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Elevated CO₂ effects on semi-arid grassland plants in relation to water availability and competition

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Summary

1. It has been suggested that much of the elevated CO₂ effect on plant productivity and N cycling in semi-arid grasslands is related to a CO₂-induced increase in soil moisture, but the relative importance of moisture-mediated and direct effects of CO₂ remain unclear.

2. We grew five grassland species common to the semi-arid grasslands of northern Colorado, USA, as monocultures and as mixtures of all five species in pots. We examined the effects of atmospheric CO₂ concentration (ambient vs. 780 p.p.m.) and soil moisture (15 vs. 20% m/m) on plant biomass and plant N uptake. Our objective was to separate CO₂ effects not related to water from water-mediated CO₂ effects by frequently watering the pots, thereby eliminating most of the elevated CO₂ effects on soil moisture, and including a water treatment similar in magnitude to the water-savings effect of CO₂.

3. Biomass of the C₃ grasses *Hesperostipa comata* and *Pascopyrum smithii* increased under elevated CO₂, biomass of the C₄ grass *Bouteloua gracilis* increased with increased soil moisture, while biomass of the forbs *Artemisia frigida* and *Linaria dalmatica* had no or mixed responses. Increased plant N uptake contributed to the increase in plant biomass with increased soil moisture while the increase in plant biomass with CO₂ enrichment was mostly a result of increased N use efficiency (NUE). Species-specific responses to elevated CO₂ and increased soil moisture differed between monocultures and mixtures. Both under elevated CO₂ and with increased soil moisture, certain species gained N in mixtures at the expense of species that lost N, but elevated CO₂ led to a different set of winners and losers than did increased water.

4. Elevated CO₂ can directly increase plant productivity of semi-arid grasslands through increased NUE, while a CO₂-induced increase in soil moisture stimulating net N mineralization could further enhance plant productivity through increased N uptake. Our results further indicate that the largest positive and negative effects of elevated CO₂ and increased soil moisture on plant productivity occur with interspecific competition. Responses of this grassland community to elevated CO₂ and water may be both contingent upon and accentuated by competition.

Key-words: elevated atmospheric CO₂, forbs, C₃ and C₄ grasses, greenhouse experiment, inter- and intraspecific competition, nitrogen dynamics, semi-arid grassland, water availability

Introduction

Both empirical and modelling studies indicate that semi-arid grasslands show some of the largest increases in plant productivity in response to atmospheric CO₂ enrichment (Melillo *et al.* 1993; Morgan *et al.* 2004b). Not all plant species respond in the same way to elevated CO₂. For instance, the C₃ grass *Hesperostipa comata* and the

sub-shrub *Artemisia frigida* showed strong increases in above-ground biomass with elevated CO₂ in a 5-year open-top-chamber experiment at the shortgrass steppe in Colorado, while above-ground biomass of the C₃ grass *Pascopyrum smithii* and the C₄ grass *Bouteloua gracilis* was not affected (Morgan *et al.* 2004a, 2007). Differences in photosynthetic pathways between C₃ and C₄ plants or the ability to fix N are important species traits that affect how plant species respond to elevated CO₂ (Johnson, Polley & Mayeux 1993; Lüscher & Nösberger 1997; Reich *et al.*

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2001b). Because soil resources such as water and nitrogen (N) are affected by elevated CO₂, the ability to compete for these resources is another factor that could cause variation in plant species growth responses to elevated CO₂ (Bernston, Rajakaruna & Bazzaz 1998; Derner *et al.* 2003; Maestre, Bradford & Reynolds 2005). Soil moisture in particular is an important resource in semi-arid grasslands that could be critical for species-specific responses to elevated CO₂. Indeed, it was suggested that the increased growth of certain semi-arid grassland species under elevated CO₂ was a result of improved soil moisture conditions (because of decreased stomatal conductance) more than direct effects of elevated CO₂ on photosynthesis (Lecain *et al.* 2003; Morgan *et al.* 2007).

Increased plant growth under elevated CO₂ coincides with increased plant N uptake as well as increased N use efficiency (NUE, Soussana *et al.* 2005; Norby & Iversen 2006; Finzi *et al.* 2007). The extent to which increased plant growth under elevated CO₂ involves changes in NUE or plant N uptake depends on how much N is available in the soil for plant growth, which itself is influenced by CO₂. Elevated CO₂ could reduce soil N availability because of increased microbial immobilization (Díaz *et al.* 1993; Gill *et al.* 2002). Initial increases in plant N uptake could reduce soil N availability in the long-term because of increased storage of N in long-lived plant biomass and soil organic matter (Luo *et al.* 2004; Reich, Hungate & Luo 2006). In systems where soil N availability is reduced by elevated CO₂, increases in plant growth under elevated CO₂ may therefore only be possible when plants increase their NUE. On the other hand, in dry ecosystems elevated CO₂ can significantly improve soil moisture conditions, thereby increasing N mineralization and plant N uptake (Hungate *et al.* 1997; Dijkstra *et al.* 2008). Therefore, increased plant N uptake under elevated CO₂ may be more important for increased plant growth in dry than in wet ecosystems.

Here we studied the effects of atmospheric CO₂ (ambient vs. 780 p.p.m.) and soil moisture (15 vs. 20% m/m) on plant growth and plant N uptake of five species common to the semi-arid grasslands in northern Colorado, in an environmentally controlled greenhouse experiment. We tried to keep soil moisture levels constant throughout the experiment to separate soil moisture effects from direct effects of elevated CO₂ not related to soil moisture, such as effects on photosynthesis and rhizosphere processes affecting nutrient cycling (Dijkstra & Cheng 2008). Many studies using greenhouse and growth chambers to test elevated CO₂ effects on plant growth and plant N uptake have been done by growing plants as monocultures or in isolation as single plants (e.g., Morgan *et al.* 1994, 1998; Dijkstra & Cheng 2008). However, plant growth responses to elevated CO₂ grown in isolation or as monocultures may be very different from plant growth responses when grown in mixtures (Navas 1998; Poorter & Navas 2003). Large variation in species-specific plant growth responses to elevated CO₂ could change competitive interactions within plant communities (Bazzaz & McConnaughey 1992; Körner & Bazzaz 1996). Indeed, above-ground plant

biomass of the C₃ grass *P. smithii* and the C₄ grass *B. gracilis* significantly increased under elevated CO₂ when grown as monocultures in growth chambers (Morgan *et al.* 1994, 1998; Hunt *et al.* 1996), whereas the same species showed no or very little response to elevated CO₂ when growing in a natural plant community (Morgan *et al.* 2004a). To elucidate the role of inter- and intraspecific competition for resources, we compared CO₂ and soil moisture treatment effects on plant species grown as monocultures with their effects on the same plant species grown in mixtures.

We asked the following questions.

1. Is the stimulatory effect of elevated CO₂ on the growth of five semi-arid grassland species caused by improved water conditions, or also by other CO₂ effects?
2. What are the roles of increased plant N uptake and increased NUE in the stimulatory effects of elevated CO₂ and increased soil moisture?
3. Do plant growth and N uptake responses to elevated CO₂ and increased water availability differ between inter- and intraspecific competitive interactions among plants?

Materials and methods

The soil we used for our experiment came from a semi-arid grassland at the USDA-ARS Central Plains Experimental Range (CPER), northeastern Colorado (lat. 40°50', long. 104°47'). The soil is a sandy loam of the Ascalon series (Aridic Argiustolls). The top 20-cm of the soil was scraped from the surface with a backhoe and dumped on a large metal sieve (mesh size 4 mm) to remove large plant parts and to homogenize the soil. The soil had 0.95% C and 0.09% N, and a pH of 6.6. We filled 120 polyvinyl chloride (PVC) pots (diam. 20 cm, height 40 cm) with sieved soil (c. 14 kg of air-dry soil per pot). The pots were capped at the bottom and no leaching occurred during the experiment. The initial inorganic N content (NH₄⁺ + NO₃⁻) of the soil was 23 mg N kg⁻¹ soil or ~0.3 g N pot⁻¹. The pots were then watered to field capacity or 30% m/m. We transplanted seedlings of the perennial grasses *Bouteloua gracilis* (BOGR, C₄ grass), *Hesperostipa comata* (HECO, C₃ grass), and *Pascopyrum smithii* (PASM, C₃ grass), the sub-shrub *Artemisia frigida* (ARFR), and the invasive forb *Linaria dalmatica* (LIDA) as monocultures (five seedlings per pot, 20 pots per species). In the other 20 pots we transplanted all five species as mixtures (one seedling of each species per pot).

We grew the plants in two greenhouses located at the USDA-ARS Crops Research Laboratory, Fort Collins, CO, USA. Half of all the pots (10 replicates of each monoculture/mixture or species composition) were placed in one greenhouse that was kept under ambient atmospheric CO₂ (400 ± 40 p.p.m., average ± standard deviation), and the other half in a greenhouse kept under elevated CO₂ (780 ± 50 p.p.m.). The CO₂ concentration was continuously monitored and the CO₂ supply was computer-controlled (Argus Control Systems Ltd, White Rock, BC¹). The added CO₂ entered the greenhouse through a ventilation system ensuring uniform distribution of the CO₂ concentration inside the greenhouse. Air temperature in both greenhouses was kept between 27 and 29 °C during the day and

¹Trade and company names are given for the reader's benefit and do not imply endorsement or preferential treatment of any product by the USDA.

between 16 and 18 °C during the night. Temperature was regulated by computer-controlled air conditioners and heaters (York International, York, PA). Both greenhouses were equipped with 600 W lights (P.L. Light Systems, Beamsville, ON) that were on during the day for 12 h. During the day the light intensity in each greenhouse was $\sim 200 \text{ W m}^{-2}$. The relative humidity in each greenhouse was $24 \pm 5\%$ during the day and $30 \pm 5\%$ during the night. To reduce greenhouse effects not related to the CO_2 treatment, we swapped the pots once a week between the two greenhouses during our experiment (12 weeks, Heijmans *et al.* 2002; Goverde & Erhardt 2003). The CO_2 treatment was swapped concurrently so that the same plants received the same CO_2 treatment throughout the experiment.

During the first week of the experiment the pots were watered frequently to maintain soil water content near 30% to enhance seedling growth. After that, watering was discontinued until half of the pots (five replicates for each monoculture/mixture and each CO_2 treatment) dried down to 15% gravimetric soil moisture (low water) and the other half to 20% gravimetric soil moisture (high water). The 15 and 20% soil moisture contents correspond to 50 and 67% of field capacity respectively. The relative difference between the two water treatments is 33%. At CPER, elevated CO_2 (720 p.p.m.) increased soil moisture on average from 11.4 to 12.9% (increase of 14% compared with ambient CO_2) in the upper metre, while the relative difference between ambient and elevated CO_2 was sometimes as much as 45% (Lecain *et al.* 2003). Thus, the magnitude of our water treatment was not unrealistic compared with the water savings effect of elevated CO_2 under field conditions. We maintained the low and high soil water levels by watering the pots three times per week with DI water. Once a week, the pots were weighed and watered to their target soil moisture levels, while during the other two times of the week, the amount of water that was added was estimated based on previous water loss from each pot. Pots inside each greenhouse were placed in five blocks of twelve pots (one replicate of each of the six species composition and two water treatments).

With our frequent watering we tried to maintain constant soil moisture levels during the experiment, thereby eliminating potential CO_2 effects on soil water content. However, between watering periods, pots under ambient CO_2 dried out faster than pots under elevated CO_2 (Fig. 1). On average, soil moisture of the low water treatment was 12.6% and 13.1% under ambient and elevated CO_2

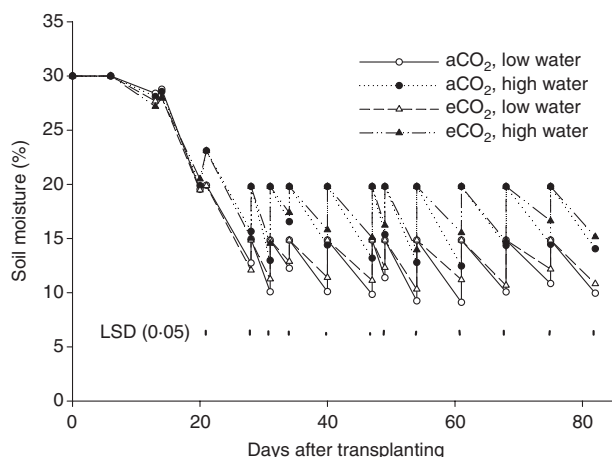


Fig. 1. Average gravimetric soil moisture content during the experiment for each of the CO_2 and water treatments (averaged across species identity and species number, a CO_2 = ambient CO_2 , e CO_2 = elevated CO_2).

respectively, and soil moisture of the high water treatment was 16.9% and 17.7% under ambient and elevated CO_2 respectively (averaged for 25–85 days after transplanting).

We harvested all pots 85 days after transplanting. Plants were separated into shoots and roots, dried (65 °C) and weighed. The plant material was then ground and analysed for N on a mass spectrometer (20–20 Stable Isotope Analyzer, Europa Scientific, Cheshire, UK). We were unable to separate root biomass in the mixtures by species and the data reported are for the combined roots from all species. The soil in each pot was thoroughly mixed and a 25 g subsample was extracted with 60 ml 2 M KCl, filtered (using pre-cleaned Whatman No. 1 filter paper) and frozen until analyses for NH_4^+ and NO_3^- on a flow injection analyzer (QuickChem FIA+, Lachat Instruments, Milwaukee, WI). We assumed that the difference between the final soil inorganic N amount (NH_4^+ and NO_3^-) and the initial amount at the start of the experiment was taken up by the plant. Note that this is a potential amount, and that the amount of initially available N that was actually taken up was somewhat lower because some of the initial inorganic N was lost as gaseous N during the experiment (Dijkstra *et al.* 2010). We then compared this amount to the total amount of N in plant biomass to deduce plant N supply through decomposition during the experiment.

We calculated the absolute change in shoot biomass in response to elevated CO_2 and high water for each species grown in monoculture and in mixture. Because there was only one plant for each species in the mixtures, but five plants in each of the monoculture pots, we multiplied the absolute responses in the mixtures by five for plant density-independent comparison with the monocultures. We also calculated the Shoot Biomass Enhancement Ratio (BER) and shoot N uptake Enhancement Ratio (NER, Berntson, Rajakaruna & Bazzaz 1998; Poorter & Navas 2003) to elevated CO_2 and high water for each species in monoculture and mixture. BER_{CO_2} was calculated as the ratio of the average shoot biomass of the elevated CO_2 treatment divided by the average shoot biomass of the ambient CO_2 treatment, while $\text{BER}_{\text{water}}$ was calculated as the ratio of the average shoot biomass of the high water treatment divided by the average shoot biomass of the low water treatment. NER_{CO_2} and $\text{NER}_{\text{water}}$ were calculated similarly, but using shoot N content (in g pot^{-1}) rather than shoot biomass. BER and NER values greater than one indicate positive effects of elevated CO_2 or high water on shoot biomass and shoot N content (increased N uptake). Further, if BER and NER are the same, then the positive effect of elevated CO_2 or high water on shoot biomass is accompanied by increased N uptake alone, but not by increased N Use Efficiency (NUE, shoot biomass/shoot N content). If BER is higher than NER, then the positive effect of elevated CO_2 or high water on shoot biomass involves increased NUE. We further defined the N Use Efficiency Enhancement Ratio (NUE-ER) as the ratio of the NUE of the elevated CO_2 or high water treatments divided by the NUE of the ambient CO_2 or low water treatments ($\text{NUE-ER}_{\text{CO}_2}$ and $\text{NUE-ER}_{\text{water}}$ respectively).

For the monocultures we used ANOVA to test for main effects of CO_2 (ambient and elevated CO_2), water (low and high water), and species (ARFR, LIDA, BOGR, HECO, and PASM), as well as their interactions, on shoot, root, and total biomass and their N contents. For each species we used the Tukey's HSD test to compare the means of the four CO_2 by water treatment combinations. We did the same analyses with the mixtures, but then only for shoot biomass and N content (we were unable to separate root biomass by species in the mixtures). For root and total biomass and their N contents in the mixtures we left the factor species out of the ANOVA, and only tested for CO_2 , water, and $\text{CO}_2 \times$ water effects. Using all pots, we tested for main effects of CO_2 , water, and species number (monocultures and

mixtures), and their interactions on shoot, root, and total biomass and their N contents. For these last analyses we first averaged the five monoculture species in each block to create equal sample sizes compared with the mixtures. We also used Tukey's HSD tests to compare the means of the four CO₂ by water treatment combinations for monocultures and mixtures separately. Finally we used ANOVA to test for main effects of CO₂, water, species, species number, and all their interactions, on shoot biomass, shoot N content, and shoot NUE. In all ANOVAs we included block as a random effect. We log-transformed data when necessary to reduce heteroscedasticity. All statistical analyses were done with JMP (version 4.0.4; SAS Institute, Cary, North Carolina, USA).

Results

Total plant biomass in the monocultures increased under elevated CO₂ (by 6.8% averaged across the water treatment) and with high water (by 10.4% averaged across the CO₂ treatment, Table 1, Fig. 2a and b). In the mixtures, elevated CO₂ and high water effects on total plant biomass were similar in magnitude (average increase of 9.8% under elevated CO₂ and 6.1% with high water), but less significant for the CO₂ and not significant for the water treatment (Table 1). Individual species in monoculture showed different responses to elevated CO₂ and high water. Total biomass of the C₃ grasses HECO and PASM increased with elevated CO₂, although only significantly so in combination with high water (Fig. 2a). Total biomass of the C₄ grass BOGR was not affected by elevated CO₂, but increased with high water. The sub-shrub ARFR did not respond to elevated CO₂ or water, while the invasive

forb LIDA responded positively to elevated CO₂ with low water but negatively with high water.

In contrast to total plant biomass, total plant N (in g pot⁻¹) was not affected by elevated CO₂ in the monocultures (Table 1, Fig. 2c). On the other hand, total plant N, averaged across all species and CO₂ levels, increased significantly with high water (on average by 10.4%, Table 1). Although similar in magnitude (average increase of 9.3%), the high water treatment effect on total plant N was not significant in the mixtures (Table 1, Fig. 2d). Within the monocultures, total species-specific plant N responses to elevated CO₂ and high water were similar to species-specific plant biomass responses, with the largest increases in total plant N for HECO, only an increase with high water for BOGR, and no elevated CO₂ or high water effects for ARFR and LIDA. Unlike total plant biomass, total plant N of PASM did not respond to elevated CO₂ or high water (Fig. 2c). Soil inorganic N was depleted from 0.32 g N pot⁻¹ at the beginning of the experiment to very low concentrations in all treatments at the end of the experiment (on average to 0.017 g N pot⁻¹, Fig. 2c and d). As a result, changes in soil inorganic N during the time frame of the experiment were very similar among treatments. Thus, treatment effects on total plant N were not due to differences in plant uptake of soil inorganic N that was available at the start of the experiment, but most likely because of differences in N supply (i.e., net N mineralization, and possibly organic N uptake).

In the monocultures, effects of elevated CO₂ and high water were slightly larger for shoot biomass than for total

Table 1. Summary of ANOVA results (*P*-values) for the effects of CO₂ (ambient and elevated), water (low and high), and species identity (ARFR, LIDA, BOGR, HECO, and PASM) in the monocultures only and in the mixtures only, and for the effects of CO₂, water, and species number (monoculture and mixture) in all pots (ns = not significant, *P* > 0.1)

Effect	Shoot biomass	Root biomass	Total biomass	Shoot N	Root N	Total N
Monocultures						
CO ₂	0.0003	ns	0.05	0.10	ns	ns
Water	0.0003	ns	0.001	0.0006	ns	0.005
CO ₂ × water	ns	0.02	ns	ns	ns	ns
Sp	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0004
CO ₂ × sp	ns	ns	ns	ns	ns	ns
Water × sp	0.02	0.01	0.001	0.005	ns	0.08
CO ₂ × water × sp	ns	< 0.0001	0.002	ns	0.03	ns
Mixtures						
CO ₂	ns	0.05	0.08	0.04	0.09	ns
Water	ns	ns	ns	0.08	ns	ns
CO ₂ × water	0.05	ns	ns	0.10	ns	0.06
Sp	< 0.0001	–	–	< 0.0001	–	–
CO ₂ × sp	0.06	–	–	0.10	–	–
Water × sp	0.09	–	–	ns	–	–
CO ₂ × water × sp	ns	–	–	ns	–	–
All pots						
CO ₂	0.02	0.05	0.008	0.02	ns	ns
Water	0.004	ns	0.007	0.003	ns	0.005
CO ₂ × water	0.05	ns	ns	0.01	ns	0.03
Sp#	ns	< 0.0001	0.007	0.0009	ns	0.06
CO ₂ × sp#	ns	0.04	ns	ns	ns	ns
Water × sp#	ns	ns	ns	ns	ns	ns
CO ₂ × water × sp#	ns	ns	ns	ns	ns	ns

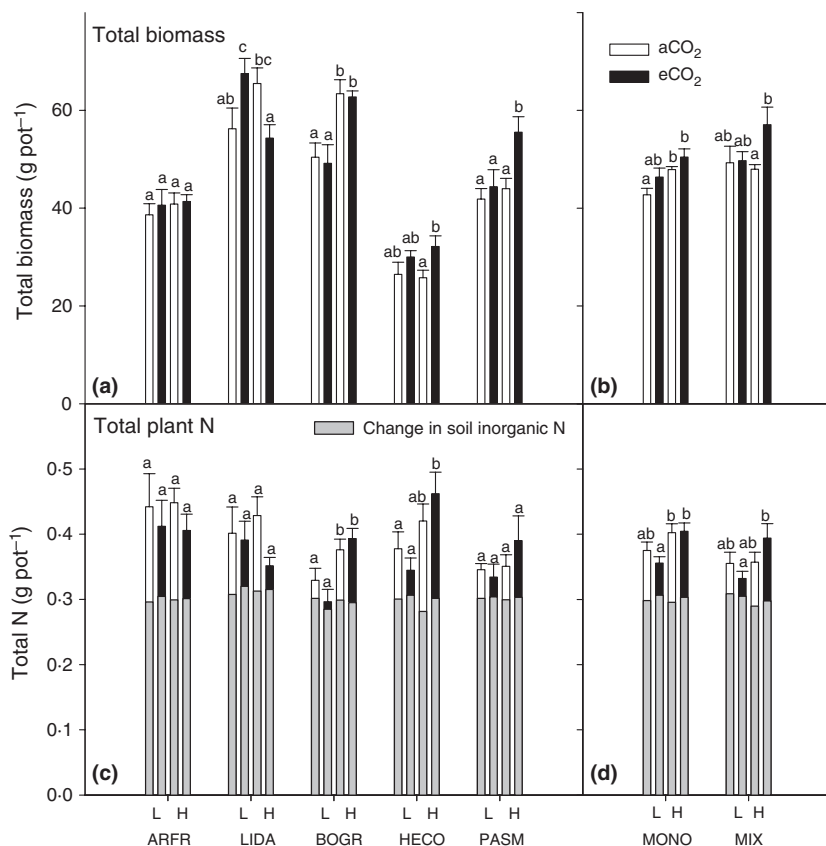


Fig. 2. Average total biomass (a and b) and total plant N content (c and d) for each of the CO₂, water, and species identity treatments within the monocultures (a and c), and for each of the CO₂, water and species number treatments for all pots (b and d, aCO₂ = ambient CO₂, eCO₂ = elevated CO₂, L = low water, H = high water, ARFR = *A. frigida*, LIDA = *L. dalmatica*, BOGR = *B. gracilis*, HECO = *H. comata*, PASM = *P. smithii*, MONO = monocultures, MIX = mixtures, error bars indicate 1 SE). Panel C and D also include the change in soil inorganic N between the end and start of the experiment. Different letters above bars indicate significant differences among the CO₂ and water treatments for each species or species number separately ($P < 0.05$, Tukey's HSD test).

biomass (average increase of 14.1% under elevated CO₂ and 14.0% with high water). Also, the increase in shoot N in response to high water was slightly larger than for total plant N (average increase of 14.3%). While larger in magnitude, individual species shoot biomass and shoot N responses to elevated CO₂ and high water in monoculture showed a similar pattern as individual species total biomass and total plant N responses, with the exception that LIDA shoot biomass did not respond to elevated CO₂ or high water and that BOGR shoot biomass increased under elevated CO₂ with high water (Fig. 3a). When all five species were grown in mixtures, elevated CO₂ and high water had no effect on shoot biomass, while shoot N significantly decreased under elevated CO₂ (on average by 16.8%) and increased with high water (on average by 8.7%, Table 1, Fig. 3b and d). Also, responses of the individual species to elevated CO₂ and high water changed compared with their responses in monoculture (Fig. 3b and d). For instance, when grown in mixtures, shoot biomass and shoot N of LIDA was negatively affected by elevated CO₂, particularly with low water, while shoot biomass of BOGR was negatively affected by high water under ambient CO₂. The CO₂ × sp × sp# and Water × sp × sp# effects on shoot biomass and shoot N were marginally significant (Table 2). Absolute differences in shoot biomass responses to elevated CO₂ and high water (net change in shoot biomass) for monocultures and mixtures are shown in Fig. 4. To compare net changes in shoot biomass between monocultures and mixtures we multiplied the net changes in the mixtures by five (see Methods). Here it becomes particularly clear that species

responses to elevated CO₂ and high water depended on whether these species were grown in monoculture or in mixture. In particular, LIDA responses to elevated CO₂ were positive in monoculture but negative in mixtures, and BOGR responses to high water were positive in monoculture but negative in mixtures (particularly under ambient CO₂).

The N uptake Enhancement Ratio (NER) was plotted as a function of the Biomass Enhancement Ratio (BER) to investigate the association of shoot biomass responses to elevated CO₂ and high water with N uptake (expressed by NER), and to evaluate changes in NUE (expressed by the deviation from the 1 : 1 line in Fig. 5). BER_{CO₂} values (BER in response to elevated CO₂, Fig. 5a) were generally greater than 1, except for some species grown in mixtures. On the other hand, NER_{CO₂} values (NER in response to elevated CO₂) were mostly smaller than 1, except for some species under high water. Further, NER_{CO₂} values were always lower than BER_{CO₂} values, indicating that the NUE increased for all treatments under elevated CO₂ ($P < 0.0001$, Table 2). The NUE Enhancement Ratios in response to elevated CO₂ (NUE-ER_{CO₂}) ranged between 1.02 and 1.42. Most of the NER_{water} values were greater than 1, and NER_{water} values were sometimes lower and sometimes higher than BER_{water} (Fig. 5b). The NUE-ER_{water} ranged between 0.78 and 1.16 and on average, the increase in NUE with high water was only marginally significant ($P = 0.09$, Table 2). Most of the treatments that had a NUE-ER_{water} smaller than 1 were under elevated CO₂. There was also a marginally significant CO₂ × water interaction for NUE ($P = 0.06$, Table 2).

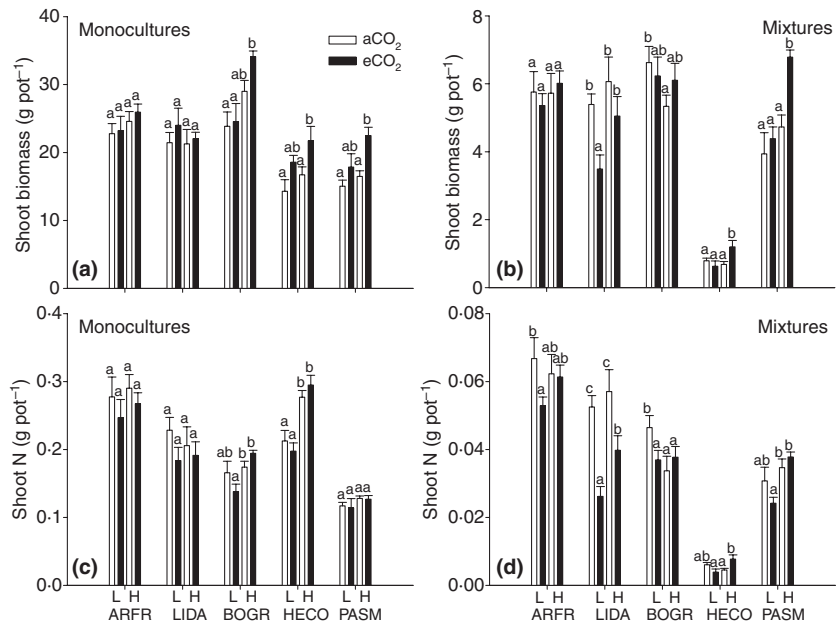


Fig. 3. Average shoot biomass (a and b) and shoot N content (c and d) for each of the CO₂, water, and species identity treatments in the monocultures (a and c) and in the mixtures (b and d), for explanation of abbreviations see Fig. 2, error bars indicate 1 SE). Different letters above bars indicate significant differences among the CO₂ and water treatments for each species separately ($P < 0.05$, Tukey's HSD test).

Discussion

IS THE STIMULATORY EFFECT OF ELEVATED CO₂ ON PLANT GROWTH CAUSED BY IMPROVED WATER CONDITIONS, OR ALSO BY OTHER CO₂ EFFECTS?

By frequently watering the pots up to their target levels, we tried to remove elevated CO₂-induced soil moisture effects on plant growth and N uptake (Dijkstra & Cheng 2008). We did not fully succeed in this in that between watering events, pots under ambient CO₂ dried out slightly faster than pots under elevated CO₂ (Fig. 1). Thus, we cannot rule out potential effects of CO₂-induced increases in soil moisture on plant

growth and N uptake. Because soil moisture differences between the ambient and elevated CO₂ treatment were quite small relative to soil moisture differences between the low and high water treatment (Fig. 1), any effects of CO₂-induced increases in soil moisture should also be relatively small compared with soil moisture effects induced by the water treatment. Thus, elevated CO₂ effects on plant growth and N uptake were most likely caused primarily by direct effects not related to soil moisture, such as effects on photosynthesis and rhizosphere processes affecting nutrient cycling.

Our results suggest that, when grown in monoculture, direct effects of elevated CO₂ and effects of increased soil moisture stimulated plant growth. The magnitude of these effects was relatively small (each less than 14%). In a Colorado shortgrass steppe field experiment with similar plant species and soil, doubling of the CO₂ concentration caused an increase in shoot biomass between 16 and 93% (with greater responses during dry years, Morgan *et al.* 2004b). However, under these field conditions, direct effects of elevated CO₂ and CO₂-induced increases in soil moisture operate simultaneously. Indeed, we observed the largest responses when direct effects of elevated CO₂ and high water were combined, indicating the importance of both effects. On average shoot biomass in the monocultures increased by 30% in the elevated CO₂-high water treatment compared with the ambient CO₂-low water treatment, not very different from field observations during a normal precipitation year. While elevated CO₂ and high water effects on plant growth were smaller in the mixtures, the largest effects also occurred in the elevated CO₂-high water treatment (average increase of 11.8% compared with the ambient CO₂-low water treatment).

It is not clear why plant growth responses to elevated CO₂ and high water in mixtures were smaller than in monocultures. Reich *et al.* (2001a) found that the increase in plant growth under elevated CO₂ was less in species-poor than in

Table 2. Summary of ANOVA results (P -values) for the effects of CO₂ (ambient and elevated), water (low and high), species (ARFR, LIDA, BOGR, HECO, and PASM), species number (monocultures and mixtures), and their interactions on shoot biomass, shoot N pool, and shoot NUE (ns = not significant, $P > 0.1$)

Effect	Shoot biomass	Shoot N	Shoot NUE
CO ₂	0.06	0.01	< 0.0001
Water	0.002	0.009	0.09
Sp	< 0.0001	< 0.0001	< 0.0001
Sp#	0.04	< 0.0001	< 0.0001
CO ₂ × water	ns	0.01	0.06
CO ₂ × sp	0.04	0.09	< 0.0001
CO ₂ × sp#	ns	ns	ns
Water × sp	ns	ns	ns
Water × sp#	ns	ns	ns
Sp × sp#	< 0.0001	< 0.0001	< 0.0001
CO ₂ × water × sp	ns	ns	ns
CO ₂ × water × sp#	ns	ns	ns
CO ₂ × sp × sp#	0.07	0.10	ns
Water × sp × sp#	0.04	0.08	ns
CO ₂ × water × sp × sp#	ns	ns	ns

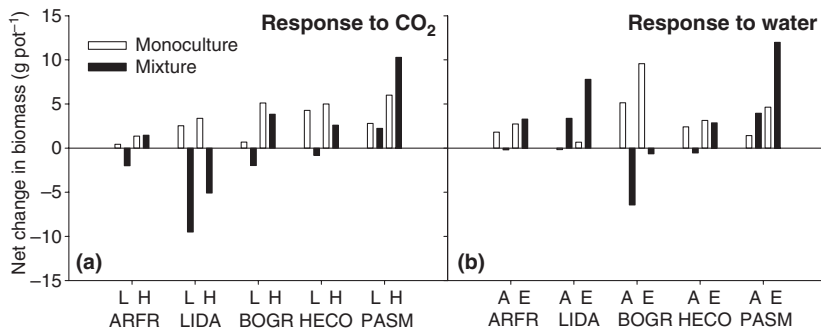


Fig. 4. Net change in shoot biomass in response to elevated CO₂ (a) and increased soil moisture (b) for each species and water treatment (a) or each species and CO₂ treatment (b) grown in monoculture and in mixture (for explanation of abbreviations see Fig. 2, and for CO₂, water, species, and species number treatment effects on shoot biomass see Table 2).

species-rich assemblages, because of multiple-species sampling effects, niche complementarity and positive species interactions. Possibly, changes in relative growth among species in mixtures may have affected the overall responses to elevated CO₂ and water in our experiment. For instance, greatly reduced growth of HECO in mixtures, a species that responded strongly to elevated CO₂ in monoculture, may

have reduced the overall CO₂ response in mixtures. HECO also responded strongly to elevated CO₂ in the Colorado shortgrass steppe field experiment, which could explain why our overall lower elevated CO₂ and high water responses in mixtures were low relative to those observed in the field (Morgan *et al.* 2004a).

The elevated CO₂ effect on plant growth was relatively strong in the C₃ grasses HECO and PASM (Fig. 4). Because elevated CO₂ did not increase plant N uptake (see below), elevated CO₂ may have increased the photosynthetic capacity of these species. On the other hand, plant growth of the C₄ grass BOGR responded to high water only, suggesting that this species did not increase its photosynthetic capacity under elevated CO₂. Others have observed increased plant growth of BOGR in response to elevated CO₂ (Morgan *et al.* 1994, 1998; Hunt *et al.* 1996). Our results suggest that this increase may have occurred because of CO₂-induced increases in soil moisture. It is noteworthy that total and shoot biomass of the sub-shrub ARFR did not respond to elevated CO₂ or high water when grown in monoculture or in mixture. Morgan *et al.* (2007) reported a 40-fold increase in above-ground biomass of this species after 5 years of elevated CO₂ in the shortgrass steppe field experiment, and suggested that CO₂-induced changes in soil/plant water relations were involved in that response. Direct photosynthetic and growth responses to CO₂ tend to be strong and expressed readily in young C₃ plants (Long *et al.* 2004), while the more indirect, secondary responses of plants to CO₂ through changes in water relations may require years to develop under more realistic field environments where species differences in traits like rooting morphology and competition for soil water come into play. Lack of a biomass response of ARFR in the present experiment suggests that the direct photosynthetic response of this species to CO₂ may be limited, and that improved water relations may have played an important role in its substantial growth response to CO₂ reported in the 5-year open top chamber experiment.

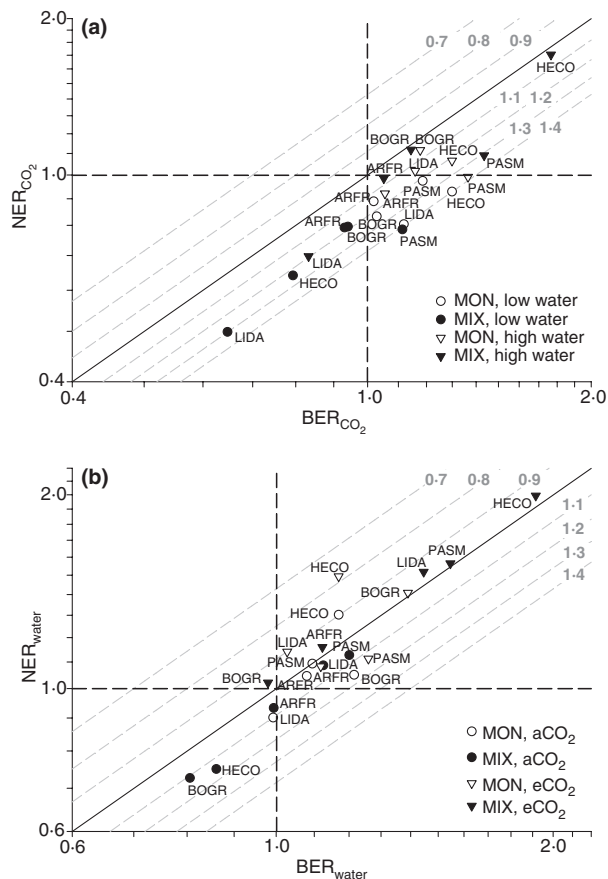


Fig. 5. The shoot N uptake enhancement ratio as a function of shoot biomass enhancement ratio in response to elevated CO₂ (a, NER_{CO₂} and BER_{CO₂} respectively) and in response to increased soil moisture (b, NER_{water} and BER_{water} respectively) for each species and water treatment (a) and for each species and CO₂ treatment (b) grown in monoculture and in mixture (for explanation of abbreviations see Fig. 2). The black solid 1:1 line indicates a NUE-ER of 1. Data points above this line have a NUE-ER < 1 and below this line have a NUE-ER > 1. The grey dashed lines indicate different values of NUE-ER. Note that BER and NER are presented on a natural log scale.

WHAT ARE THE ROLES OF INCREASED PLANT N UPTAKE AND INCREASED NUE IN THE STIMULATORY EFFECTS OF ELEVATED CO₂ AND INCREASED SOIL MOISTURE?

Total plant N uptake (plant N content in g pot⁻¹) was not affected by elevated CO₂ but increased with high water in the

monocultures (Table 1, Fig. 2). Shoot N content often tended to decrease under elevated CO₂, particularly in the low water treatment (i.e., NER_{CO_2} smaller than 1, Fig. 5a), while shoot N content mostly increased with high water (NER_{water} larger than 1, Fig. 5b). Consequently, the often greater shoot biomass under elevated CO₂ was mostly a result of increased NUE. On the other hand, the greater shoot biomass in the high water treatment was accompanied by correspondingly greater plant N uptake. In fact, the NUE decreased sometimes in response to high water, particularly under elevated CO₂. We should note that the frequent watering of our pots may have caused different effects on N dynamics than when water becomes available in pulses (Collins *et al.* 2008). However, the purpose of our study was not to explicitly predict how the five plant species in this experiment respond to elevated CO₂ and high water in field situations, but to better understand potential mechanisms causing the responses.

These results suggest that the high water treatment increased plant N supply in the soil (i.e., through increased net N mineralization, but possibly also through increased uptake of organic N). Increased soil moisture often increases net N mineralization in semi-arid grasslands (Burke, Lauenroth & Parton 1997; Austin & Sala 2002; Yuan *et al.* 2006). We did not directly measure net N mineralization in our experiment, but the increase in total plant N uptake with high water, without greater depletion of soil inorganic N during the experiment suggest that the high water treatment increased net N mineralization (Fig. 2c and d). Five years of elevated CO₂ in an open top chamber experiment in a semi-arid grassland in northern Colorado caused increased plant N uptake and N mineralization (Dijkstra *et al.* 2008; Dijkstra 2009). There is no evidence that elevated CO₂ increased N supply in our greenhouse experiment, likely because changes in soil moisture were limited. It is therefore likely that the increase in N cycling under field conditions may have been a result of CO₂ improved soil moisture conditions.

Although a pot study such as ours creates several artifacts, soil N availability in our experiment was comparable to field conditions. Average extractable inorganic N pools reported for the USDA-ARS Central Plains Experimental Range (the site where our soil came from) during the growing season was around 1.4 g N m⁻² in the top 15 cm of the soil (McCulley, Burke & Lauenroth 2009). The initial soil inorganic N concentration in our pots was 0.32 g N pot⁻¹, or 10.2 g N m⁻², higher than the average field observation (most likely because of soil disturbance), but not unusually high under certain field conditions (e.g., after long dry spells followed by a large precipitation event). Plant N uptake during the experiment reduced the soil inorganic N concentration to 0.017 g N pot⁻¹, or 0.54 g N m⁻², by the end of the experiment, lower than average field observations. However, temporal extremes in soil inorganic N in our pots most likely covered the range of soil inorganic N concentrations that can be observed in the field. A low soil inorganic N concentration does not necessarily reflect low net N mineralization rates. Indeed, the large variability in plant N uptake among treatments, but similar low soil inorganic N concentrations at the end of the experi-

ment, suggest that net N mineralization rates were not related to soil inorganic N concentration. Nevertheless, by the end of the experiment plant growth responses to elevated CO₂ may have been constrained by N availability (Reich, Hungate & Luo 2006a, Reich *et al.* 2006b) as the increase in plant growth under elevated CO₂ was associated with an increased NUE, not with increased plant N uptake.

DO PLANT GROWTH AND N UPTAKE RESPONSES TO ELEVATED CO₂ AND INCREASED WATER AVAILABILITY DIFFER BETWEEN INTER- AND INTRASPECIFIC COMPETITIVE INTERACTIONS AMONG PLANTS?

Our results show that species-specific shoot biomass and shoot N content responses to elevated CO₂ and increased water availability depend on inter- and intraspecific competitive interactions among plants. BOGR responded positively to high water when grown as a monoculture, but when competing for resources with other species, its response to high water was negative. Five years of water additions in a semi-arid grassland in northern Colorado also resulted in the replacement of warm season grasses (pre-dominantly BOGR) by introduced subordinate species (Lauenroth, Dodd & Sims 1978). The increased BOGR shoot biomass when grown in monoculture under elevated CO₂ in other growth chamber studies (Morgan *et al.* 1994, 1998; Hunt *et al.* 1996) could have been due to a lack of interspecific competition for water (and possibly other resources), because when grown in a plant community under field conditions, BOGR shoot biomass did not respond to elevated CO₂ (Morgan *et al.* 2004a). LIDA shoot biomass was unaffected by high water in monoculture, but increased with high water in mixtures. Similarly, experimental water addition greatly increased the ability of LIDA to invade native mixed-grass prairie (Blumenthal *et al.* 2008). In contrast, elevated CO₂ decreased the growth of LIDA in mixtures, perhaps because of stronger direct photosynthetic responses to CO₂ among its competitors than in LIDA itself.

Species-specific shoot biomass responses to elevated CO₂ and high water were sometimes negative in the mixtures. These negative responses also coincided with large negative responses of shoot N. Some of the strongest positive species-specific shoot biomass and shoot N responses also occurred in the mixtures (particularly in response to high water). This suggests that shifts in N uptake among plant species may explain the highly variable species-specific shoot biomass responses in mixtures to elevated CO₂ and high water. That is, increased N uptake by 'winners' under elevated CO₂ or high water may have reduced N availability to 'losers'. Others have also suggested that the ability of plants to increase N uptake may be an important determinant of which species in an assemblage will be able to respond to elevated CO₂ (Berntson, Rajakaruna & Bazzaz 1998; Maestre, Bradford & Reynolds 2005). Our results show that changes in water availability also cause shifts in N uptake among plant species within mixtures, but that the 'winners' and 'losers' may not be the same as those resulting from direct effects of elevated CO₂. As discussed above,

unlike the CO₂ treatment, the water treatment itself increased plant N availability for plant uptake. It is likely that this increase in overall soil N availability with increased soil moisture affected interspecific competition for N as well (Wedin & Tilman 1993; Clark *et al.* 2007), which may partially explain why the winners and losers with high water and elevated CO₂ were not always the same.

Conclusions

Increases in plant productivity with elevated CO₂ in semi-arid grasslands have been related to CO₂-induced increases in soil moisture (Morgan *et al.* 2004a, 2007). In this greenhouse study we tried to separate the effect of soil moisture from direct CO₂ effects on plant productivity. We found that productivity of certain grassland species responded more strongly to direct effects of elevated CO₂ (the C₃ grasses HECO and PASM), while others responded more strongly to increased soil moisture (the C₄ grass BOGR). The CO₂ and soil moisture treatments also had differential effects on N cycling. We observed greater overall plant N uptake and most likely increased net N mineralization with increased soil moisture. In contrast, increased productivity under elevated CO₂ could to a larger degree be explained by increased NUE. These findings suggest that elevated CO₂ may increase plant productivity of certain species (C₃ grasses) by increasing their photosynthetic capacity, but also confirm the notion that elevated CO₂-induced increases in soil moisture are important for sustained increases in plant productivity, N uptake, and N cycling in semi-arid grasslands (Dijkstra *et al.* 2008). We further found that both plant biomass and plant N responses to CO₂ and water depended on whether plants were grown in monoculture or mixture. The largest responses, both positive and negative, were observed with interspecific competition. Thus responses of this grassland community to CO₂ and water may be both contingent upon and accentuated by competition. While current ecosystem models predicting effects of global change incorporate indirect effects of elevated CO₂ on soil moisture and N cycling (Coughenour & Chen 1997; Parton *et al.* 2007), here we emphasize the need for models to incorporate interspecific competition for N and other resources.

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