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CO_2 enrichment increases element concentrations in grass mixtures by changing species abundances

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Abstract Atmospheric carbon dioxide (CO_2) enrichment may increase plant growth more than the uptake of chemical elements from soil. Increased CO_2 also may alter element levels in biomass from multi-species vegetation by changing plant species abundances. We measured concentrations of ten elements in aboveground tissues of three C_4 grasses that had been exposed for 2–3 growing seasons to a continuous gradient in CO_2 from 250 to 500 μmol mol^{-1}. The grasses, *Bouteloua curtipendula*, *Schizachyrium scoparium*, and *Sorghastrum nutans*, are competitive dominants in assemblages of tallgrass prairie vegetation growing on each of three soil types along a field CO_2 gradient in central Texas, USA. Our objective was to determine whether CO_2 influences element concentrations in grass mixtures by changing concentrations in individual species or shifting species abundances. Increased CO_2 had little effect on element concentrations in grasses compared to differences observed among grass species and soils. Increasing CO_2 from the pre-Industrial to elevated levels reduced the phosphorus concentration in grasses grown on a clay and sandy loam soil. Concentrations of most other elements did not respond to CO_2 treatment. Cover of the mid-grass *Bouteloua* declined at higher CO_2 levels as cover of the taller grass *Sorghastrum* increased. Concentrations of several elements were lower in *Bouteloua* than *Sorghastrum*; hence, this exchange of species at higher CO_2 increased element concentrations in grass assemblages. Potential consequences include an improvement in the nutritional quality of plants for herbivores. Results highlight the underappreciated impact that CO_2 enrichment may have on ecosystem functioning by changing plant composition.

Keywords *Bouteloua curtipendula* · C_4 grasses · Soil type · *Sorghastrum nutans* · Subambient CO_2 concentration · Tallgrass prairie

Introduction

Atmospheric carbon dioxide (CO_2) concentration has increased by about 40% since the nineteenth century, from the pre-Industrial level of 270 μmol mol^{-1} to

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the current level of 385 μmol mol^{-1} (Petit et al. 1999; Keeling et al. 2009). Atmospheric CO_2 is expected to reach double the pre-Industrial concentration during this century (IPCC 2007). Experimentally doubling the current CO_2 concentration increases biomass in natural and semi-natural grassland ecosystems by as much as 95% (Morgan et al. 2004a). Proportional increases in biomass may have been even greater over subambient to current CO_2 concentrations (Polley et al. 2003). Evidence is accumulating, however, that plant growth may increase disproportionately compared to tissue levels of chemical elements that are essential both for plants and the herbivores they support (Overdieck 1993; Loladze 2002). Greater CO_2 has been shown to reduce concentrations of plant macroelements, like N and P (Cotrufo et al. 1998; Loladze 2002), and microelements, like iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu) (Penuelas and Matamala 1993; Loladze 2002). Growth responses to CO_2 also may differ among species, leading to changes in the relative abundances of plant species in mixed-species assemblages (Polley et al. 2003; Morgan et al. 2004b). Should element concentrations also differ among species, vegetative change could affect element levels in and, potentially, the nutritional value of tissue from plant assemblages.

Several factors may contribute to an elemental imbalance in plants grown under CO_2 enrichment. First, growth at higher CO_2 may simply outpace the capacity of plants to acquire mineral elements from soil (Taub and Wang 2008). Element uptake may lag growth when element levels in soil are small or for elements with restricted mobility in the soil (i.e., P, Zn, Cu) or limited solubility in soil solution (i.e., P, Mn, Zn, Cu in calcareous soils). Second, CO_2 enrichment may reduce plant demand for certain elements (Conroy 1992). For example, higher CO_2 may reduce plant demand for N by increasing the efficiency with which C is fixed per unit of N invested in photosynthetic enzymes (Taub and Wang 2008). Third, CO_2 enrichment may reduce mass flow of elements in soil by reducing transpiration. Fourth, increased CO_2 may change the rate at which organically bound elements in soil are released during microbial decomposition. Decomposition may be slowed if CO_2 enrichment increases C/element ratios in plant litter (Cotrufo et al. 1998; but see, Gill et al. 2006) or enhanced if CO_2 enrichment slows transpiration and increases soil water content (Dijkstra et al. 2008). Owing to the multiple effects of CO_2 on element uptake, uptake rates likely will not change uniformly among elements as CO_2 rises, likely altering element ratios in plants.

Rising CO_2 also may alter the elemental composition and, potentially, nutritional quality of biomass derived from multi-species vegetation by changing plant composition. Tissue concentrations of mineral elements may differ substantially among plant species growing in the same environment, even when species are closely related (White and Broadley 2009). Element concentrations differ among species partly because root morphological and physiological traits important in element uptake differ among species. Element uptake from soil depends on root length, rooting depth, the association of roots with mycorrhizal fungi, and rates at which roots secrete organic acids and enzymes capable of degrading organic compounds (White and Broadley 2009). We know of no published evidence that CO_2 enrichment affects the elemental composition of species assemblages by influencing species abundances.

We measured concentrations of several plant-essential and/or nutritionally important elements in aboveground tissues of three C_4 grasses that had been exposed for two to three growing seasons to a continuous gradient in atmospheric CO_2 spanning pre-Industrial to elevated concentrations. The grasses, *Bouteloua curtipendula* (Michx.) Torr. [side-oats grama], *Schizachyrium scoparium* (Michx.) Nash [little bluestem], and *Sorghastrum nutans* (L.) Nash [Indian grass], are competitive dominants in a 7-species assemblage of tallgrass prairie vegetation established before CO_2 treatment. Grasses were sampled from assemblages growing on three soil types along the field CO_2 gradient (250–500 μmol mol^{-1}). Our objective was to determine whether CO_2 influenced element concentrations in grass mixtures by changing concentrations in individual species or shifting species abundances. Consistent with current evidence, we predicted that increasing CO_2 would reduce tissue concentrations of each of the ten chemical elements we analyzed, with the exception of C. Although CO_2 effects vary among studies, element concentrations are lower on average at elevated than lower CO_2 concentrations (Overdieck 1993; Loladze 2002). Concentrations of each of the 12 elements measured in herbarium specimens of 13 plant species also were lower at today's CO_2 concentration than when CO_2 was increasing from the pre-Industrial level (Penuelas and Matamala 1993). Specifically, we predicted that increasing CO_2 would: (1) reduce tissue concentrations of N and elements like P, Zn, and Cu with restricted mobility and limited solubility in calcareous soils and (2) mitigate stand-level effects of the predicted decrease in N, P, Zn, and Cu concentrations in individual species by favoring grasses with relatively high concentrations of these elements. This prediction is based on the premise that CO_2 enrichment would favor plants with root traits important to the uptake of limiting elements, such as CO_2 enrichment sometimes relieves the N limitation on biomass response by favoring greater abundances of legumes among plant functional groups (Edwards et al. 2001; Joel et al. 2001).

Materials and methods

CO_2 chambers

We used elongated field chambers to expose assemblages of tallgrass prairie species to a continuous gradient in CO_2 spanning pre-Industrial to elevated concentrations (Polley et al. 2008; Fay et al. 2009). The CO_2 facility is located in central Texas, USA (31°05′ N, 97°20′ W) and consists of two transparent, tunnel-shaped chambers each 1.2 m wide and tall and 60 m long, aligned parallel along a north–south axis. Each chamber is divided into ten consecutive compartments each 5 m long. Aerial growth of chambered vegetation is enclosed in a transparent polyethylene film which transmits >90% of incident irradiance with minimal effects on spectral quality. Chambers depend upon photosynthesis during daylight and upon respiration at night to create CO_2 gradients. Air introduced into chambers during daylight is progressively depleted of CO_2 by photosynthesizing plants as it is moved by blowers toward the air outlet of the system. The direction of air flow is reversed at night, and respiration by the enclosed ecosystem progressively increases the CO_2 concentration of chamber air. Desired CO_2 concentration gradients are maintained by automatically varying the rate of air flow through chambers in response to changes to photosynthetic (daylight) or respiration rates (night). During daylight, pure CO_2 is injected into air introduced into the south end of one chamber (elevated chamber) to elevate the CO_2 concentration to 500 $\mu mol\ mol^{-1}$. The CO_2 level is depleted to 370 $\mu mol\ mol^{-1}$ at the north end of the chamber. Ambient air is introduced into the south end of the second chamber (subambient chamber) to initiate an ambient to subambient gradient in CO_2 (370–250 $\mu mol\ mol^{-1}$). Night-time CO_2 concentrations are regulated at about 130 $\mu mol\ mol^{-1}$ above daytime values along each chamber. Air temperature and vapor pressure deficit are regulated near ambient values by cooling and dehumidifying air at 5-m intervals along chambers. CO_2 treatments have been maintained each growing season from early April through mid-November since CO_2 control was initiated in 2006.

Soil monoliths

We installed four steel boxes containing intact soil monoliths (each 1 m wide and long × 1.5 m deep) beneath each of the twenty 5-m long compartments in chambers. Three soil types of contrasting physical and hydrological properties are represented. These include a Mollisol from the Austin series (Udorthentic Haplustolls), a Vertisol from the Houston Black series (Udic Haplusterts), and a sandy loam Alfisol from the Bastrop (or, Bastsil) series (Udic Paleustalfs). Each soil type is alkaline (pH 7.60, 8.02, and 8.06 for Houston, Austin, and Bastrop soils, respectively). Two monoliths of each of two soil types were randomly placed beneath each 5-m long compartment. Twelve compartments of chambers contain two monoliths each of the clay Houston Black and Austin series soils. Remaining compartments contain two monoliths of the sandy Bastrop soil and two monoliths of either the Houston soil (4 compartments) or Austin soil (4 compartments).

Perennial species characteristic of tallgrass prairie in central Texas were transplanted into 60 monoliths in June 2003, 3 years before CO_2 treatment was initiated. Eight plants of each of four C_4 grass species [*Bouteloua curtipendula*, *Schizachyrium scoparium*, *Sorghastrum nutans*, *Tridens albescens* (Vasey) Wooton& Standl. (white tridens)] and three forb species [*Salvia azurea* Michx. Ex Lam. (pitcher sage), *Solidago canadensis* L. (Canada goldenrod), and the legume *Desmanthus illinoensis* (Michx.) MacMill. ex B.L. Rob. & Fernald (Illinois bundleflower)] were transplanted into each monolith (total density = 56 plants/m^2). Monoliths received ambient precipitation prior to CO_2 treatment, with two exceptions. We irrigated monoliths to maintain soil water content near field capacity during the summer following planting (2003) and added 150 mm of water to each monolith during the dry summer of 2005. Precipitation during 2005 was 71% of the 97-year mean for the site (878 mm). After initiating CO_2 control, we excluded precipitation and irrigated each monolith (2006, 2007, 2008, and 2009) to simulate the average of growing season precipitation in central Texas.

Analysis of chemical elements

We collected vegetative modules (tillers) of each of the three dominant grass species (*Bouteloua curtipendula, Schizachyrium scoparium, Sorghastrum nutans*; hereafter referenced by genus) from each prairie monolith on June 10, 2008 and June 4, 2009. Two to three vegetative tillers were clipped at crown level from each of the four to eight plants of each species in each monolith. For each monolith, tillers from a given species were pooled, dried at 60°C, and ground for element analyses. Remnant prairies in central Texas typically are hayed each year in June to provide fodder for cattle; hence, element concentrations in grasses at this time of year are of practical importance. Total carbon (C) and nitrogen (N) concentrations in tissues of each species from each monolith were determined using a combustion gas chromatograph (Variomax CN, Elementar Instruments, Hanau, Germany). Larger samples were required for analysis of plant microelements and other macroelements. The composite samples for each species were created by combining an equal mass of ground plant material collected from each of the two monoliths of a given soil type in each 5-m compartment of chambers. Aliquots of tissues were prepared for analysis of potassium (K), calcium (Ca), phosphorus (P), magnesium (Mg), zinc (Zn), manganese (Mn), copper (Cu), and iron (Fe) by refluxing with nitric acid over a 3-day period. Digests were reconstituted with 2% nitric acid and analyzed using inductively coupled, argon plasma emission spectrometry (3100XL; Perkin Elmer Corp., Wellsley, MA) equipped with automated sample injection. Analytes were monitored at the following wavelengths: Ca, 315.9 nm; Cu, 324.8 nm; Fe, 259.9 nm; K, 766.5 nm; Mg, 279.1 nm; Mn, 257.6 nm; P, 214.9 nm; Zn, 213.9 nm; and Sc (internal standard), 361.4 nm. The minimum detection limits (MDL) were: Ca 101 ppb, Cu 10.4 ppb, Fe 11 ppb, K 11.6 ppm, Mg 131 ppb, Mn 11 ppb, P 784 ppb, and Zn 16 ppb. Samples were analyzed at the USDA Agricultural Research Service's Grand Forks Human Nutrition Research Center, Grand Forks, North Dakota, USA. All elemental data are reported per gram dry weight of tissue.

Vegetation sampling and data analysis

Vegetation in each monolith was photographed from above the plant canopy with a digital camera suspended 1.5 m above the soil surface of each monolith. Vegetation was photographed on the day tiller samples were collected. We applied a grid intersection technique to photographs to determine the contribution of each species to vegetative cover. We placed a 12 × 12 grid on each photograph, counted the number of grid intersections at which each species was encountered, and divided counts for each species by the total number of intersections at which vegetation was encountered in order to determine the contribution of each species to plant cover.

Higher CO_2 may change the mean concentration of elements in the biomass of grass mixtures by changing species abundances. For each year, we calculated the effect of CO_2-caused changes in grass abundances on the average concentration of each element in the biomass of dominant grasses by weighting element concentrations of *Bouteloua, Schizachyrium*, and *Sorghastrum* by the contribution of each species to the total cover of grasses. Together, the three dominant grasses contributed 69–76% of vegetative cover on the three soils. The measured contribution of each grass to cover along the CO_2 gradient in June 2008 was strongly and linearly correlated with each species' contribution to aboveground biomass harvested the previous November ($r^2 = 0.70$, $P < 0.0001$), evidence that cover data by species provide a reliable estimate of species' contributions to June biomass each year.

The leaf area index (LAI) in each monolith also was measured using an AccuPAR LP-80 ceptometer (Decagon Devices, Inc., Pullman, WA). The LAI was used as a surrogate of aboveground biomass because a June destructive harvest was not possible. Aboveground biomass harvested from monoliths at the end of the growing season was strongly and linearly correlated with LAI measured with the

LP-80 ceptometer at that time ($r^2 = 0.68$, $P < 0.0001$, $n = 60$).

We used a mixed-model analysis of covariance (ANCOVA) to determine treatment effects on LAI, the contribution of each species to vegetative cover, and element concentrations in tillers for each year (2008, 2009). Analyses were conducted with SAS (Littell et al. 2002). Soil type and species identity were considered fixed effects. The assignment of soil types to 5-m lengths of chambers was considered a random effect. The mean CO_2 concentration per chamber length was treated as a covariate in each analysis. Prior to analysis, we averaged values of LAI, cover, and C and N concentration of each species for the two monoliths of each soil type in each 5-m length of chambers.

We measured concentrations of each of ten elements on a common set of plant samples from each year. The greater the number of element comparisons from a given set of samples, the greater the possibility that the concentration of at least one element will appear to differ among treatments. The error rate for each comparison should be more stringent than 0.05 in order to retain a prescribed familywise error rate in analyses involving multiple comparisons. One way to maintain an overall error rate of $\alpha = 0.05$ is to test for treatment effects on each element using a significance level of α/n (Bonferroni correction) or, for this analysis, $P = 0.05/10 = 0.005$.

Results

Element concentrations

Element concentrations usually differed among the C_4 grass species, among plants grown on different soils, or both, but differences were most consistent among species (Tables 1, 2; complete data are listed in Online Resource 1). Of the elements measured, only [Fe] did not differ significantly among species ($P = 0.10$) or soils ($P = 0.30$) in 2008. Concentrations of many of the elements analyzed were lower in *Bouteloua* than the other grasses. Concentrations of C, K, Mg, and Mn all were significantly lower for *Bouteloua* than *Sorghastrum* and *Schizachyrium* when 2008 data were pooled across soils (Table 1). Concentrations of K, P, Mg, and Mn were lowest in *Bouteloua* in 2009. Zn in 2008 and N in 2009 were the only elements for which the concentration was significantly greater in *Bouteloua* than another of the grasses. Importantly, several of the elements for which concentrations were smallest in *Bouteloua* occurred at levels deemed inadequate to support the growth of beef cattle (NRC 1996). Tissue concentrations of P, Mg, Zn, and Cu all were lower than required to support cattle growth in one or more species. Concentrations of three of these elements, P, Mg, and Cu, were least in *Bouteloua*.

Not surprisingly, element levels often differed between plants grown on the sandy Bastrop soil and the two clay soils. Concentrations of N, K, Zn, and Cu all were lower in plants grown on the Bastrop soil than Austin and Houston soils in one or both years, whereas tissue [Mn] was greater for plants on the sandy soil than clay soils in both years.

Species effects on [P] and [Zn] in 2008, [Fe] in 2009, and [Mn] in both years differed among soil types, as indicated by significant Species X Soil interaction terms in the ANCOVA ($P < 0.05$). Differences among species usually were greater on one soil than the other two soils (Fig. 1). Manganese concentrations were greater for *Schizachyrium* and *Sorghastrum* than *Bouteloua* on the Bastrop soil only in 2008. Species differences in [P] also were magnified on the Bastrop soil, whereas species differences in [Fe] were greatest on the Austin soil in 2009.

Concentrations of fewer elements differed as a function of CO_2 treatment than among grass species and soils. There was a weak tendency across data from all species and soils combined in 2008 for tiller [Fe] to increase at higher CO_2 levels ($P = 0.08$; not shown), but CO_2 effects on element concentrations usually depended on species identity or soil type. For example, the [Ca] in tillers of *Schizachyrium* and *Bouteloua* declined by about 560 $\mu g\ g^{-1}$ per 100 $\mu mol\ mol^{-1}$ increase in CO_2 concentration in 2008 (Fig. 2), whereas CO_2 had no effect on the [Ca] of *Sorghastrum* tillers in 2008 ($P = 0.54$) or on any species in 2009. Increasing CO_2 from 280 to 480 $\mu mol\ mol^{-1}$ reduced the [P] of *Bouteloua* tillers by 22% (200 $\mu g\ g^{-1}$) in 2008 and of *Schizachyrium* by 18% (201 $\mu g\ g^{-1}$) in 2009, but did not affect the [P] of other species. Increasing CO_2 from 280 to 480 $\mu mol\ mol^{-1}$ increased tiller [C] from 41.5 to 42.0% and from 42.0 to 42.8% in *Sorghastrum* and *Schizachyrium*, respectively, in 2008 (not shown; CO_2 * species interaction; $P < 0.0001$). Contrary to results from most CO_2 studies, CO_2 treatment had no

Table 1 Mean values (SE) of element concentrations in tillers harvested in June 2008 from three species of C_4 grasses grown along a subambient to elevated gradient in atmospheric CO_2 concentration on soils of each of three types

Element	Soil			*P*-value	Species			*P*-value	Element requirement
	Austin	Bastrop	Houston		*Bouteloua curtipendula*	*Schizachyrium scoparium*	*Sorghastrum nutans*		
Carbon (%)	41.9^{a} (0.1)	41.2^{b} (0.2)	41.6^{ab} (0.1)	0.02	40.5^{c} (0.2)	42.4^{a} (0.2)	41.9^{b} (0.2)	<0.0001	–
Nitrogen (μg g^{-1})	9932^{a} (233)	8666^{b} (303)	10505^{a} (271)	0.0004	9468^{b} (215)	10483^{a} (212)	9153^{b} (210)	<0.0001	–
Potassium (μg g^{-1})	9870^{bc} (243)	9172^{c} (281)	10120^{ab} (259)	0.05	7987^{b} (213)	10570^{a} (221)	10605^{a} (208)	<0.0001	6000
Calcium (μg g^{-1})	5279^{a} (180)	5497^{a} (207)	5540^{a} (192)	0.58	5140^{b} (193)	5334^{ab} (201)	5842^{ab} (188)	0.04	2500
Phosphorus* (μg g^{-1})	816^{b} (31)	939^{a} (36)	909^{ab} (33)	0.04	828^{b} (27)	973^{a} (28)	863^{b} (27)	0.0003	1500
Magnesium (μg g^{-1})	885^{a} (41)	997^{a} (47)	975^{a} (43)	0.17	693^{c} (38)	1153^{a} (39)	1011^{b} (37)	<0.0001	1000
Zinc* (μg g^{-1})	23.7^{a} (0.8)	19.1^{b} (0.9)	23.9^{a} (0.8)	0.0005	22.7^{a} (0.7)	19.6^{b} (0.7)	24.4^{a} (0.7)	<0.0001	30
Manganese* (μg g^{-1})	26.7^{b} (3.3)	68.2^{a} (3.8)	13.6^{c} (3.5)	<0.0001	28.2^{b} (2.7)	39.6^{a} (2.8)	40.7^{a} (2.7)	0.0004	20
Copper (μg g^{-1})	3.6^{a} (0.1)	3.0^{b} (0.1)	3.9^{a} (0.1)	0.0002	3.3^{b} (0.1)	3.9^{a} (0.1)	3.3^{b} (0.1)	0.003	10

Values for a given element do not differ significantly among soils or among species if followed by the same letter ($n = 23$–33; $P < 0.05$). The effect of soil type depended on species identity (significant Soil × Species interaction) for those elements labeled with an asterisk. Note that most differences among treatments remain significant when tested using a more stringent significance level of $P < 0.005$ (Bonferroni correction for tests involving multiple comparisons). Also shown are the approximate element concentrations required by growing beef cattle (NRC 1996)

significant effect on tissue [N] across data for all species and soils combined ($n = 91$, $P = 0.47$ in 2008; $n = 90$, $P = 0.28$ in 2009). When analyzed using data from all grasses, tiller [P] declined on the Bastrop and Houston soils in 2008 and Houston soil in 2009 (Fig. 3). The decline in [P] was a linear function of CO_2 for the Bastrop soil in 2008 and Houston soil in 2009, but [P] changed relatively little above 400 μmol mol^{-1} CO_2 for the Houston soil in 2008.

LAI and species abundances

The LAI measured in June of each year was greater on average on the Bastrop and Houston soils than on the Austin soil (1.46, 1.24, and 0.97, respectively, in 2008 and 1.03, 1.13, and 0.72, respectively, in 2009). LAI was not significantly correlated with CO_2 treatment ($P > 0.10$) on any soil type or in either year, implying that CO_2 had no consistent effect on aboveground biomass at the June sampling.

The CO_2 treatment had a dramatic effect on species relative abundances, as evidenced by the contrasting responses of *Bouteloua* and *Sorghastrum* to CO_2 enrichment in both 2008 and 2009 (Fig. 4). The contribution of *Sorghastrum* to vegetative cover increased markedly at higher CO_2 each year, especially on Austin and Bastrop soils. As estimated from regression equations, cover of *Sorghastrum* increased from near 0 to 37% on the Austin soil as CO_2 rose from 280 to 480 μmol mol^{-1} and was greater by almost a factor of five at the highest than lowest CO_2 level on the Bastrop soil. An increase in the cover of the tallgrass *Sorghastrum* usually was accompanied by a decrease in cover of the mid-grass *Bouteloua*. The contribution of *Bouteloua* to plant cover declined by 82% on average across years (from 78 to 14%) on the Austin soil as CO_2 increased from 280 to 480 μmol mol^{-1}. Cover of *Bouteloua* declined from about 32 to 1% on the Bastrop soil and from 45 to 20% on the Houston soil as CO_2 increased.

Table 2 Mean values (SE) of element concentrations in tillers harvested in June 2009 from three species of C_4 grasses grown along a subambient to elevated gradient in atmospheric CO_2 concentration on soils of each of three types

Element	Soil			*P*-value	Species			*P*-value	Element requirement
	Austin	Bastrop	Houston		*Bouteloua curtipendula*	*Schizachyrium scoparium*	*Sorghastrum nutans*		
Carbon (%)	43.1^{a} (0.1)	42.6^{b} (0.1)	42.7^{b} (0.1)	0.0007	42.0^{b} (0.1)	44.0^{a} (0.1)	42.3^{b} (0.2)	<0.0001	–
Nitrogen ($\mu g\ g^{-1}$)	9106^{ab} (322)	8249^{b} (399)	10072^{a} (360)	0.008	9258^{a} (286)	9853^{a} (281)	8315^{b} (283)	<0.0001	–
Potassium ($\mu g\ g^{-1}$)	9558^{b} (288)	9325^{b} (288)	10867^{a} (288)	0.002	8123^{b} (293)	11121^{a} (310)	10507^{a} (275)	<0.0001	6000
Calcium ($\mu g\ g^{-1}$)	4928^{a} (189)	5518^{a} (194)	5460^{a} (194)	0.07	4779^{b} (196)	4986^{b} (200)	6142^{a} (184)	<0.0001	2500
Phosphorus ($\mu g\ g^{-1}$)	912^{a} (29)	1007^{a} (30)	985^{a} (29)	0.08	900^{b} (25)	1016^{a} (26)	987^{a} (24)	0.003	1500
Magnesium ($\mu g\ g^{-1}$)	919^{b} (36)	1202^{a} (37)	990^{b} (36)	<0.0001	829^{b} (35)	1189^{a} (37)	1092^{a} (33)	<0.0001	1000
Iron* ($\mu g\ g^{-1}$)	187^{b} (10)	141^{b} (11)	149^{b} (11)	0.01	105^{b} (13)	288^{a} (14)	84^{b} (12)	<0.0001	50
Zinc ($\mu g\ g^{-1}$)	25.0^{a} (0.8)	20.9^{b} (0.8)	24.2^{a} (0.9)	0.004	20.9^{a} (1.2)	25.6^{a} (1.3)	23.7^{a} (1.2)	0.09	30
Manganese* ($\mu g\ g^{-1}$)	17.9^{b} (3.0)	64.8^{a} (3.2)	18.3^{b} (3.3)	<0.0001	22.4^{b} (4.0)	42.4^{a} (4.3)	36.2^{ab} (3.9)	0.01	20
Copper ($\mu g\ g^{-1}$)	3.7^{a} (0.2)	3.5^{a} (0.2)	4.2^{a} (0.2)	0.07	3.3^{b} (0.2)	4.7^{a} (0.2)	3.5^{b} (0.2)	<0.0001	10

Values for a given element do not differ significantly among soils or among species if followed by the same letter ($n = 22$–23; $P < 0.05$). The effect of soil type depended on species identity (significant Soil × Species interaction) for those elements labeled with an asterisk. Note that most differences among treatments remain significant when tested using a more stringent significance level of $P < 0.005$ (Bonferroni correction for tests involving multiple comparisons). Also shown are the approximate element concentrations required by growing beef cattle (NRC 1996)

Increasing CO_2 concentration favored *Sorghastrum* at the expense of *Bouteloua* apparently by reducing rates of evapotranspiration and soil water depletion. Both the contribution of *Sorghastrum* to vegetative cover in 2008 and 2009 (Fig. 4) and the mean of soil water content to 50 cm depth during the two preceding years (averaged for June through September) increased linearly at higher CO_2 on Austin and Bastrop soils [not shown: $r^2 = 0.40$, $P = 0.01$, $n = 13$ and $r^2 = 0.48$, $P = 0.04$, $n = 8$, respectively]. Cover of *Sorghastrum* increased exponentially as soil water content to 50 cm depth (cm water) increased on the Austin soil [fraction *Sorghastrum* cover = $(5.36 \times 10^{-9}) \times e^{(1.087 \times \text{soil water})}$; $r^2 = 0.65$, $P = 0.0005$]. Vegetative cover of *Sorghastrum* was a weak linear function of soil water content on the Bastrop soil. The contribution of *Sorghastrum* to total cover increased by a fraction of 0.1 for each 1 cm increase in soil water content ($r^2 = 0.28$, $P = 0.10$). Neither CO_2 concentration ($P = 0.21$) or the fraction of *Sorghastrum* cover was correlated with soil water content for the Houston soil ($P = 0.21$ and 0.20, respectively, $n = 10$), on which CO_2 effects on vegetation were smallest.

Elements in the combined biomass of dominant grasses

Higher CO_2 may change the concentration of elements in the combined biomass of dominant grasses by changing species relative abundances. CO_2 effects on grass abundances were especially large on the Austin soil. Cover of *Bouteloua* declined, whereas that of *Sorghastrum* increased as CO_2 rose from subambient to elevated concentrations (Fig. 4). CO_2 enrichment did not affect concentrations of most elements, including K, Mg, and Mn, in *Bouteloua, Schizachyrium*, or *Sorghastrum* in either 2008 or

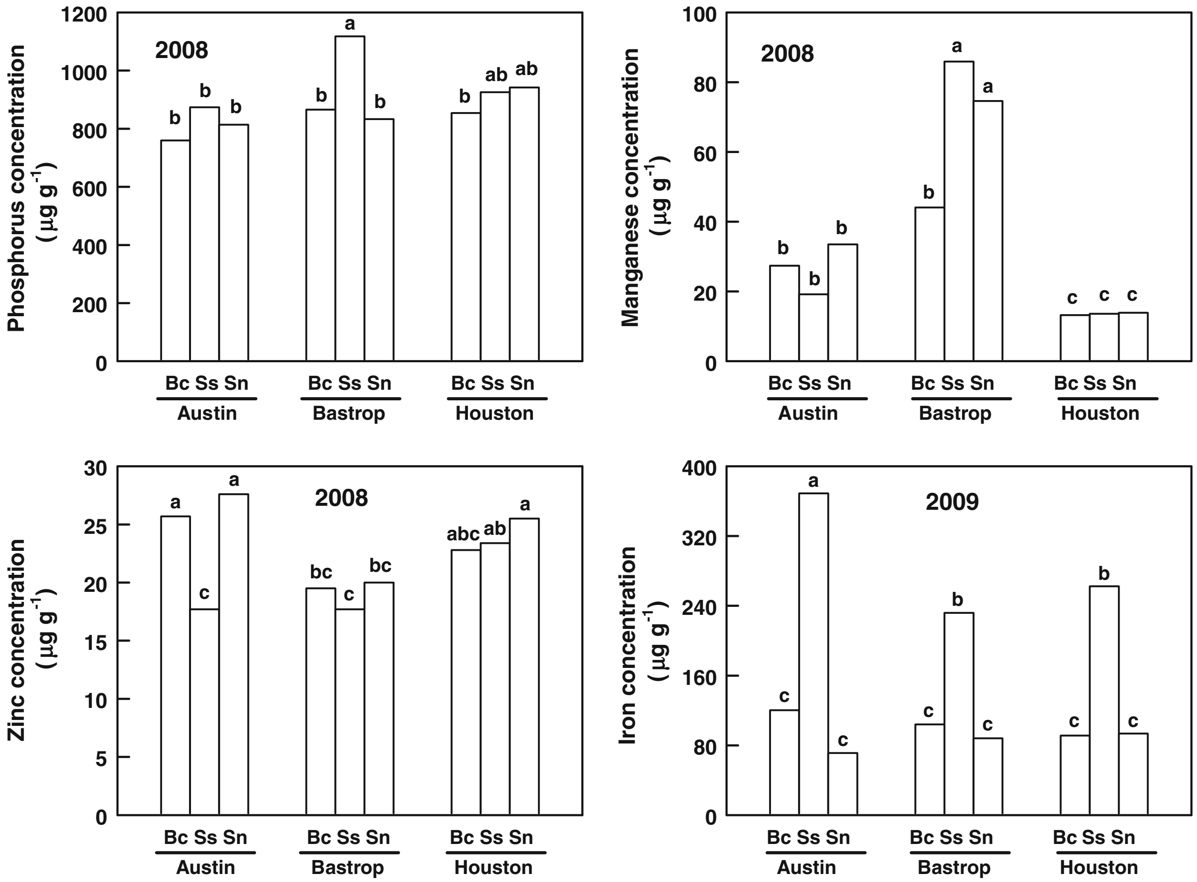

Fig. 1 Mean values of element concentrations in tillers of the C_4 grasses *Bouteloua curtipendula* (Bc), *Schizachyrium scoparium* (Ss), and *Sorghastrum nutans* (Sn) grown along a subambient to elevated gradient in atmospheric CO_2 concentration on soils of each of three types (Austin, Bastrop, Houston). Values for a given element are averaged over CO_2 treatments during a given year (2008, 2009) and do not differ significantly if labeled with the same letter (n = 6–11)

2009 (Tables 1, 2), but changed the mean level of these elements in biomass of the three grasses combined by changing species abundances. We calculate that increasing CO_2 from 280 to 480 µmol mol^{-1} increased average concentrations of K, Mg, and Mn in grass biomass by 18, 31, and 7%, respectively, on the Austin soil during the first year of 2008 (Table 3) by increasing cover of *Sorghastrum* at the expense of *Bouteloua*, the grass with the lowest concentration of each element. Similarly, CO_2 enrichment increased average concentrations of K, Mg, P, and Ca in grass biomass by 20, 35, 10, and 12%, respectively, on the Austin soil during the second year of measurements (2009) by favoring *Sorghastrum* over *Bouteloua*. By contrast, higher CO_2 reduced [N] by 6% in biomass of grasses combined on the Austin soil in 2009 (not shown). CO_2 enrichment reduced the [P] of *Bouteloua* and [Ca] of both *Schizachyrium* and *Bouteloua* across all soils combined in 2008 (Fig. 2). Effects of these trends on the average [P] and [Ca] of dominant grasses growing on the Austin soil were partially offset by the decline in *Bouteloua* cover at higher CO_2. The [P] and [Ca] of dominant grasses declined by 9% and 7% from 280 to 380 µmol mol^{-1} CO_2 in 2008 (Table 3; from 913 to 828 µg g^{-1} and 5,706 to 5,362 µg g^{-1}, respectively), but changed little at elevated CO_2.

Discussion

Element concentrations

Concentrations of fewer elements differed as a function of CO_2 treatment than among grass species

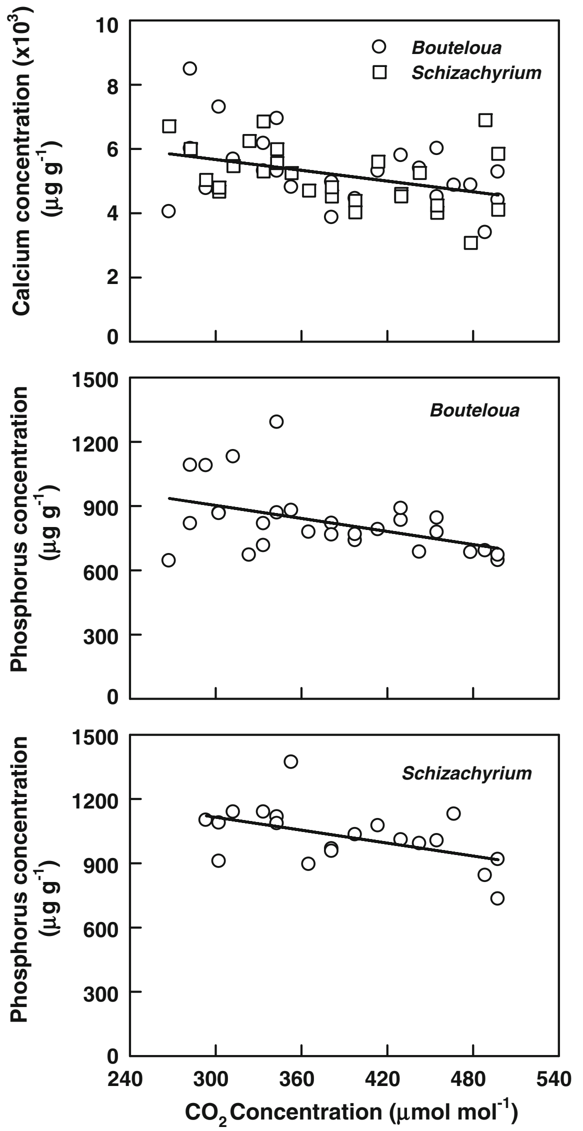

Fig. 2 Significant relationships between element concentrations in tillers of the C_4 grasses *Bouteloua curtipendula* and *Schizachyrium scoparium* and CO_2 treatment. Lines are linear regression fits to data combined across three soil types and two grass species for calcium in 2008 ($n = 53$, $P = 0.003$) and three soil types for phosphorus in *Bouteloua* in 2008 ($n = 28$, $P = 0.02$) and *Schizachyrium* in 2009 ($n = 20$, $P = 0.02$)

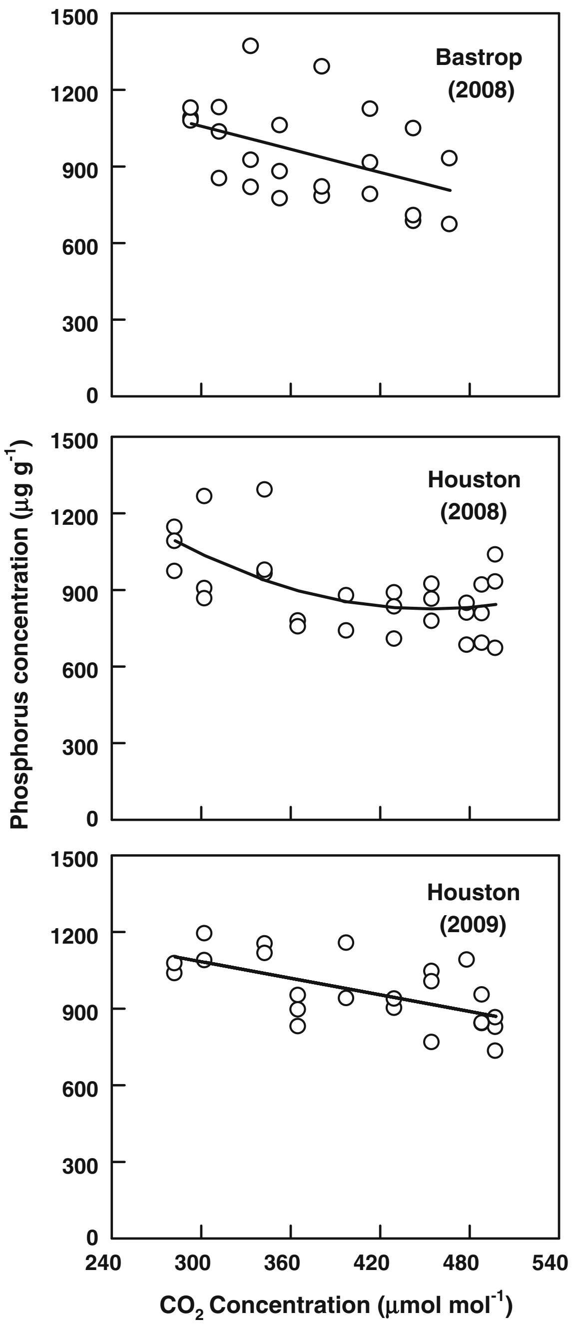

Fig. 3 Relationships between the phosphorus concentration in tillers of C_4 grasses and CO_2 concentration. Lines are regression fits to data combined across three grass species for the Bastrop soil ($n = 23$, $P = 0.01$) and Houston soil ($n = 28$, $P = 0.004$) in 2008 and Houston soil in 2009 ($n = 23$, $P = 0.002$)

and soils. Increasing CO_2 from pre-Industrial to elevated levels reduced tiller [P] in grasses grown on the Houston and Bastrop soils. Concentrations of most of the other elements analyzed did not respond to CO_2 treatment, contrary to our prediction. The limited response of most elements, including N, to CO_2 may reflect the absence of a CO_2 effect on LAI and, by inference, on aboveground biomass at the June sampling. The effect of CO_2 on species relative abundances was far more important than CO_2-caused

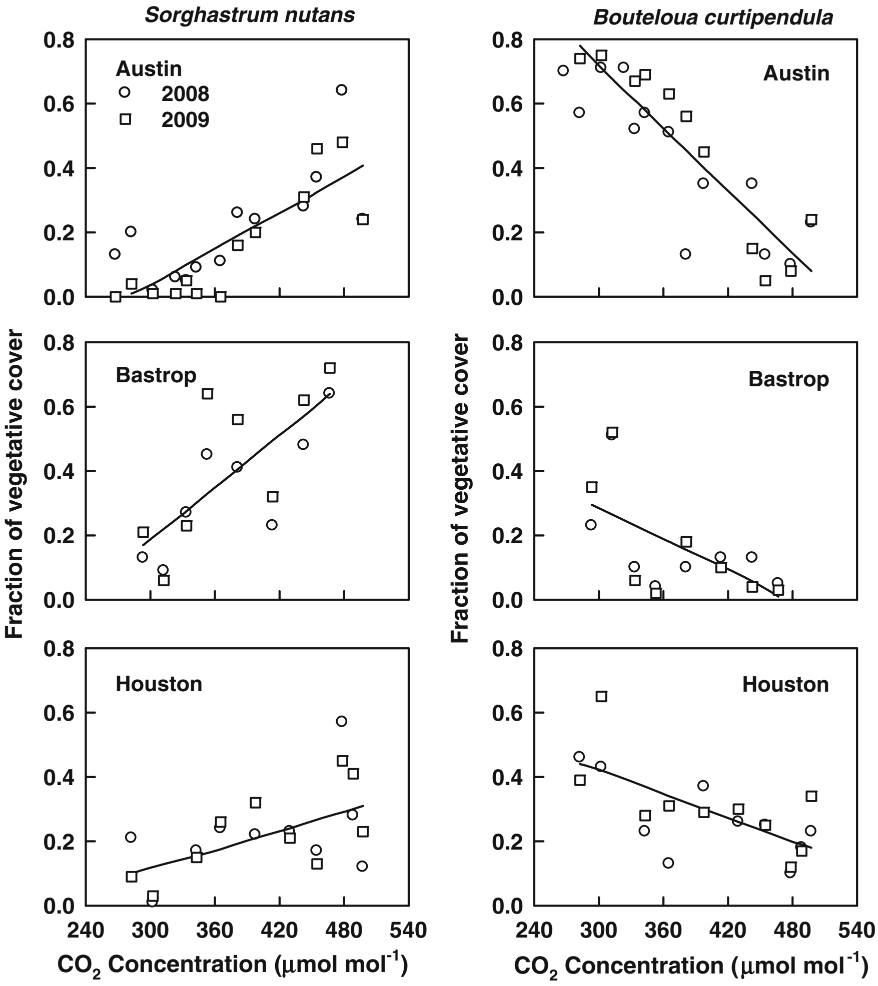

Fig. 4 The contribution of the C$_4$ grasses *Sorghastrum nutans* and *Bouteloua curtipendula* to cover of tallgrass prairie vegetation in June of the third (2008) and fourth (2009) years of exposure along a subambient to elevated gradient in CO$_2$ concentration. Lines are regression fits to data from both years combined for each of three soil types for each species, *Sorghastrum* [$n = 26$, $P < 0.0001$ (Austin); $n = 16$, $P < 0.0001$ (Bastrop); $n = 20$, $P = 0.03$ (Houston)] and *Bouteloua* [$n = 26$, $P < 0.0001$ (Austin); $n = 16$, $P = 0.008$ (Bastrop); $n = 20$, $P = 0.005$ (Houston)]

changes in element levels of individual species to the nutritional status of grasses in these tallgrass prairie assemblages. Cover of the mid-grass *Bouteloua* declined markedly at higher CO$_2$ levels as cover of the taller grass *Sorghastrum* increased. Concentrations of several elements were smaller in *Bouteloua* than *Sorghastrum*, hence this exchange of species as CO$_2$ increased likely improved the overall nutritional status of grasses, as predicted. For example, we calculated that the shift in relative dominance from *Bouteloua* to *Sorghastrum* at higher CO$_2$ increased tissue concentrations of K, Mg, and Mn (2008) and K, Mg, P, and Ca (2009) in the combined biomass of grasses grown on the Austin soil.

We did not observe the general decrease in element concentrations often reported with elevated atmospheric CO$_2$ (Overdieck 1993), especially when results from multiple studies are averaged (Loladze 2002). Contrary to our prediction that CO$_2$ enrichment would reduce element concentrations, we found that CO$_2$ affected tiller levels of only one of the elements (P) that was present in grasses at concentrations considered inadequate to support cattle. Somewhat surprisingly, CO$_2$ enrichment did not affect the [N] of grass tillers on any of the three soil types studied. A decrease in the amount of N present per unit of leaf or aboveground biomass is one of the more repeatable effects of CO$_2$ enrichment, although the relative decrease in [N] at elevated CO$_2$ is smaller for C$_4$ than C$_3$ plants (Cotrufo et al. 1998). Indeed, elevated CO$_2$ did not significantly affect the leaf [N] of the wild C$_4$ grasses included in the meta-analysis of Wand et al. (1999). A decrease in [N] at higher CO$_2$ usually is associated with an increase in plant biomass and attributed to N dilution by the additional carbohydrates accumulated

Table 3 Effects of CO_2 treatment on concentrations of selected chemical elements in the combined biomass of three species of C_4 grasses (*Bouteloua, Schizachyrium*, and *Sorghastrum*) grown on the Austin soil

CO_2 concentration (μmol mol^{-1})	Chemical element				
	Potassium (μg g^{-1})	Calcium (μg g^{-1})	Phosphorus (μg g^{-1})	Magnesium (μg g^{-1})	Manganese (μg g^{-1})
	2008				
280	8603	5706	913	705	26.9
330	8952	5502	864	755	27.3
380	9322	5362	828	809	27.7
430	9715	5298	809	865	28.2
480	10132	5291	808	925	28.7
	2009				
280	8009	4827	835	705	18.2
330	8195	4888	845	733	18.2
380	8542	5022	863	787	18.2
430	8989	5195	885	856	18.1
480	9585	5427	916	948	18.1

The concentration of each element in grass biomass was calculated for each year (2008, 2009) at each CO_2 concentration by weighting measured element concentrations of *Bouteloua, Schizachyrium*, and *Sorghastrum* by the contribution of each species to the total cover of grasses. The cover of each species was calculated as a function of CO_2 using linear regressions fit to cover–CO_2 relationships for each year. The element concentrations used for each species in calculations were derived by either of two methods. We used the mean element concentration from all CO_2 treatments combined when CO_2 had no effect on element levels. Alternatively, element concentrations were calculated using regressions fit to element–CO_2 relationships (Fig. 2)

at high CO_2 (Loladze 2002; Taub and Wang 2008). CO_2 did not affect LAI and, by inference, aboveground biomass of the prairie assemblages we studied, at least at the June sampling. Similarly, elevated CO_2 had no effect on photosynthesis, biomass, or the N status of maize (*Zea mays*) in the absence of water stress (Leakey et al. 2006). We sampled tillers during the period when grasslands in our area typically are mowed for hay and before reproductive culms of C_4 species elongate. Grasslands in central Texas usually approach maximal physiological activity during June (Mielnick et al. 2001), but aboveground biomass in June is only about 50% of annual biomass production for tallgrass prairies in the area (Polley et al. 2007). Perhaps, CO_2 effects on element concentrations would have been larger had we harvested tillers late in the season after soils dried and the positive effect of CO_2 on water use efficiency had been expressed as greater C_4 biomass (Cunniff et al. 2008; Gerhart and Ward 2010). Alternatively, CO_2 effects on element concentrations simply may vary among years because of inter-annual variation in factors other than CO_2 that affect plant growth and element availability in soil. For example, higher CO_2 reduced the [Ca] in tillers of two grasses in 2008, but did not affect the [Ca] of any species in 2009. Similarly, an increase in CO_2 from subambient to elevated levels reduced the [N] of aboveground tissues of the dominant C_4 grass in mesic grassland during 2 years of treatment, but did not affect [N] during two additional years (Polley et al. 2003). The response to CO_2 also may vary among elements, depending on soil characteristics and plant demand. For instance, CO_2 enrichment reduced concentrations of K and Na in aboveground material from fertilized mixtures of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.), but increased tissue [Ca] and had no effect on [P], [Mg], and [S] in mixtures (Schenk et al. 1997).

Species abundances

The CO_2 effect on relative abundances of *Bouteloua* and *Sorghastrum* had far greater impact on element concentrations in grass stands than did change in element levels of individual species. Elements that were most limiting relative to the nutritional requirements of cattle generally occurred at lowest concentrations in *Bouteloua*, the species most strongly disadvantaged at elevated CO_2. Consistent with our prediction, CO_2 enrichment favored a grass, *Sorghastrum*, with relatively high concentrations of these elements, thereby increasing mean concentrations of several elements in grass assemblages. The tradeoff in cover of *Bouteloua* and *Sorghastrum* as CO_2 increased was best expressed on the Austin soil where tiller concentrations of K, Mg, and Mn were, on average, >30% greater in *Sorghastrum* than *Bouteloua*. The exchange of *Bouteloua* for *Sorghastrum* at high CO_2 also offset effects of the CO_2-caused decrease in [P] of *Bouteloua* in 2008 on the average [P] of dominant grasses growing on the Austin soil. It is not clear why element concentrations often were smallest in *Bouteloua*. Element levels per unit of biomass in *Bouteloua* may have been reduced if the mid-grass accumulated silica to higher concentrations than other grasses. The silica content of forage grasses averages about 2–3% of plant biomass (Vicari and Bazely 1993), but can be much greater in some grasses (McNaughton et al. 1985). In addition, some of the elements present at relatively low levels in *Bouteloua* have limited mobility or solubility in calcareous soils (Mengel and Kirkby 1982). These elements include P, K, and Mn. Plants may improve acquisition of immobile elements by developing a more extensive root system and longer and thinner roots. Mycorrhizal associations have been shown to increase the concentration of P and other elements in crop plants (White and Broadley 2009). Data on rooting patterns of *Bouteloua* and *Sorghastrum* are limited, especially for plants grown on the same soil type. Wilsey and Polley (2006), however, found that root biomass over the deepest soil increment sampled (20–45 cm) was much greater for *Sorghastrum* than *Bouteloua* during the second year of growth in field plots on the Austin series soil. The fraction of end-of-season plant biomass in roots was similar to or slightly smaller for *Bouteloua* than both *Schizachyrium* and *Sorghastrum*.

Increasing CO_2 concentration favored *Sorghastrum* over *Bouteloua* apparently by reducing rates of soil water depletion. CO_2 enrichment has been shown to change species abundances in ecosystems as diverse as Swiss grassland and semi-arid shortgrass steppe by slowing evapotranspiration and the rate at which soil water content declines (Niklaus et al. 2001; Morgan et al. 2004a).

Our results highlight the importance of accounting for change in species abundances and composition when predicting CO_2 effects on ecosystem functioning and services (Polley et al. 2010). By favoring one grass species over another, CO_2 enrichment from pre-Industrial to elevated levels increased concentrations of several nutritionally important elements in prairie grasses.

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