian grassland (Joel *et al.* 2001).

High NPK supply mainly enhanced the biomass response to CO₂ in grasses, with N supposedly being the essential nutrient for this effect, as shown in different grasslands (Daepf *et al.* 2000; Niklaus *et al.* 1998a; Owensby *et al.* 1994). Alternatively, it could be the consequence of N plus P addition, as P limitation reduced biomass production at elevated CO₂ in a Californian grassland fertilized with N (Shaw *et al.* 2002). Legume growth was suppressed at high NPK probably because of competition from grasses. This also suggests that a long-term enhancement of legumes by CO₂ enrichment under natural conditions is unlikely, as stimulation of legumes would ultimately enhance N-supply to grasses (Grünzweig & Körner 2001b; Vitousek & Field 1999).

The CO₂-induced higher dead-leaf and lower green-leaf fraction in grasses indicated earlier leaf senescence and suggested an advancement of plant development. This is in line with earlier flowering and increased reproductive fraction in *Bromus fasciculatus* (the only grass species that flowered prior to harvest). Plants tend to show a functional equilibrium response in the sense that they reduce the size or amount of structures that produce a surplus of resources. Green-leaf fraction is reduced at elevated CO₂ because of excessive production of non-structural carbohydrates (Grünzweig & Körner 2001a). More rapid plant development at elevated CO₂ was obvious only in grasses in our study, and was also observed in wheat (Kimball *et al.* 1995), barley (Fangmeier *et al.* 2000) and several Mediterranean grasses (Navas *et al.* 1997). Enhanced development and earlier flowering is common in dry ecotypes of annuals (Aronson *et al.* 1992; Del Pozo *et al.* 2000; Ehrman & Cocks 1996), and can be an advantage in seasonally dry grasslands because more time and water can be used for grain filling. Improved water supply increased grain mass in wheat because of a longer grain-filling period (Li *et al.* 2000). This is of particular importance in arid and semi-arid climates where large variation in precipitation within and among years can halt rainfall and result in late-season drought (Noy-Meir 1973).

SPECIES-SPECIFIC RESPONSES

Legume species differed considerably in their biomass response to CO₂ enrichment and in the effect that nutrients had on this. Two mechanisms might cause different biomass responses of species to CO₂ enrichment: (1) Species differ in their response potential. For example, *Hymenocarpus* was the least responsive

legume species under all nutrient conditions. (2) Species differ in their interaction with competitors concerning resource availability. Depending on species-specific sensitivity and competitiveness, legume species are affected to different degrees by competition for water, nutrients and light imposed by grasses.

Notably, the biomass response to elevated CO₂ in grasses was small and not species-specific, similar to the consistent zero effects on vegetative growth of grasses in larger unfertilized communities (Grünzweig & Körner 2001a). Among non-leguminous forbs, which also showed little responsiveness to CO₂, *Carriechtera annua* tended to lose terrain at elevated CO₂, in line with earlier results for several members of the Brassicaceae (Grünzweig & Körner 2001a).

Earlier work showed large variation in reproductive output, seed quality and germination success among species in the CO₂ treatment (Grünzweig & Körner 2000, 2001a), thus making future changes in species composition and biodiversity likely. These changes will depend on the CO₂ concentration, the nutrient status of the ecosystem and the species present.

Conclusions

Plant responses to CO₂ enrichment in this semi-arid community were mediated largely by water effects and were limited by nutrient availability. Therefore, the more desertification will deteriorate the nutrient status of ecosystems, the less will primary production respond to rising atmospheric CO₂. The differential CO₂ response of species, particularly the interactive one with nutrients, suggests consequences for species composition and abundance. Considering the entire postglacial period of past and future CO₂ increase, many of the responses reported here might be close to their potential realization in the wild as we rapidly approach a CO₂ concentration of 420–450 µL L⁻¹. At these concentrations most of the responses explored in this study appeared to be saturated unless significant amounts of N or NPK were added.

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