

# Interactions between elevated atmospheric CO<sub>2</sub> and defoliation on North American rangeland plant species at low and high N availability

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## Abstract

Although common disturbances of grazing lands like plant defoliation are expected to affect their sensitivity to increasing atmospheric CO<sub>2</sub> concentration, almost no research has been conducted to evaluate how important such effects might be on the direct responses of rangelands to CO<sub>2</sub>. This growth chamber experiment subjected intact plant–soil cylinders from a Wyoming, USA, prairie to a 3-way factorial of CO<sub>2</sub> (370 vs. 720  $\mu\text{L L}^{-1}$ ), defoliation (non-clipped vs. clipped) and soil nitrogen (control vs. 10 g m<sup>-2</sup> added N) under simulated natural climatic conditions. Above- and below-ground biomass and N dynamics of the functional groups C<sub>3</sub> grasses, C<sub>4</sub> grasses and forbs were investigated. CO<sub>2</sub> and defoliation had independent influences on biomass and N parameters of these rangeland plants. Growth under CO<sub>2</sub>-enriched conditions enhanced above-ground biomass 50% in C<sub>3</sub> grasses alone, while shoot N concentration declined 16% in both C<sub>3</sub> and C<sub>4</sub> grasses. Plant–soil <sup>15</sup>N uptake was unaffected by CO<sub>2</sub> treatment. In contrast, defoliation had no effect on biomass, but increased tissue N concentration 29% across all functional groups. Without additional N, forage quality, which is in direct relation to N concentration, will decline under increasing atmospheric CO<sub>2</sub>. Increased dominance of C<sub>3</sub> grasses plus reduced forage quality may necessitate changes in grazing management practices in mixed-species rangelands.

**Keywords:** semi-arid rangeland, CO<sub>2</sub>, defoliation, nitrogen, C<sub>3</sub> grass, C<sub>4</sub> grass, forb, root, biomass, <sup>15</sup>N recovery, forage quality.

## Introduction

About 40% of terrestrial ecosystems are classified as rangelands (Suttie *et al.*, 2005). Rangeland ecosystems are not characteristically productive lands, but they support most of the world's managed livestock in addition to large herds of native ungulates (Campbell *et al.*, 1997). The productivity of rangelands may be slowly increasing owing to the fertilization effects of rising levels of atmospheric CO<sub>2</sub> (Polley, 1997; Morgan *et al.*, 2001a, 2004b). However, the consequences of rising atmospheric CO<sub>2</sub> concentrations on plant–herbivore dynamics like defoliation, that influence the ecology of rangelands, are relatively unexplored.

Elevated CO<sub>2</sub> is well known to increase above-ground plant productivity (Korner, 2000; Morgan *et al.*, 2004a; De Graaff *et al.*, 2006). Owing to greater photosynthetic enhancement, C<sub>3</sub> plant growth is stimulated more by elevated CO<sub>2</sub> than C<sub>4</sub> species (Korner, 2000; Reich *et al.*, 2001). However, there are several reports of improved productivity in C<sub>4</sub> rangeland species (Read 1996; LeCain and Morgan, 1998; Wand *et al.*, 1999; Owensby *et al.*, 1999; LeCain *et al.*, 2003). This has been attributed to improved water-use efficiency and soil-water status resulting from reduced stomatal conductance and plant transpiration under elevated CO<sub>2</sub> (Volk *et al.*, 2000; Nelson *et al.*, 2003). This stomatal response occurs similarly in both C<sub>3</sub> and C<sub>4</sub> grasses (Wand *et al.*, 1999) and is a major factor boosting productivity in CO<sub>2</sub>-enriched semi-arid rangelands (Morgan *et al.*, 2011). In many temperate rangelands (such as much of the USA Great Plains), C<sub>3</sub> grasses are the major forage during spring while C<sub>4</sub> grasses are the primary mid-late-season forage; forbs are typically a less important food

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source (Milchunas *et al.*, 1995). Rangeland forb production may be increased by higher atmospheric CO<sub>2</sub>, presumably because most rangeland forbs are C<sub>3</sub> photosynthesis types (Reich *et al.*, 2001; Teyssonneyre *et al.*, 2002; Polley *et al.*, 2003). In mixed-species rangelands, CO<sub>2</sub>-induced shifts in the balance of C<sub>3</sub> and C<sub>4</sub> grasses and forbs have the potential to significantly alter community structure and ecosystem processes, including animal grazing processes (Campbell and Stafford Smith, 2000; Morgan *et al.*, 2007).

In most rangelands, there is more plant matter below than above ground (Milchunas and Lauenroth, 1993, 2001). Although there are many reports on the influence of elevated CO<sub>2</sub> on root productivity, the results vary widely between ecosystems and sampling methods and a conceptual understanding of root responses has not emerged (Hebeisen *et al.*, 1997; Wilsey *et al.*, 1997; Morgan *et al.*, 2001b; Milchunas *et al.*, 2005b; De Graaff *et al.*, 2006). In a field study on the shortgrass steppe of Colorado, we reported little change in root biomass after 5 years of elevated CO<sub>2</sub> (LeCain *et al.*, 2006). However, studies investigating the interaction of elevated CO<sub>2</sub> and grazing on root growth are rare (Augustine *et al.*, 2010).

As it is difficult to evaluate how grazing might interact with CO<sub>2</sub> levels in sparsely stocked rangelands, defoliation has been used to simulate grazing. Grazing or defoliation alone can increase or decrease plant productivity depending on grazing intensity and the grazing tolerance of species (Hendon and Briske, 2002). Forbs are generally thought to be less grazing tolerant than grasses because of herbivory-exposed meristems (Heady and Child, 1994). While CO<sub>2</sub> enrichment might be expected to enhance plant recovery from defoliation, because of more available CO<sub>2</sub> and soil water (Wand and Midgley, 2004), this has not always been found (Polley *et al.*, 2011). Elevated CO<sub>2</sub> and defoliation may have opposing as well as additive effects on plant productivity, species composition and allocation of resources (Morgan *et al.*, 2001a; Harmens *et al.*, 2004; Wand and Midgley, 2004; Lau and Tiffin, 2009; Augustine *et al.*, 2010). Consequently, it is presently unclear how defoliation interacts with rising atmospheric CO<sub>2</sub> to affect rangeland productivity and ecology, particularly in a mixed-species ecosystem.

In many arid and semi-arid rangelands, nitrogen (N) dynamics are second only to precipitation in importance for plant–ecosystem processes (Burke *et al.*, 1997). Responses of plant productivity to elevated CO<sub>2</sub> and defoliation, and the resulting changes in ecosystem functioning, are co-dependent on the cycling of N through the plant–soil–atmosphere. In the long term, plants will not sustain increased productivity under elevated CO<sub>2</sub> when other factors become growth limiting (De Graaff *et al.*, 2006). Nitrogen limitation is

expected to be a major factor in rangelands as many rangeland soils are N limiting for plant growth (Hungate *et al.*, 1997). Many studies report reduced plant-tissue N concentration under elevated CO<sub>2</sub> (Wilsey *et al.*, 1997; Morgan *et al.*, 2004a; Milchunas *et al.*, 2005a; Reich *et al.*, 2006) because of improved nitrogen-use efficiency or reduced soil-N availability (King *et al.*, 2004; Wand and Midgley, 2004). Although productivity may increase under elevated CO<sub>2</sub>, reduced tissue N and protein concentration will have negative consequences for domestic and indigenous fauna (Campbell and Stafford Smith, 2000; Milchunas *et al.*, 2005a). Conversely, defoliation can increase N concentration in regrowth tissue (Milchunas *et al.*, 1995, 2005a; Mikola *et al.*, 2005), but in the long term, reduced soil-N availability under elevated CO<sub>2</sub> may lessen this response.

Our objective was to investigate how defoliation and soil-N availability interact to affect the above- and below-ground productivity (assessed as plant biomass) and tissue N responses of a semi-arid grassland to growth under present-day [CO<sub>2</sub>] and CO<sub>2</sub>-enriched conditions. In this experiment, we utilized perennial plant-intact soil microcosms extracted from a northern USA mixed-grass prairie and subjected them to manipulations of defoliation, CO<sub>2</sub> and N. Responses were evaluated for the three major plant functional groups, C<sub>3</sub> grasses, C<sub>4</sub> grasses and forbs. Our hypotheses were as follows:

- H1: Elevated CO<sub>2</sub> will increase shoot biomass in forbs and C<sub>3</sub> grasses, with little response observed in C<sub>4</sub> grasses and roots. Defoliation will reduce shoot biomass in forbs, but elevated CO<sub>2</sub> will improve recovery from defoliation.
- H2: Enhanced shoot biomass under elevated CO<sub>2</sub> will reduce tissue N concentration and N uptake (measured by <sup>15</sup>N) in all functional groups, particularly without N addition. Defoliation and N addition will counter the effect of elevated CO<sub>2</sub> on these N parameters.
- H3: Elevated CO<sub>2</sub> will increase volumetric soil-water content (VWC) that will contribute to increased biomass of all functional groups. Defoliation will have minimal effect on VWC.

## Materials and methods

### Site characteristics

Plant–soil cylinders for this experiment were extracted from the USDA-ARS High Plains Grasslands Research Station (HPGRS), near Cheyenne, WY, USA (41°11' N lat, 104°54' W long). The HPGRS is within the northern mixed-grass prairie, with elevations ranging from 1910 to 1950 m, an average 127-d growing

season, and annual precipitation of 384 mm. Site vegetation is mostly comprised of grasses, averaging 55% C<sub>3</sub> grasses and 23% C<sub>4</sub> grasses plus a variety of forbs, sedges and sub-shrubs. In our cylinders, there were typically four C<sub>3</sub> grasses dominated by *Pascopyrum smithii* [Rydb.] A. Love, two C<sub>4</sub> grasses dominated by *Bouteloua gracilis* [H.B.K.] Lag. and five forbs dominated by *Sphaeralcea coccinea* [Nutt.] Rydb. Soils are Ascalon sandy loams. The pasture from which cylinders were extracted had been lightly grazed by cattle (annual removal of 30% vegetation) since 1982 (LeCain *et al.*, 2000).

### Experimental treatments

In early April of year one (plants dormant), forty PVC cylinders measuring 30 cm tall by 25 cm diameter were pushed into the soil, then extracted with intact plant–soil cores, capped at the bottom and transported to the USDA-ARS Crops Research Lab (Fort Collins, CO, USA). Eight cylinders were immediately harvested for plant and soil analyses (T0). The soil of each of the remaining thirty-two cylinders was injected with 1 g m<sup>-2</sup> <sup>15</sup>N (as KNO<sub>3</sub>, 99 atom% <sup>15</sup>N). The cylinders were randomly separated into four groups of eight; each group was then placed into four growth chambers (EGC, Chagrin Falls, OH, USA), two at present atmospheric CO<sub>2</sub> concentration (ACO<sub>2</sub>) and two others at 720 μL L<sup>-1</sup> (ECO<sub>2</sub>).

The eight plant–soil cylinders within each of the four growth chambers were randomly partitioned into two groups of four, one receiving supplemental N in the amount of 10 g m<sup>-2</sup> as KNO<sub>3</sub> (+N treatment), the other with no supplemental N (–N treatment). The first week of June, all vegetation in two of the cylinders from the +N and –N groups was defoliated to 2.5 cm height (+D treatment), which corresponds with typical late spring cattle grazing on this rangeland. The other two cylinders were not defoliated (–D treatment). The cylinders were destructively sampled for plant attributes at ecologically significant times: the time of typical peak green biomass, (15 July; T1), and at the end of the growing season but before leaf loss, (28 September; T2). Clipped tissue from the June defoliation treatment (+D) was dried and weighed and analysed with the same protocol as the two complete harvests (see below). To summarize, the experimental treatments consisted of two CO<sub>2</sub> × two defoliation × two nitrogen with two replications each, sampled on two harvest dates. The entire experiment was repeated the following year, extracting new plant–soil cylinders from the same pasture in early April. The T0 assessment showed that the soil texture and nutrients and initial species composition were similar between the 2 years (data not shown).

### Growth chamber conditions

Environmental conditions in the growth chambers simulated the growing season of SE Wyoming. Sodium vapour and metal halide lamps provided a realistic spectrum of light at 600 μmol m<sup>-2</sup> s<sup>-1</sup> photosynthetic photon flux density at plant height. Photoperiod and day/night temperature were adjusted weekly to mimic the seasonal climate in SE Wyoming. Long-term precipitation data were used to calculate weekly water additions similar to what occurs in the field, with most precipitation occurring in May, June and early July followed by late season drought. All cylinders were weighed weekly (prior to irrigations) to calculate VWC (m<sup>3</sup> m<sup>-3</sup>).

### Plant sampling

On the harvest dates, all plants were defoliated to 1 cm height and separated into C<sub>3</sub> grasses, C<sub>4</sub> grasses and forbs. The soil cores were pushed out of cylinders, and all visible roots were hand picked and washed. Sub-samples of all plant fractions were frozen in liquid N, lyophilized and ground to 0.5 mm for tissue analysis. The remaining shoot and root material was oven-dried at 60°C, weighed and sub-sampled for ash analysis: all data were ash-corrected. Above-ground plant biomass and nutrients collected from the June defoliation treatment were added (for biomass) and weighted (for nutrients) to the July and September data. Therefore, these data are cumulative for the +D treatment.

### Shoot and root tissue analyses

Total N and <sup>15</sup>N analyses were performed by combust-ing samples in a Carlo Erba Series II automated N/C analyzer interfaced with a Europa isotope-ratio mass spectrometer (Knowles and Blackburn, 1993).

We calculated <sup>15</sup>N recovery (% of applied <sup>15</sup>N) in the different plant N pools (<sup>15</sup>N<sub>rec, plant</sub>) by:

$$^{15}\text{N}_{\text{rec, plant}} = \text{N}_{\text{plant}} / \text{N}_{\text{label}} * (^{15}\text{N}_{\text{plant, T1}} - ^{15}\text{N}_{\text{plant, T0}}) / ( ^{15}\text{N}_{\text{label}} - ^{15}\text{N}_{\text{plant, T0}}) * 100$$

where N<sub>plant</sub> and <sup>15</sup>N<sub>plant</sub> are the total amount of N and <sup>15</sup>N atom% in the plants at T1 and T2, <sup>15</sup>N<sub>plant, T0</sub> is the average <sup>15</sup>N atom% in the plants at T0, and N<sub>label</sub> and <sup>15</sup>N<sub>label</sub> are the total amount of N and the <sup>15</sup>N atom% of the label applied.

### Statistics

The experiment had three measured or calculated parameters in both above- and below-ground tissues: biomass, %N and <sup>15</sup>N recovery. Interactions with the experimental treatments (CO<sub>2</sub>, nitrogen, defoliation)

and both 'Harvest Date' and 'Year' were rare for all experimental parameters; therefore, a conservative statistical analysis was made by pooling the data over Harvest Date and Year, using the separate growth chambers as replicates ( $n = 2$ ). The data were analysed using Proc Mixed (SAS Institute Inc., Cary, NC USA) as a split-split plot design, with CO<sub>2</sub> as main plots, N as a split plot and defoliation as a second split plot within N. Also, there were significant treatment by functional group interactions for all the experimental parameters (for biomass the functional group\* CO<sub>2</sub>  $P = <0.0001$ ); therefore, each functional group was analysed independently to investigate the unique responses of each.

## Results

### Biomass responses to CO<sub>2</sub>, defoliation and nitrogen in three functional groups

Elevated CO<sub>2</sub> significantly increased shoot biomass only in C<sub>3</sub> grasses ( $P = 0.0001$ ) and particularly when combined with the +N treatment (CO<sub>2</sub> \*N interaction ( $P = 0.02$ ); Table 1 and Figure 1). C<sub>4</sub> grass and forb shoot biomass did not respond to ECO<sub>2</sub> ( $P = 0.55$  and  $0.98$ ). The absence of a CO<sub>2</sub> effect on forb shoot biomass in particular was surprising (H1). However, forb biomass was low, and quite variable between cylinders (0–20%) suggesting that our sampling scheme may have been inadequate to detect treatment effects in this functional group. Contrary to our hypothesis (H1), there was no significant effect of the defoliation treatment on the total amount of shoot biomass that accrued over the growing season in forbs, or any functional group (Table 1); biomass was nearly equal in +D and –D. We observed no significant interaction between the CO<sub>2</sub> and defoliation treatments on above-ground biomass in any functional group. Nitrogen addition increased shoot biomass in both grass groups (C<sub>4</sub> grasses  $P = 0.03$ ; Figure 1), but for C<sub>3</sub> grasses, this was significant only in combination with ECO<sub>2</sub> (CO<sub>2</sub>\*Nit  $P = 0.02$ ; Figure 1). In contrast, +N did not increase forb biomass ( $P = 0.25$ ) but again forb variability was very high.

Roots could not be separated by functional group in these cylinders. Interactions between treatments were common with root biomass. Root biomass was not significantly increased by the main effect of ECO<sub>2</sub> ( $P = 0.22$ ) but was significantly reduced with defoliation ( $P = 0.002$ ; Table 1 and Figure 1). Unlike shoots, the effects of defoliation on root biomass interacted with CO<sub>2</sub> ( $P = 0.052$ ). Defoliation reduced root biomass overall, but to a larger extent under ECO<sub>2</sub> (Figure 1). Root biomass increased under +N, but only in combination with ECO<sub>2</sub> (CO<sub>2</sub>\*Nit  $P = 0.001$ ; Table 1 and Figure 1). It is important to note that many of the collected roots had formed prior to the experiment as

**Table 1** Probabilities from Proc Mixed analysis of CO<sub>2</sub>, (elevated and ambient), nitrogen (addition and control) and defoliation (defoliated and control), and their interactions, on plant biomass and nitrogen resources of C<sub>3</sub> grasses, C<sub>4</sub> – grasses, forbs and roots from the semi-arid prairie of NE Wyoming, USA

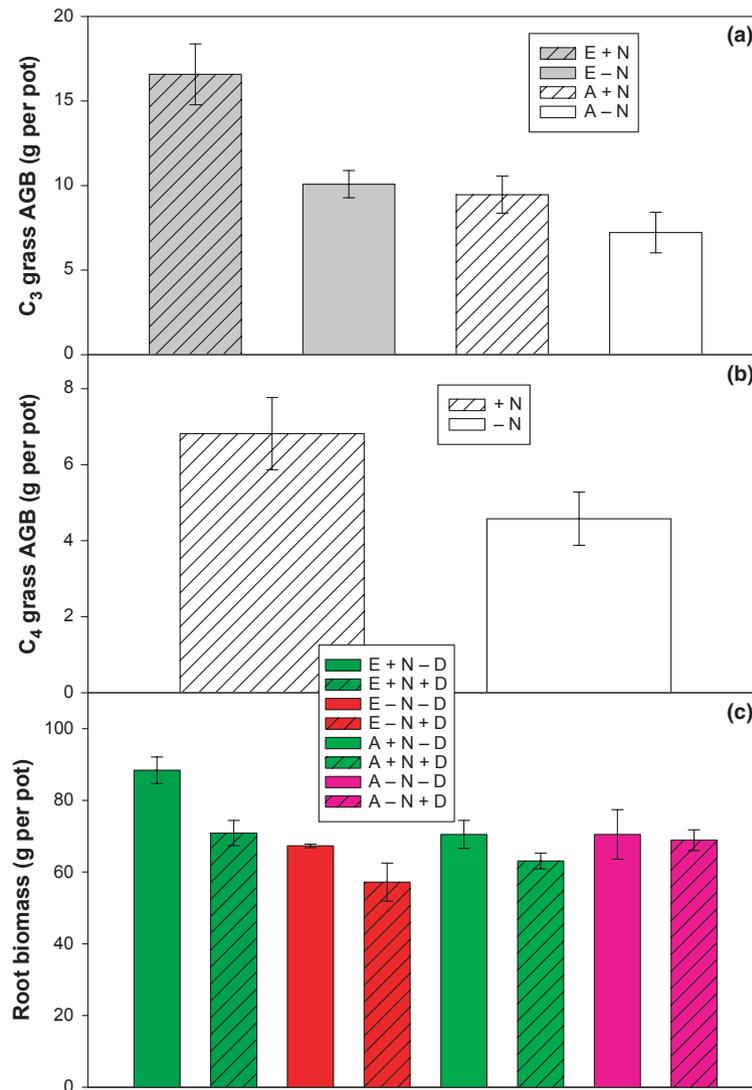
	Biomass	%Nitrogen	<sup>15</sup> N recovery
<b>C<sub>3</sub> grass</b>			
CO <sub>2</sub>	<b>0.0001</b>	<b>0.0538</b>	0.4100
NIT	<b>0.0003</b>	<b>0.0107</b>	0.3626
CO <sub>2</sub> *NIT	<b>0.0198</b>	<b>0.0357</b>	0.9324
DEFOL	0.9521	<b>0.0009</b>	<b>0.0100</b>
CO <sub>2</sub> *DEFOL	0.4118	0.6330	0.0697
NIT*DEFOL	0.6428	0.8049	0.8782
CO <sub>2</sub> *NIT*DEFOL	0.1654	0.5742	0.0835
<b>C<sub>4</sub> grass</b>			
CO <sub>2</sub>	0.5509	<b>0.0366</b>	0.3177
NIT	<b>0.0313</b>	<b>&lt;0.0001</b>	<b>0.0399</b>
CO <sub>2</sub> *NIT	0.3405	0.0772	0.1291
DEFOL	0.2047	<b>&lt;0.0001</b>	0.4113
CO <sub>2</sub> *DEFOL	0.8913	0.6961	0.3624
NIT*DEFOL	0.6314	0.7580	0.5581
CO <sub>2</sub> *NIT*DEFOL	0.1040	0.8806	0.1437
<b>Forbs</b>			
CO <sub>2</sub>	0.9894	0.5825	0.9508
NIT	0.2547	<b>0.0460</b>	0.3419
CO <sub>2</sub> *NIT	0.3841	0.3696	0.3219
DEFOL	0.1424	<b>0.0598</b>	0.0761
CO <sub>2</sub> *DEFOL	0.7072	0.3732	0.9451
NIT*DEFOL	0.5598	0.5030	0.2976
CO <sub>2</sub> *NIT*DEFOL	0.2514	0.3320	0.2619
<b>Roots</b>			
CO <sub>2</sub>	0.2198	0.1762	0.1405
NIT	<b>0.0072</b>	0.1184	<b>0.0089</b>
CO <sub>2</sub> *NIT	<b>0.0010</b>	0.1345	0.1011
DEFOL	<b>0.0020</b>	0.6311	<b>0.0002</b>
CO <sub>2</sub> *DEFOL	<b>0.0521</b>	0.5762	0.2154
NIT*DEFOL	0.1471	0.8499	0.3290
CO <sub>2</sub> *NIT*DEFOL	0.8432	0.6888	0.6810

Bold values are significant at  $P < 0.054$ .

root turnover in this ecosystem is about 5 years (Milchunas and Lauenroth, 2001). Therefore, our data show a short-term view of root responses and not a measure of actual root productivity (Milchunas, 2009).

### Nitrogen resource responses to CO<sub>2</sub>, defoliation and nitrogen

Defoliation and CO<sub>2</sub> treatments influenced shoot %N completely independently (Table 1). Supporting our hypothesis (H2), defoliation increased shoot %N in all



**Figure 1** Statistically significant effects of CO<sub>2</sub> (elevated and ambient: E and A), nitrogen (addition and control: +N and -N) and defoliation (defoliated and control: +D and -D) treatments on C<sub>3</sub> grass (a) and C<sub>4</sub> grass (b) above-ground biomass (AGB) and root biomass (c) in soil-plant cylinders extracted from a Wyoming, USA rangeland.

functional groups regardless of CO<sub>2</sub> or N treatment (C<sub>3</sub> grass, C<sub>4</sub> grass, forbs  $P = 0.0009, 0.0001, 0.059$ ; Tables 1 and 2). Also supporting H2, shoot N concentration (%N) declined in both E-CO<sub>2</sub>-grown C<sub>3</sub> and C<sub>4</sub> grasses particularly in the +N treatment (CO<sub>2</sub> \*N interaction in C<sub>3</sub> grasses  $P = 0.036$ ; Tables 1 and 2). The +N treatment increased %N in all functional groups, but in C<sub>3</sub> grasses, only significantly when grown under A-CO<sub>2</sub> (CO<sub>2</sub> \*Nit  $P = 0.036$ ; Table 2). In contrast to our hypothesis, forb %N was not reduced by E-CO<sub>2</sub> ( $P = 0.58$ ). There were no significant treatment effects on root %N (Table 1).

Similar to %N, CO<sub>2</sub> and defoliation treatments acted independently on <sup>15</sup>N recovery (Table 1). Contrary to H2, the recovery of <sup>15</sup>N was not reduced by CO<sub>2</sub> treatment in any of the functional groups or in roots (Table 1). However, defoliation reduced C<sub>3</sub> grass shoot <sup>15</sup>N recovery ( $P = 0.01$ ) (Tables 1 and 2). The +N treatment increased <sup>15</sup>N recovery in C<sub>4</sub> grasses, but not in the other groups ( $P = 0.04$ , Tables 1 and 2). Compared with controls, root <sup>15</sup>N recovery was higher under +N ( $P = 0.009$ ), but lower under +D ( $P = 0.0002$ ; Tables 1 and 2).

**Table 2** Means of significant CO<sub>2</sub> (elevated and ambient: E and A), nitrogen (addition and control: +N and -N) and defoliation (defoliated and control: +D and -D) treatment effects ( $P < 0.05$ ) for nitrogen resource parameters of functional groups and roots of the semi-arid Wyoming prairie: non-significant means in parenthesis

Treatment	C3 grass	C4 grass	Forb	Roots
%N				
A -N	1.03 b	1.07 b	1.20 b	(1.44)
A +N	1.48 a	1.32 a	1.43 a	(1.56)
E -N	0.97 b	0.92 c	1.15 b	(1.39)
E +N	1.04 b	1.08 b	1.51 a	(1.39)
-D	0.92	1.0	1.21	(1.46)
+D	1.35	1.2	1.51	(1.44)
<sup>15</sup> N recovery (%)				
-N	(10.6)	3.5	(2.1)	25.7
+N	(11.2)	5.5	(3.5)	30.3
-D	12	(4.8)	(1.45)	32.1
+D	9.69	(4.1)	(3.62)	23.9

When significant interactions occurred, Tukey's means comparison test was used; means with different letters are significantly different at  $P < 0.05$ .

### Soil water content responses to CO<sub>2</sub>, defoliation and nitrogen

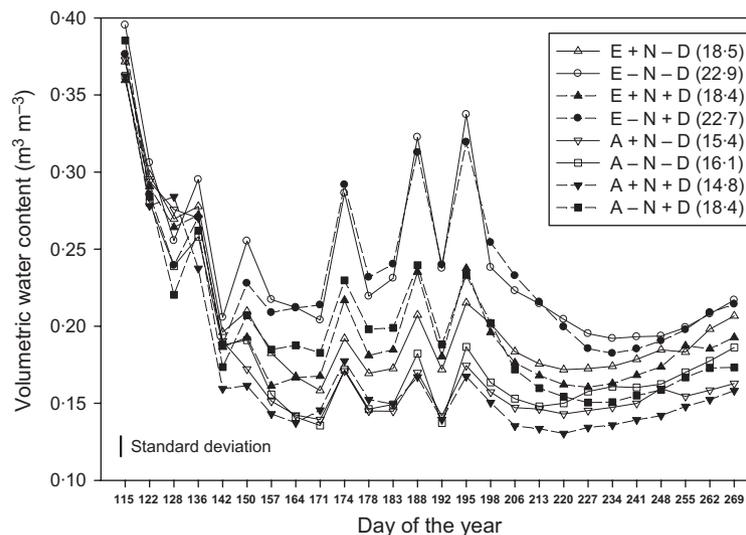
As water is the primary driver of productivity in this ecosystem, soil water was carefully monitored. Treatment differences in VWC were most apparent at certain times in the growing season. Therefore, all of the data

are shown in Figure 2, with averages in the figure legend. Supporting our hypothesis (H3), after an initial dry-down from winter-stored soil moisture, it was clear that the ECO<sub>2</sub>/-N treatment was the most conservative with soil water. The ACO<sub>2</sub>/+N treatment had the lowest VWC during much of the experiment. Therefore, ECO<sub>2</sub> promoted water conservation, while +N promoted water usage. Note that ECO<sub>2</sub> improves growth, while conserving water [improved water-use efficiency - water-use efficiency (WUE)], while +N improves growth while using more water (reduced WUE). Largely supporting our hypothesis (H3), defoliation treatment had only a small impact on VWC: average VWC was improved by +D in the ACO<sub>2</sub>/-N treatment (18.4% vs. 16.1% in +D vs. -D) but not in the other CO<sub>2</sub>/N combinations (Figure 2).

### Discussion

#### Above- and below-ground biomass responses of three functional groups to CO<sub>2</sub> and defoliation

Our most important finding was that CO<sub>2</sub> and defoliation treatments appeared to have independent influences on shoot growth (Table 1). Elevated CO<sub>2</sub> improved above-ground biomass only in the C<sub>3</sub> grass group, while there was no effect of defoliation on above-ground biomass of any functional group (defoliated and non-defoliated plants had equal biomass). This was contrary to our hypothesis that ECO<sub>2</sub> would increase forb growth and that ECO<sub>2</sub> would improve



**Figure 2** Effects of CO<sub>2</sub> (elevated and ambient: E and A), nitrogen (addition and control: +N and -N) and defoliation (defoliated and control: +D and -D) on volumetric soil water content in soil-plant cylinders extracted from a Wyoming, USA rangeland. The average volumetric soil water content (VWC) from day of year 142–269 for each treatment is shown in parenthesis in the legend.

recovery from defoliation. Our results differ from reports that forb production can be stimulated more than grasses by elevated CO<sub>2</sub> (Reich *et al.*, 2001; Teyssonneyre *et al.*, 2002; Polley *et al.*, 2003). However, forbs averaged <10% of the shoot biomass and were extremely variable between pots, potentially making detection of treatment effects difficult. Although the lack of a forb response was unexpected, it is not unique; in a California grassland, where annual plants dominate, ECO<sub>2</sub> 'alone' also had no effect on forb productivity. This was speculated to result from increased shading by the dominant grasses. However, the combination of the global changes ECO<sub>2</sub> + warming + precipitation increased the relative abundance of forbs (Zavaleta *et al.*, 2003a,b). A 9-year Free Air CO<sub>2</sub> Enrichment (FACE) study in Minnesota grassland, with highly mixed species composition, reported a strong reduction in photosynthetic capacity of forbs under ECO<sub>2</sub> but not in C<sub>3</sub> grasses, suggesting the potential for major shifts in species composition and diversity in mixed functional group grasslands (Crous *et al.*, 2010). More work is needed to establish these treatment responses of forbs in mixed-species grasslands. It is likely that grouping forbs into a single functional group is not appropriate, but future research might consider multiple 'forb' groups, such as annual forbs, perennial forbs, woody forbs, invasive forbs, etc. (Suding *et al.*, 2005).

Contrary to this study, we previously reported improved growth in C<sub>4</sub> grasses in controlled environment elevated CO<sub>2</sub> studies (Read and Morgan, 1996; LeCain and Morgan, 1998; Morgan *et al.*, 1998). However, those earlier experiments were performed in monoculture. The more realistic prairie microcosms used in this experiment represent natural plant communities and soils where muted responses to CO<sub>2</sub> sometimes occur because of competition for soil and climate resources (Campbell *et al.*, 1997; Owensby *et al.*, 1999; Korner, 2000; Morgan *et al.*, 2004b, 2011). Further, the sensitivity of C<sub>4</sub> grasses as well as intact grassland ecosystems to CO<sub>2</sub> is inversely proportional to the supply of soil water available to plants (Wand *et al.*, 1999; Joel *et al.*, 2001; Morgan *et al.*, 2004b; Wand and Midgley, 2004). Although CO<sub>2</sub> did induce water savings in this experiment and realistic field 'precipitation' resulted in terminal water stress in our microcosms (Figure 2), the potential benefit to the C<sub>4</sub> grasses was apparently insufficient to elicit a water relations-growth response. We conclude that the indirect effects of water savings on elevated CO<sub>2</sub>-improved plant growth depend on threshold-dependent soil moisture level as well as complicated interactions of multiple species with other climate and nutrient factors (Campbell *et al.*, 1997; Owensby *et al.*, 1999; Korner, 2000; Morgan *et al.*, 2004b, 2011).

Root biomass was not significantly increased under ECO<sub>2</sub>. While increased root productivity under elevated CO<sub>2</sub> has been observed in other experiments (Hebeisen *et al.*, 1997; Wilsey *et al.*, 1997; Morgan *et al.*, 2001b; De Graaff *et al.*, 2006), our results are consistent with other reports from near by semi-arid grasslands in which 5 years of CO<sub>2</sub> enrichment caused little change in root biomass in shortgrass steppe (LeCain *et al.*, 2006), and 4 years of CO<sub>2</sub> enrichment were required to detect significant changes in root biomass of a northern mixed-grass prairie (Morgan *et al.*, 2011). While exposure of grasslands to CO<sub>2</sub>-enriched atmospheres tends to enhance productivity and root growth, it also speeds up root turnover (Allard *et al.*, 2005; Milchunas *et al.*, 2005b). Thus, the biomass of perennial root systems may not change much over time, and multiple years of ECO<sub>2</sub> might be needed to observe any significant change.

Reduced root biomass, but not shoot biomass, with defoliation suggests resource translocation from roots to shoots after defoliation. This translocation may have been stronger under ECO<sub>2</sub>, where we observed greater reductions in root biomass with defoliation (CO<sub>2</sub>\*D  $P = 0.05$ ; Figure 1). We suspect the domination of this grassland community by C<sub>3</sub> grasses may be involved in this interaction. Remobilization of below-ground reserves to shoots after defoliation appears to be a more important response mechanism of *Pascopyrum smithii*, one of the dominant C<sub>3</sub> grasses in the mixed-grass prairie, compared with the C<sub>4</sub> dominant *Bouteloua gracilis* (Skinner *et al.*, 1999; Augustine *et al.* 2010). Greater remobilization of root reserves plus stronger responses of C<sub>3</sub> plants in general to CO<sub>2</sub> may tend to amplify this apparent defoliation response of plant community root biomass.

### The influence of available soil N on biomass responses to CO<sub>2</sub> and defoliation

CO<sub>2</sub> by N interactions on above-ground biomass only occurred in C<sub>3</sub> grasses. Our result of a stronger C<sub>3</sub> grass biomass response to CO<sub>2</sub> when more N is available agree with many reports that CO<sub>2</sub>-induced increases in plant productivity are often constrained by soil-N availability (Hebeisen *et al.*, 1997; Joel *et al.*, 2001; Grunzweig and Korner, 2003; De Graaff *et al.*, 2006). In our study, the amount of N uptake (<sup>15</sup>N) did not increase in conjunction with increased C resources (ECO<sub>2</sub>), resulting in reduced tissue %N in plants under elevated CO<sub>2</sub> (see below). We conclude that while CO<sub>2</sub>-enhanced water relations is a major factor behind the positive production effects of elevated CO<sub>2</sub> in dry ecosystems (Morgan *et al.*, 2004b, 2011), soil N may constrain those responses. There were no defoliation by N interactions in any of the experimental parameters,

and defoliation did not reduced above-ground biomass. We therefore conclude that above-ground productivity is not reduced by defoliation even under naturally low soil N availability.

Although not directly investigated in this study, increasing N deposition because of human activity is another important global change. Reports from other grasslands show that increased plant productivity, whether through ECO<sub>2</sub> or increased N deposition, can reduce plant diversity, primarily through elimination of rare species (Zavaleta *et al.*, 2003b; Suding *et al.*, 2005). Although our forb results were variable, the lack of a ECO<sub>2</sub> and +N response in forb biomass suggests that, similar to a native California grassland, forbs will be losers under multiple global changes as the dominant C<sub>3</sub> grasses out-compete them (Zavaleta *et al.*, 2003b).

### The influence of CO<sub>2</sub> and defoliation on plant N dynamics

Shoot %N was higher after defoliation in all functional groups, while ECO<sub>2</sub> reduced %N in grasses (Table 2), similar to results reported in an earlier open-top-chamber (OTC) experiment on the Colorado short-grass steppe (Milchunas *et al.*, 2005a). Thus, these observations support our second hypothesis that defoliation may ameliorate the ECO<sub>2</sub> effects on tissue N. Defoliation can improve tissue N through remobilization of N resources from roots and crowns and/or physiological signals to roots that temporarily improve root N uptake (Skinner *et al.*, 1999). Leaf tissue N may also be higher owing to younger leaves after defoliation (Milchunas *et al.*, 1995). <sup>15</sup>N recovery was reduced with defoliation in roots and C<sub>3</sub> grasses, suggesting that N uptake from the soil did not improve with defoliation and implying that improved shoot tissue %N was primarily from N remobilization after defoliation.

CO<sub>2</sub> by N interactions on shoot %N also only occurred in C<sub>3</sub> grasses (although a trend was seen for C<sub>4</sub> grasses:  $P = 0.077$ ). Reduced %N in plant tissues is a common response to elevated CO<sub>2</sub> (Morgan *et al.*, 2001b; Reich *et al.*, 2001; King *et al.*, 2004), particularly under limited soil-N availability and especially in C<sub>3</sub> plants, but we observed that ECO<sub>2</sub> reduced %N in C<sub>3</sub> plants only in the +N treatment (Tables 1 and 2), which was unexpected. Forb %N was not reduced by ECO<sub>2</sub>, which was also unexpected. Our results differ from those of a Minnesota prairie FACE study, where leaf N<sub>mass</sub> was strongly reduced in forbs but not in C<sub>3</sub> grasses (Crous *et al.*, 2010). The forb response was attributed to lesser roots and consequent nutrient foraging. It seems clear that comparisons between studies based on functional groups may be too simplistic (Suding *et al.*, 2005). C<sub>4</sub> grasses did not have a significant growth response to ECO<sub>2</sub>, but improved growth in the C<sub>3</sub> grasses likely

depleted N in the entire root zone, thereby reducing %N in C<sub>4</sub> grasses too (Table 2). Our results agree with an OTC study with similar species composition, which reported reduced %N in the dominant grass species under elevated CO<sub>2</sub> (King *et al.*, 2004). As with biomass, there were no interactions between CO<sub>2</sub> and defoliation on important N resources. Elevated CO<sub>2</sub> quite consistently reduced shoot tissue 'quality' of grasses (reduced %N). This will have negative consequences for indigenous and domestic grazing animals in world-wide grasslands. In this and other rangelands, grasses are the major forage for grazing animals. In N-limited soils, increased grass biomass may not compensate for reduced grass protein contents, and therefore, animal nutrition and landscape carrying capacity will suffer (Milchunas *et al.*, 2005a).

Many studies have investigated how 'global change' might alter nutrient cycling. Increased atmospheric CO<sub>2</sub> can alter soil N availability, potentially in several ways. Improved soil water content can improve microbial activity and N mineralization rates (Hungate *et al.*, 1997; Dijkstra and Cheng, 2008). Soil microbial activity could be improved by 'priming' with more abundant C from roots (Reich *et al.*, 2006). However, De Graaff *et al.* (2006) performed a meta-analysis on field studies (FACE and OTC) and concluded that gross and net N mineralization were not affected by ECO<sub>2</sub>, but that gross N immobilization increased by 22%. In a recent report from our same growth chamber study, we showed increased N immobilization into the soil under elevated CO<sub>2</sub>, which may further have reduced %N in both C<sub>3</sub> and C<sub>4</sub> plants (Dijkstra *et al.*, 2011).

From our study, it appears that 'grazing' is likely to have as much impact on some grassland ecosystem functions as increasing atmospheric CO<sub>2</sub>. Defoliation consistently increased shoot %N but reduced below-ground biomass. Also, defoliation reduced <sup>15</sup>N recovery by roots without improving recovery in shoots. Of course our experiment was short-term and long-term speculation should be made cautiously.

In this semi-arid and typically grazed ecosystem, plant processes are very much driven by soil water availability. Therefore, it is important to note that there were no apparent interactions between CO<sub>2</sub> and defoliation treatments on VWC. Overall, defoliation had little effect on VWC (Figure 2). Elevated CO<sub>2</sub> improved VWC an average of 27% over the growing season, while N addition reduced VWC by an average of 17%. Elevated CO<sub>2</sub> increased biomass (in C<sub>3</sub> grasses) while reducing water use; therefore, WUE was improved (~23%). In the field, we expect water savings owing to ECO<sub>2</sub> to significantly impact plant growth and nutrient cycling, primarily during below-normal precipitation years (Morgan *et al.*, 2004b).

## Conclusions

From our study, we conclude that in mixed-grass prairie ecosystems of the world, there will be little interaction between appropriate grazing practices and increasing atmospheric CO<sub>2</sub> on many important ecosystem processes. However, over the long-term, increasing atmospheric CO<sub>2</sub> will cause changes in ecosystem functions. Our results support other reports that show a shift in species composition to more C<sub>3</sub> grass dominance as atmospheric CO<sub>2</sub> increases (Korner, 2000; Reich *et al.*, 2001; Polley *et al.*, 2003; Morgan *et al.*, 2004a, 2007; Crous *et al.*, 2010). Increased competition with C<sub>4</sub> grasses would have negative consequences on availability of forage late in the growing season when C<sub>3</sub> grasses are dormant. We also show limited evidence that forbs will be out-competed in this grassland under global change. The resulting reduced plant diversity will reduce ecosystem adaptability to global changes (Reich *et al.*, 2004), suggesting the potential for a decline in the health and adaptability of mixed-species grasslands around the world. Improved available soil moisture under elevated CO<sub>2</sub> should increase plant productivity, but only near a critically low soil moisture threshold. Morgan *et al.* (2011) reported that water conservation under elevated CO<sub>2</sub> can compensate for desiccation under global warming and that combined elevated CO<sub>2</sub> and warming might favour some C<sub>4</sub> grasses. Therefore, ultimate species composition will depend on complex interactions of multiple biotic and abiotic factors. Elevated CO<sub>2</sub> quite consistently reduced forage 'quality' in this study. As it is impractical to add fertilizer to most rangelands, changes in grazing management strategies or supplemental feed may eventually be needed to cope with reduced forage quality and changing species composition.

This ecosystem has a long evolutionary history of grazing by large mammals and has been proven to be well adapted to grazing (Milchunas and Lauenroth, 1993). This and other rangeland ecosystems are less well adapted to global change induced perturbations on ecosystem processes. Understanding and planning for these perturbations remains a challenge for scientists and land managers. Not surprisingly, plant and ecosystem responses to atmospheric CO<sub>2</sub>, defoliation and nutrient resources depend on environmental thresholds that reflect many thousands of years of evolution.

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