Oecologia (2011) 165:755–770
DOI 10.1007/s00442-010-1845-4

ECOSYSTEM ECOLOGY - ORIGINAL PAPER

Rhizosphere interactions, carbon allocation, and nitrogen acquisition of two perennial North American grasses in response to defoliation and elevated atmospheric CO_2

David J. Augustine · Feike A. Dijkstra · E. William Hamilton III · Jack A. Morgan

Received: 17 February 2010 / Accepted: 4 November 2010 / Published online: 27 November 2010
© Springer-Verlag (outside the USA) 2010

Abstract Carbon allocation and N acquisition by plants following defoliation may be linked through plant–microbe interactions in the rhizosphere. Plant C allocation patterns and rhizosphere interactions can also be affected by rising atmospheric CO_2 concentrations, which in turn could influence plant and microbial responses to defoliation. We studied two widespread perennial grasses native to rangelands of western North America to test whether (1) defoliation-induced enhancement of rhizodeposition would stimulate rhizosphere N availability and plant N uptake, and (2) defoliation-induced enhancement of rhizodeposition, and associated effects on soil N availability, would increase under elevated CO_2. Both species were grown at ambient (400 $\mu L\ L^{-1}$) and elevated (780 $\mu L\ L^{-1}$) atmospheric [CO_2] under water-limiting conditions. Plant, soil and microbial responses were measured 1 and 8 days after a defoliation treatment. Contrary to our hypotheses, we found that defoliation and elevated CO_2 both reduced carbon inputs to the rhizosphere of *Bouteloua gracilis* (C_4) and *Pascopyrum smithii* (C_3). However, both species also increased N allocation to shoots of defoliated versus non-defoliated plants 8 days after treatment. This response was greatest for *P. smithii*, and was associated with negative defoliation effects on root biomass and N content and reduced allocation of post-defoliation assimilate to roots. In contrast, *B. gracilis* increased allocation of post-defoliation assimilate to roots, and did not exhibit defoliation-induced reductions in root biomass or N content. Our findings highlight key differences between these species in how post-defoliation C allocation to roots versus shoots is linked to shoot N yield, but indicate that defoliation-induced enhancement of shoot N concentration and N yield is not mediated by increased C allocation to the rhizosphere.

Keywords Global change · Grazing tolerance · Pulse dynamics · Rhizodeposition · Water relations

Introduction

The capacity for a plant species to persist in the presence of herbivores (grazing resistance) is related both to traits that prevent or minimize plant tissue loss to herbivores (grazing avoidance) and traits that contribute to rapid post-defoliation recovery of photosynthetic capacity (grazing tolerance; reviewed by Briske and Richards 1995). Grazing tolerance may be conferred by plant carbon allocation patterns that facilitate regrowth (e.g., Caldwell et al. 1981; Briske et al. 1996; Skinner et al. 1999) and the capacity for plants to rapidly acquire and allocate nutrients to leaves (e.g., Chapin and McNaughton 1989; Hamilton et al. 1998; Morgan et al. 2001). While C allocation and N acquisition are often studied separately, ecologists have increasingly recognized that linkages between these processes may underlie feedbacks between aboveground herbivores and belowground microbial activity, with significant implications for ecosystem function (Bardgett et al. 1998; Bardgett and Wardle 2003).

Plant C allocation and N acquisition can also be strongly affected by climate change. In semi-arid grasslands of

Communicated by Christian Körner.

D. J. Augustine (✉) · F. A. Dijkstra · J. A. Morgan
Agricultural Research Service, Rangeland Resources Research Unit, USDA, 1701 Centre Ave, Fort Collins, CO 80526, USA
e-mail: David.Augustine@ars.usda.gov

E. William Hamilton III
Department of Biology, Washington and Lee University, Lexington, VA 24450, USA

central North America, elevated [CO_2] can substantially increase plant productivity but also reduce grass leaf nitrogen concentration (Morgan et al. 2004a, b; Milchunas et al. 2005a). Increased plant production under elevated [CO_2] can arise in part from increases in water use efficiency, but soil N availability may not increase at the same rate (Luo et al. 2004; Morgan et al. 2004b; Dijkstra et al. 2008). Soil N availability and N concentrations in plant tissues frequently decline under elevated CO_2 in grasslands, which may be linked to microbial N immobilization following changes in the quality and quantity of exudate and litter inputs to the soil (e.g., Diaz et al. 1993; Hungate et al. 1997; Hu et al. 2001; Dijkstra et al. 2010). Soil N availability can strongly influence plant regrowth following defoliation, such that increasing [CO_2] could potentially limit aboveground plant regrowth and N concentration following defoliation (Morgan et al. 2001). Feedbacks between grazed grasses and soil microbial communities represent one way that plants could influence soil N availability following defoliation (Bardgett et al. 1998; Hamilton and Frank 2001; Bardgett and Wardle 2003), but little is known about how rhizosphere processes may respond to both grazing and climate change.

Some grazing-tolerant grass species have been shown to increase C inputs to the soil immediately following defoliation, which in turn stimulates microbial activity within the rhizosphere of the plant (Mawdsley and Bardgett 1997; Hamilton and Frank 2001; Paterson et al. 2003; Henry et al. 2008). Such a short-term (1- to 2-day) pulse in microbial activity and biomass has the potential to increase soil N mineralization (several days to a week following defoliation), thereby enhancing N uptake and regrowth by the plant (Hamilton and Frank 2001). However, the magnitude and direction of herbivore effects on rhizosphere process can vary with plant species identity and defoliation intensity (e.g., Guitian and Bardgett 2000; Mikola et al. 2001; Mikola and Kytoviita 2002; Dilkes et al. 2004; Fu and Cheng 2004). Many studies have documented declines in root growth and respiration following defoliation (Briske and Richards 1995), suggesting that defoliation may often reduce rhizodeposition. However, few studies have examined rhizosphere processes following defoliation, and it remains unclear what species or environmental conditions are likely to generate positive versus negative changes in post-defoliation rhizodeposition. The direction of this response may depend on soil N availability and the magnitude, location and composition of storage reserves in the plant, all of which can be influenced by rising atmospheric [CO_2] in semi-arid grasslands (Read and Morgan 1996; Morgan et al. 1998; Wilsey et al. 1997; Dijkstra et al. 2008). Thus, studies are needed that measure patterns of C allocation and N in parallel, and that test for potential interactive effects of defoliation and elevated [CO_2].

Grasslands in central North America have a long evolutionary history of grazing by large herbivores, and the dominant grasses in this region can persist under higher grazing pressure than dominant species in many other grasslands worldwide (Milchunas et al. 2008). In the semi-arid portion of the North American Great Plains, two of the most widespread grasses are blue grama (*Bouteloua gracilis*, C_4 perennial grass) and western wheatgrass (*Pascopyrum smithii*, C_3 perennial grass). Blue grama dominates much of the shortgrass steppe in the southwestern Great Plains, and western wheatgrass is often a dominant species in mixed-grass prairies of the northwestern Great Plains (Lauenroth et al. 1999). Both species are relatively palatable to domestic livestock and can undergo rapid aboveground growth after defoliation (Detling et al. 1979; Detling and Painter 1983). However, they respond differently to long-term grazing in field studies, with *B. gracilis* increasing and *P. smithii* decreasing (Milchunas et al. 2008).

Previous studies emphasized the importance of root carbohydrate remobilization and allocation aboveground to support leaf regrowth (e.g., Detling et al. 1979; Menke and Trlica 1981; Detling and Painter 1983), but have given less attention to rhizosphere processes and their effects on N acquisition for regrowth. Root exudates comprise 15–17% of total C fixed by *B. gracilis* and *P. smithii* under hydroponic conditions (Biondini et al. 1988) and at least 17% in native shortgrass steppe (Milchunas and Lauenroth 1992), but the effect of grazing on rhizodeposition has not been evaluated. Field studies suggest grazing does not influence the amount of N mineralized during laboratory incubations of soils from the shortgrass steppe (Burke et al. 1999), but have not examined grazing effects on microbes and N dynamics in soils with plant roots.

We studied *B. gracilis* (C_4) and *P. smithii* (C_3) because they differ in photosynthetic pathway, which is likely to influence their response to rising atmospheric CO_2 and potentially interact with their response to defoliation (Wilsey et al. 1997). We measured C allocation and rhizosphere processes (C deposition in the rhizosphere, changes in microbial C and N biomass, changes in soil inorganic N pools, and plant N uptake) following defoliation, and tested whether rhizosphere responses to defoliation interact with elevated atmospheric [CO_2]. Specifically, we hypothesized that (1) defoliation will stimulate C rhizodeposition compared to unclipped plants, which (2) will induce a short-term (24-h) increase in microbial biomass in the rhizosphere, and (3) increase subsequent N release from the microbial biomass and uptake of N by plants 1–8 days after defoliation. We also hypothesized that (4) elevated atmospheric [CO_2] will increase C rhizodeposition following defoliation, with a stronger response for the C_3 than the C_4 grass, and (5) this response will be associated with a

larger defoliation effect on plant N acquisition under elevated [CO_2] and for the C_3 compared to the C_4 grass.

Materials and methods

Soil was collected from the USDA-ARS Central Plains Experimental Range (CPER) in the shortgrass steppe of north-eastern Colorado. The soil was a sandy loam of the Ascalon series (Aridic Argiustolls) taken to 20-cm depth, was carbonate-free, had a pH of 6.6, and contained 0.95% total C and 0.09% total N. The soil was homogenized by sieving (4 mm) and air-dried before use. Polyvinylchloride cylinders 15 cm diameter by 45 cm deep were filled with a 33:33:33 mixture of the CPER soil, washed sand, and calcined clay. This mixture allowed for more effective separation of bulk soil from plant roots and rhizosphere soil (soil clinging to roots; see below) at the time of plant harvest compared to the use of pure grassland soil.

We used a factorial design with two grass species, two levels of atmospheric CO_2, and two levels of defoliation (clipped vs unclipped). Seeds of *P. smithii* ($n = 40$ columns) or *B. gracilis* ($n = 40$ columns) were sown in the soil mixture and columns were placed in a greenhouse at the USDA-ARS Crops Research Laboratory in Fort Collins, CO, USA, for establishment at ambient CO_2 and daytime temperatures of approximately 25°C. At 7 days after planting, the columns were divided between two greenhouses. Given that the Intergovernmental Panel on Climate Change predicts the earth's atmospheric CO_2 concentration will exceed 700 $\mu L\ L^{-1}$ by the end of the century, we raised the atmospheric CO_2 concentration in one greenhouse to a constant level of $780 \pm 50\ \mu L\ L^{-1}$ (average + standard deviation), while the other greenhouse was kept near ambient level ($400 \pm 40\ \mu L\ L^{-1}$). The CO_2 concentration was continuously monitored and the CO_2 supply was computer-controlled (Argus Control Systems, White Rock, BC, Canada). Air temperature was 27–29°C during the day and 16–18°C at night. Temperature was regulated by computer-controlled air conditioners and heaters (York International, York, PA, USA). Both greenhouses were equipped with 600-W lights (P.L. Light Systems, Beamsville, ON, USA) that were on during the day for 12 h. The light intensity in each greenhouse at plant height was $\sim 500\ \mu mol\ m^{-2}\ s^{-1}$ during the day. Relative humidity was $24 \pm 5\%$ during the day and $30 \pm 5\%$ during the night. Throughout the experiment, we switched the ambient and elevated CO_2 treatments and associated columns between the two greenhouses every 7–8 days. Thus, we note that the CO_2 treatment was not randomized at the level of individual columns, but columns were independent with respect to the soil–plant interactions that were the focus of our measurements. In addition, switching the CO_2 treatment between greenhouses weekly insured that any unknown variation between the greenhouses other than [CO_2] was experienced by both CO_2 treatments (Goverde and Erhardt 2003; Clark et al. 2010). Seedlings were thinned to eight per pot at 14 days after planting, and to six per pot at 22 days after planting.

Elevated CO_2 is known to influence plant growth in semi-arid grasslands by enhancing soil moisture availability during dry periods between precipitation events (LeCain et al. 2003; Morgan et al. 2004a). To evaluate the effect of elevated CO_2 on plant C allocation patterns under the type of water-limited conditions that these grass species experience in the shortgrass steppe, we applied a deficit irrigation regime to all treatments following Morgan et al. (1998). Columns were watered daily during seedling establishment (0–15 days after planting), and watered to field capacity 16 days after planting. No water was added for the subsequent 19 days. A clipping treatment (described below) was applied 62 days after planting. For consistency in terminology, hereafter we refer to dates in terms of the number of days before clipping (DBC) or days after clipping (DAC). Our deficit watering regime began 17 days after planting, which was equivalent to 44 DBC. Soil moisture was measured in the columns approximately weekly, and limited watering resumed at 27 DBC. Because these species can experience multiple defoliation events during the growing season under field conditions, all plants were defoliated to 10 cm height at 24 DBC. A total of 1,310 mL water per column (equivalent to 74 mm cumulative rainfall) was added during 44–10 DBC (Fig. 1). Leaf water potential was measured 3 DBC (prior to the watering event that day) with a Plant Measurement System (Corvallis, OR) pressure chamber using a recently expanded leaf from ten replicates of each treatment combination.

We used pulse additions of ^{15}N and ^{13}C to examine plant C and N allocation patterns in response to the clipping treatment. Plants in the clipped treatment were defoliated to a height of 10 cm to apply a moderate level of defoliation that approximated the recommended grazing intensity for the shortgrass steppe (Bement 1969). Defoliation to a constant height also allowed for differences in shoot morphology (more prostrate leaf angles in *B. gracilis* compared to *P smithii*) to influence defoliation intensity (see "Results"). All columns received the equivalent of a 48-mm rainfall event at 3 DBC and a 36-mm rainfall event at 4 DAC to provide sufficient moisture for plant regrowth after the clipping treatment.

Columns were labeled at 2 DBC with the equivalent of 0.5 g $^{15}N\ m^{-2}$ as $K^{15}NO_3$ (98 atom% ^{15}N) dissolved in 30 mL distilled water. The ^{15}N label was applied at this time to allow for partial uptake of ^{15}N by the soil microbial biomass and plants prior to the application of the clipping treatment. ^{15}N was added via five injections at a depth of

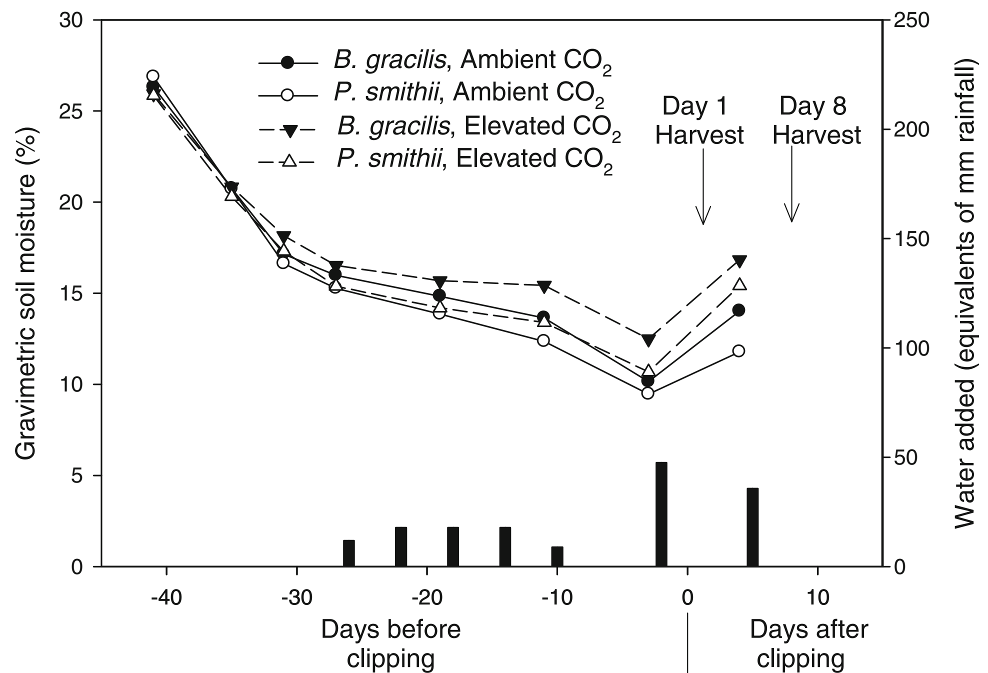

Fig. 1 Changes in soil moisture for two grass species and two CO_2 treatments over the course of the experiment in relation to amounts of water added to columns. *Error bars* are not shown for soil moisture to enhance visibility of treatment differences; soil moisture was significantly lower for *P smithii* compared to *B. gracilis* and for ambient compared to elevated CO_2 treatment during 30 DBC until 4 DAC, with a significant species $\times CO_2$ interaction only at 3 DBC due to a greater CO_2 effect on *B. gracilis* compared to *P. smithii* at that time (Table 1)

5 cm and five injections at a depth of 15 cm. Immediately after the clipping treatment, plants were placed within a sealed, solid polycarbonate chamber. Once atmospheric CO_2 in the chamber declined to 200 μL L^{-1}, we injected sufficient $^{13}CO_2$ (99 atom% ^{13}C) to raise atmospheric CO_2 in the chamber to 800 μL L^{-1} and kept plants in the chamber for 1 h after $^{13}CO_2$ addition. Two small fans operated continuously in the chamber, and internal temperature never exceeded 28°C.

Four replicate columns per each treatment were harvested at the following times: (1) immediately prior to the clipping treatment and ^{13}C labeling (only unclipped columns harvested at this time), which is referred to as day 0 in treatment analyses (because it was the day the clipping treatment was applied), (2) 24 h after application of the clipping treatment and 72 h after ^{15}N labelling (4 replicates from each of 8 treatment combinations), referred to as day 1 in treatment analyses, and (3) 8 days after the clipping treatment (4 replicates from each of 8 treatment combinations).

At the time of harvest, we carefully removed the cylinder of soil from the PVC column, and while holding the plants near the base of the crown, we gently shook the bulk soil away from the plant root mass. Remaining soil clinging to the roots was designated as rhizosphere soil, and was brushed into a collection pan. After brushing as much rhizosphere soil from the roots as possible, the roots were rinsed in 100 mL distilled water, and a subsample of the rinse water was retained for laboratory analysis. Rhizosphere soil was refrigerated until analysis for moisture content and microbial biomass within 24 h, and any visible roots were removed by hand prior to subsampling for analyses. Plant biomass from each harvested column was separated into root, crown and aboveground components, oven-dried at 55°C, and weighed. Plant biomass samples from the harvests, plus the biomass removed by the clipping treatment, were ground and analyzed for total C and N content with a CE Instruments NA-2100 autoanalyzer (Thermoquest, Milan, Italy), and were analyzed for ^{13}C and ^{15}N with a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon, Cheshire, UK) at the University of California, Davis Stable Isotope Facility.

Immediately prior to the harvests at 8 DAC, we measured photosynthesis rates for one leaf from a plant in each pot. Leaf assimilation of CO_2 (μmol m^{-2} s^{-1}) was measured using a portable gas analysis system (CIRAS-1 with PLC[N] leaf cuvette; PP Systems, Hitchin, UK) at out-going cuvette CO_2 concentrations of the growth environment (400 and 780 μL L^{-1}). Healthy, recently expanded leaves were measured in both clipped and unclipped plants. Assimilation rate was calculated on a leaf area basis (leaf area measured with an area meter; LI-COR 3000A; Lincoln, NE, USA), and converted to a leaf dry weight basis after drying and weighing the portion of the leaf in the cuvette.

We measured microbial biomass C (MBC) and N (MBN) and ^{13}C and ^{15}N in rhizosphere soil using fumigation-extraction (Bruulsema and Duxbury 1996). After thoroughly homogenizing the sample, we added a 25-g sub-sample to 40 mL of 0.05 M K_2SO_4. Another 25-g subsample was fumigated with chloroform for 2 days in a vacuum dessicator and then also added to 40 mL of 0.05 M K_2SO_4. Samples were shaken for 1 h and filtered through

pre-leached Whatman No. 1 filter paper. We analyzed aliquots of the extracts for total organic C and total N on a TOC analyzer with an N measuring unit attached (Shimadzu TOC-V_{CPN}; Shimadzu Scientific Instruments, Wood Dale, IL, USA). Another aliquot of 6 mL was freeze-dried and analyzed for ^{15}N on a mass spectrometer. Gravimetric soil moisture content was measured on a 15-g sub-sample by oven drying (105°C). Soil sub-samples were dried at 65°C, ground, and analyzed for total N and ^{15}N on a mass spectrometer.

Calculations

We calculated microbial N as the difference between N in the fumigated and non-fumigated samples divided by 0.54 (Brookes et al. 1985), and microbial C as the difference in C in fumigated and non-fumigated samples multiplied by 2.64 (Vance et al. 1987). For ^{13}C, we calculated the net increase in the amount of ^{13}C in soils, microbes and plant tissues on days 1 and 8 as compared to pre-labelling amount of ^{13}C on day 0 (referred to as the amount of ^{13}C label hereafter), and we calculated the relative distribution in the amount of ^{13}C label in soils, microbes and different plant tissues in order to account for potential differences among plants in different treatments in their C assimilation rate when the ^{13}C label was applied. We tested for effects of defoliation, elevated CO_2 and plant species on (1) absolute amount of C and MBC in rhizosphere soil, (2) the amount of ^{13}C label in rhizosphere soil and MBC, and (3) the relative distribution of ^{13}C label in rhizosphere soil, microbial biomass, roots, crowns, and aboveground plant tissues. For ^{15}N, we examined total and relative amounts of ^{15}N in rhizosphere soil, microbial biomass, and plant biomass at 0, 1 and 8 days after clipping. All analyses of variance were based on a completely randomized experimental design (destructive harvests did not allow for repeated measures on the same subjects), and were implemented in Proc GLIMMIX of SAS System for Windows, v9.2.

Results

Soil and plant–water responses to elevated CO_2

The watering regime produced a rapid decline in gravimetric soil moisture content during 46–27 DBC (from a mean of 31% at 46 DBC to 16% at 27 DBC), and a more gradual decline in soil moisture during 27–3 DBC (to 11%; Fig. 1). Soil moisture was still similar across treatments at 35 DBC, but thereafter was consistently lower for *P. smithii* compared with *B. gracilis*, and lower for plants grown at ambient versus elevated CO_2 (Table 1; Fig. 1). The only

Table 1 Results of analyses of variance for the influence of elevated CO_2 and plant species on soil moisture measured at different dates during the experiment, and for the influence of defoliation, elevated CO_2, species and date of measurement (0, 1 and 8 days after clipping) on plant biomass production

Response variable	Factor	*df*	*F*	*P*
Gravimetric soil moisture, 31 DBC	CO_2	1, 76	24.57	<0.0001
	Species	1, 76	15.96	0.0001
Gravimetric soil moisture, 27 DBC	CO_2	1, 76	27.04	<0.0001
	Species	1, 76	3.31	0.0727
Gravimetric soil moisture, 19 DBC	CO_2	1, 76	9.67	0.0026
	Species	1, 76	41.54	<0.0001
Gravimetric soil moisture, 3 DBC	CO_2 × species	1, 76	5.96	0.017
Gravimetric soil moisture, 4 DAC	CO_2	1, 28	73.49	<0.0001
	Species	1, 28	23.97	<0.0001
Leaf water potential, 3 DBC	CO_2	1, 36	150.20	<0.0001
	Species	1, 36	261.20	<0.0001
Defoliation intensity	CO_2	1, 28	0.01	0.92
	Species	1, 28	5.09	0.032
Total plant production	Clipping × species × date	1, 60	5.81	0.019
	CO_2 × species	1, 60	8.44	0.0051
	CO_2 × date	1, 60	4.39	0.017
Aboveground production	CO_2 × species	1, 60	3.79	0.056
	Clipping × date	1, 60	4.28	0.043
Crown production	CO_2 × species	1, 60	2.84	0.097
	Clipping	1, 60	0.35	0.56
	Date	1, 60	1.62	0.21
Root production	Clipping × species × date	1, 60	13.32	0.0006
	CO_2 × species × date	1, 60	4.17	0.020

For each response variable, we report *F* and *P* statistics for those higher-order interactions for which $P \leq 0.10$, and for those main factors for which $P > 0.10$ for all higher-order interactions. Degrees of freedom (*df*) are given as (numerator, denominator) for each *F* statistic

DBC Days before clipping, *DAC* Days after clipping

significant species CO_2 × interaction occurred at 3 DBC, when soil moisture was 2.3% greater for *B. gracilis* at elevated versus ambient CO_2, and 1.2% greater for *P. smithii* at elevated versus ambient CO_2 (Table 1; Fig. 1). Thus, the watering regime allowed the CO_2 treatment to influence plant–water relations in a manner consistent with previous field studies in shortgrass steppe (LeCain et al. 2003; Morgan et al. 2004b). Water limitation was most severe during 31–3 DBC, and then was partially alleviated by a large watering event at 3 DBC. Our intensive measurements of plant and rhizosphere responses to defoliation therefore occurred when plants were responding to a simulated thunderstorm preceded by drought conditions, which is a pattern characteristic of semiarid ecosystems (Sala et al. 1992). Despite increased soil moisture when we measured rhizosphere responses to defoliation, differences in soil moisture between species and CO_2 treatments were still evident at this time (e.g., 4 DAC in Fig. 1). Measurements of leaf water potential at 3 DBC confirmed that moisture was more limiting for *P. smithii* compared with *B. gracilis* ($\bar{X} = -1.93$ vs -1.42 MPa), and more limiting for plants at ambient compared with elevated CO_2 ($\bar{X} = -2.18$ vs -1.16 MPa; Table 1).

Plant production

The defoliation treatment removed an average of 45% of the shoot biomass from *P. smithii*, and 36% of shoot biomass from *B. gracilis*, and defoliation intensity was similar for the two CO_2 treatments (Table 1). For total plant production, the effect of CO_2 varied by species, and effect of clipping varied with species and date of harvest (Table 1). Given these interactions, we focused on contrasts examining (1) the effects of CO_2 on each species averaged across harvest dates (because the CO_2 treatment was applied throughout the experiment), and (2) the effect of defoliation on plants of each species harvested 8 days after the defoliation treatment. Elevated CO_2 increased production of *P. smithii* by 20%, but had no influence on *B. gracilis* (Fig. 2a). Defoliation significantly reduced *P. smithii* production by 17% on day 8, but had no effect on *B. gracilis* (Fig. 2b).

For aboveground plant production, no three- or four-way interactions were detected (Table 1). Elevated CO_2 increased aboveground production for *P. smithii* (by 26%), but not *B. gracilis* (Fig. 2a). Defoliation had no effect on aboveground production of either species on day 8 (Fig. 2b). Crown biomass exhibited a marginal interaction between species and CO_2 treatment (Table 1), but contrasts for the effect of CO_2 by species were not significant ($P = 0.18$ for *P. smithii* and $P = 0.52$ for *B. gracilis*, data not shown). The largest clipping and CO_2 effects were observed for root production in *P. smithii*. Elevated CO_2

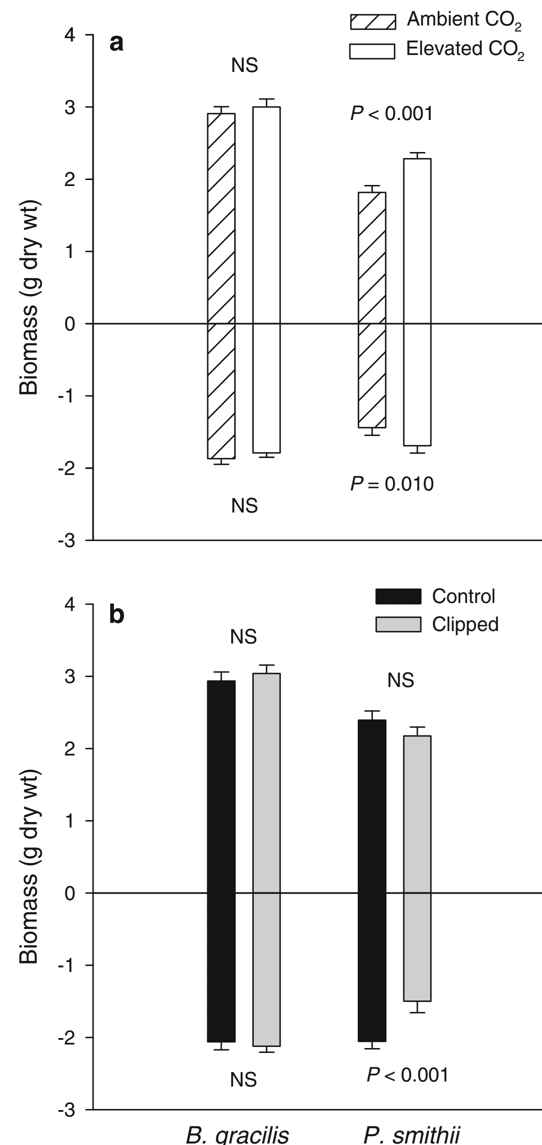

Fig. 2 Shoot (positive values) and root (negative values) biomass of *B. gracilis* and *P. smithii* as affected by elevated versus ambient CO_2, averaged across all three harvest dates (**a**) and in clipped versus unclipped treatments 8 days after clipping (**b**). Crown biomass was unaffected by CO_2 or clipping treatments and is not shown. Shoot biomass includes biomass removed by the clipping treatment. *NS* indicates contrasts with $P > 0.10$

increased *P. smithii* root biomass by 17%, and had no influence on *B. gracilis* roots (Fig. 2a). Defoliation reduced root biomass of *P. smithii* by 43% on day 8, but did not affect *B. gracilis* root biomass (Fig. 2b).

Carbon dynamics

Contrary to our original hypothesis, we found no evidence that defoliation enhanced short-term C inputs to the soil for either grass species. Effects of CO_2 on soil C did not interact with other factors, but elevated CO_2 reduced soil C

by 10% (Table 2; Fig. 3a). Effects of clipping varied by date and species (Table 2). For *P. smithii*, clipping had no effect on soil C on day 1, but reduced soil C by day 8 (Fig. 3b). For *B. gracilis*, clipping had no influence on soil C on either date (Fig. 3b). Clipping reduced microbial biomass carbon (MBC) by 12% across both species and dates (Fig. 4), and did not interact with other factors (Table 2). Effects of elevated CO_2 depended on species and date because there was no CO_2 effect for *P. smithii* soils on day 0, but the CO_2 effect was consistently negative for all other dates, species and defoliation combinations (data not shown). Hence, we focused on the main effect of CO_2. Elevated CO_2 reduced MBC by 20% across species and dates (Fig. 4).

The ^{13}C pulse addition assessed changes in allocation of current assimilate above versus belowground following clipping. Elevated CO_2 did not interact with other factors to influence ^{13}C inputs to the rhizosphere (Table 2), and reduced ^{13}C inputs in the rhizosphere soil and root wash solution (Table 2; Fig. 5a, c). In addition, effects of clipping differed between species. For *B. gracilis*, clipping reduced ^{13}C inputs to rhizosphere soil by 34% and had no effect on 13C in root wash solution (Fig. 5b, d). For *P. smithii*, defoliation reduced ^{13}C inputs to the soil by 55% (averaged across dates; Fig. 5b); defoliation also reduced ^{13}C in the root wash solution by 55% on day 1, with the effect dissipating by day 8 (Fig. 5d). These results tracing short-term assimilate inputs to the soil were consistent with the results for total C inputs to the soil in terms of the reduced rhizosphere C at elevated CO_2 and the negative effect of clipping on rhizosphere C for *P. smithii* 8 days after defoliation (Fig. 3).

Because clipped plants assimilated less ^{13}C label than unclipped plants, we controlled for the amount of label uptake by examining the relative distribution of ^{13}C allocated to roots, shoots and crowns in each treatment, and the ratio of the amount of ^{13}C label detected in the rhizosphere to the amount of ^{13}C label in plant tissues. Most variation in ^{13}C relative distribution was between the two species and dates of measurement (Fig. 6). The effect of defoliation on ^{13}C allocation to shoots varied by species and date of measurement (Table 2), and analysis of the simple effects by date and species showed that clipping slightly reduced allocation of ^{13}C to shoots for *B. gracilis* on day 8, and had no effect otherwise (Fig. 6). Elevated CO_2 did not interact with other treatments or directly affect the relative allocation of ^{13}C to shoots (Table 2). Relative allocation of ^{13}C to crowns did not vary between treatments or species. Relative allocation of ^{13}C to roots exhibited patterns inverse to allocation to shoots. Most important, clipping effects on roots varied with species and date (Table 2) because clipping had no effect on day 1, but clipping enhanced relative ^{13}C distribution in roots of *B. gracilis* by day 8 ($t_{48} = 2.3$, $P = 0.0258$) and reduced relative ^{13}C

Table 2 Results of analyses of variance for the influence of defoliation, elevated CO_2, species and date of measurement (0, 1 and 8 days after clipping) on plant and rhizosphere carbon dynamics

Variable	Term	*df*	*F*	*P*
Rhizosphere soil carbon	Clipping × date	1, 60	4.14	0.0463
	Clipping × species	1, 60	5.96	0.0176
	CO_2	1, 60	37.61	<0.0001
Microbial biomass C	CO_2 × species × date	1, 60	4.09	0.022
	Clipping	1, 60	4.98	0.029
Rhizosphere soil ^{13}C	Clipping × species	1, 48	2.83	0.099
	CO_2	1, 48	18.16	< 0.0001
	Date	1, 48	10.05	0.0026
Rootwash ^{13}C	Clipping × species × date	1, 47	3.75	0.059
	CO_2	1, 47	3.00	0.089
^{13}C relative distribution in shoots	Clipping × species × date	1, 47	6.16	0.017
	CO_2	1, 48	0.06	0.8124
^{13}C relative distribution in crowns	CO_2	1, 48	0.03	0.8655
	Clip	1, 48	0.29	0.5953
	Date	1, 48	14.90	0.0003
	Species	1, 48	1.73	0.1944
^{13}C relative distribution in roots	Clipping × species × date	1, 48	6.36	0.015
	CO_2 × species	1, 48	4.09	0.0487
^{13}C in rhizosphere:^{13}C in plant	Species × date	1, 48	12.23	0.0004
	CO_2	1, 48	4.88	0.032
	Clipping	1, 48	1.00	0.3225

For each response variable, we report *F* and *P* statistics for those higher-order interactions for which $P \leq 0.10$, and for those main factors for which $P > 0.10$ for all higher-order interactions. Degrees of freedom (*df*) are given as (numerator, denominator) for each *F* statistic

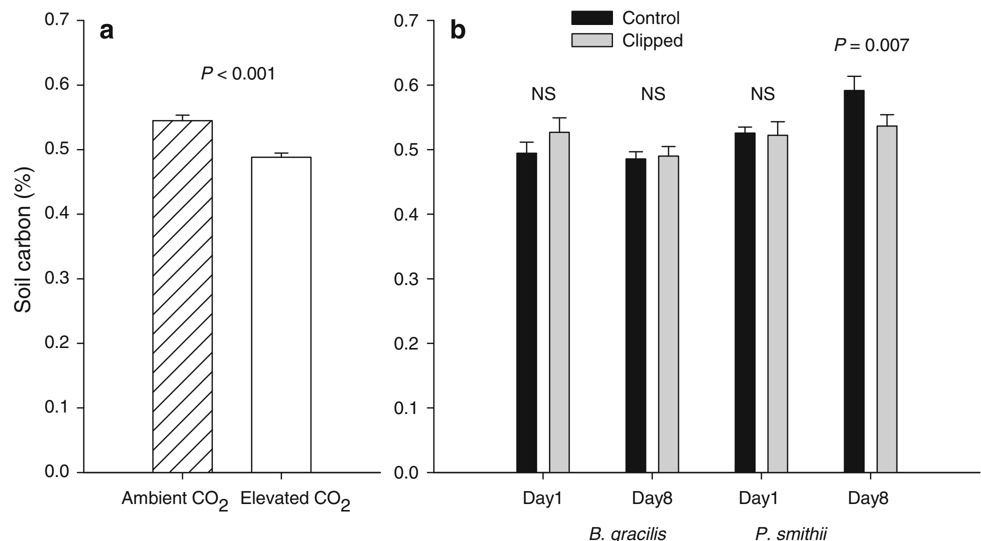

Fig. 3 Carbon content of rhizosphere soil for *B. gracilis* and *P. smithii* as affected by elevated versus ambient CO_2 (**a**) and defoliation (**b**). Days in (**b**) refer to number of days after the clipping treatment. *NS* indicates contrasts with $P > 0.10$

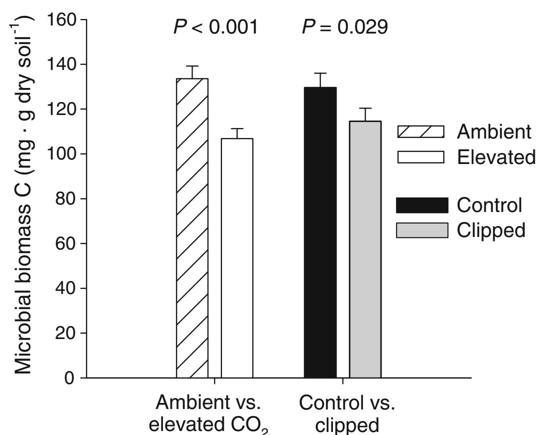

Fig. 4 Effects of elevated CO_2 and defoliation on microbial biomass carbon in rhizosphere soils of *B. gracilis* and *P. smithii*. The magnitude of effects were consistent across species, so only main effects of CO_2 and defoliation treatments are presented

distribution in roots of *P. smithii* ($t_{48} = 2.14$, $P = 0.0379$; Fig. 6). We also examined the ratio of the amount of ^{13}C label in rhizosphere soil versus plant tissues. Surprisingly, and in contrast to our original hypothesis, this ratio was unaffected by clipping (Table 2). The ratio did vary by species and date, and was significantly higher at ambient compared to elevated CO_2 (Table 2).

Nitrogen dynamics

Total dissolved nitrogen (TDN) in rhizosphere soil was reduced by 20% at elevated compared to ambient CO_2 for *P. smithii*, but was not affected by CO_2 for *B. gracilis* (Table 3; Fig. 7a). TDN also declined over time to a greater degree in the *P. smithii* compared to the *B. gracilis* rhizosphere, but was not affected by clipping (Table 3; Fig. 7a). *B. gracilis* maintained substantially lower extractable TDN concentrations in the soil than *P. smithii* on all dates. We detected large declines in TDN for *P. smithii* soil between days 0–1 and 1–8, and a smaller decline for *B. gracilis* primarily between days 1–8 (Fig. 7b). For ^{15}N in the TDN pool, trends over time and response to treatments showed similar patterns as TDN (Table 3; data not shown). Inorganic N accounted for 95% of soil TDN, with 43% as NH_4^+ and 57% as NO_3^-. Extractable NH_4^+ did not vary by treatment or plant species; extractable NO_3^- showed similar patterns as TDN, with differences between plant species in the size of the NO_3^- pool, and the rate of decline of that pool over time (Table 3; Fig. 7d). Elevated CO_2 substantially reduced extractable NO_3^- for *P. smithii*, but not for *B. gracilis* (Table 3; Fig. 7c). Elevated CO_2 also reduced N in microbial biomass for both species (Table 3; Fig. 7e). Microbial biomass N remained relatively constant over time (Table 3; Fig. 7f), but ^{15}N in microbial biomass averaged across all treatments declined significantly (Table 3) from a mean of 0.41 μg g dry soil^{-1} on day 0–0.23 μg g dry soil^{-1} on day 8. Thus, the decline in TD^{15}N between days 0 and 8 was not associated with microbial ^{15}N uptake. Furthermore, contrary to our original hypothesis, the clipping treatment did not affect the rate of loss of ^{15}N from the microbial biomass for either species or CO_2 treatment (Table 3), i.e. we found no evidence that clipping stimulated release of ^{15}N from the microbial biomass between days 0, 1 and 8 under any of the treatment combinations.

Defoliation affected plant N uptake (net change in plant N between day 0 and 8) differently for the two species (Fig. 8). Following defoliation, more N and ^{15}N accumulated in the shoots of clipped versus unclipped plants of both species (Table 3; Fig. 8). Defoliation did not influence crown N or ^{15}N (Table 3). Defoliation

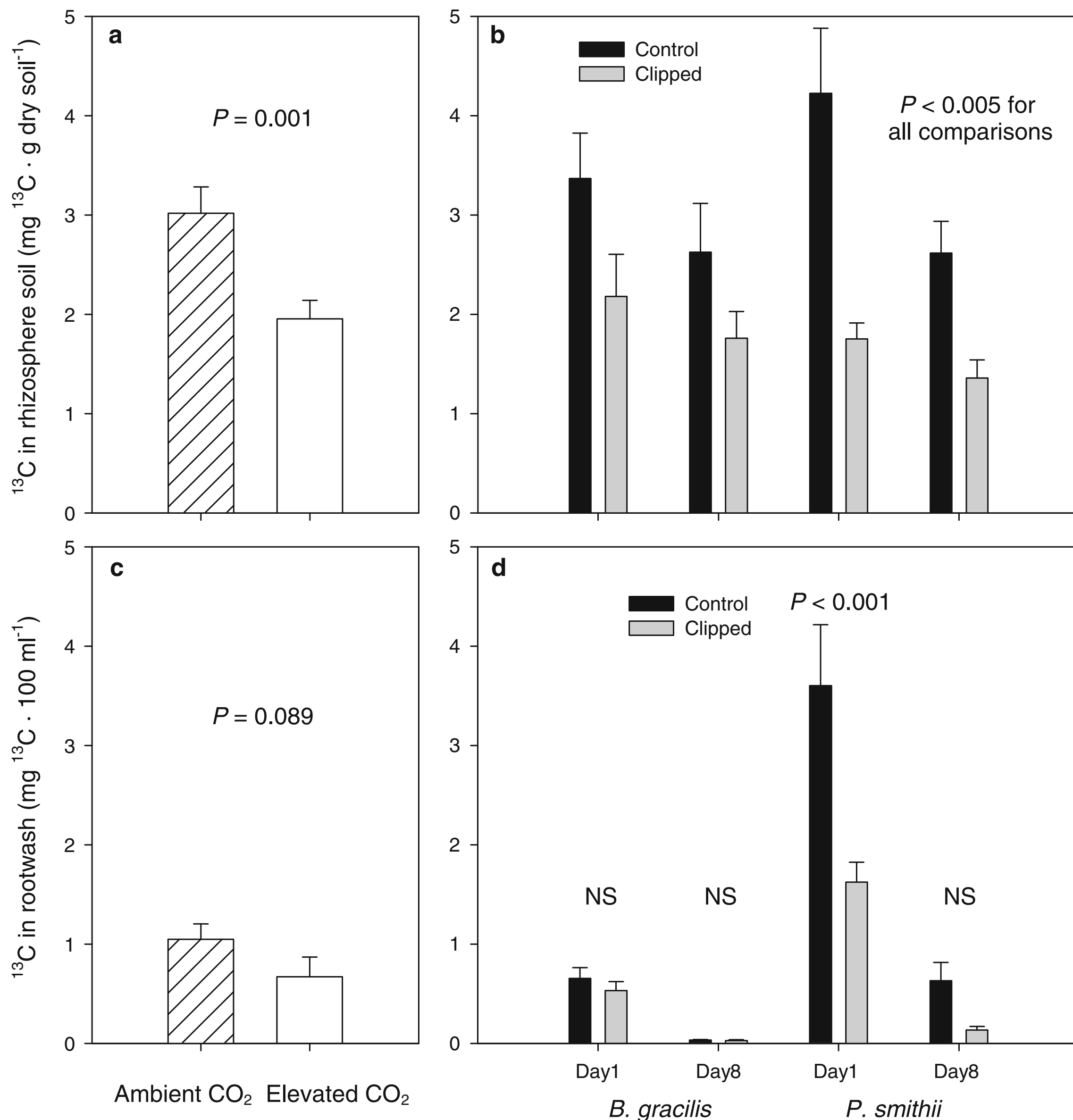

Fig. 5 Effects of elevated CO_2 (**a**, **c**) and defoliation (**b**, **d**) on the amount of ^{13}C label measured in rhizosphere soil (**a**, **b**) and in the solution used to rinse the root surface immediately after removing the rhizosphere soil (**c**, **d**) for *B. gracilis* and *P. smithii*. Main effects of CO_2 and defoliation are presented because we found no significant interactions between the two treatments. *NS* indicates contrasts with $P > 0.10$

effects on root N differed between the species (Table 3), with root N and ^{15}N uptake unaffected by clipping for *B. gracilis*, but negatively affected for *P. smithii* (Fig. 8). In other words, the clipping-induced enhancement of shoot N occurred at the expense of root N for *P. smithii*, but not for *B. gracilis*. Clipping effects on the net change in shoot and root N and ^{15}N did not vary with CO_2 treatment (Table 3).

Finally, we measured shoot [N] and photosynthesis rates 8 days after the defoliation treatment to examine recovery from defoliation under ambient and elevated CO_2. For shoot [N], effects of defoliation and CO_2 differed between the species, but effects of CO_2 and clipping did not interact (Table 3; Fig. 9a). For *P. smithii*, the magnitude of the increase in shoot [N] caused by clipping was equal to the reduction caused by elevated CO_2 (Fig. 9a). For photosynthesis, the effect of defoliation varied with CO_2 treatment and species (Table 3). *B. gracilis* showed no response to treatments ($F_{3,24} = 1.22$, $P = 0.32$), while *P. smithii* photosynthesis showed a significant interaction between

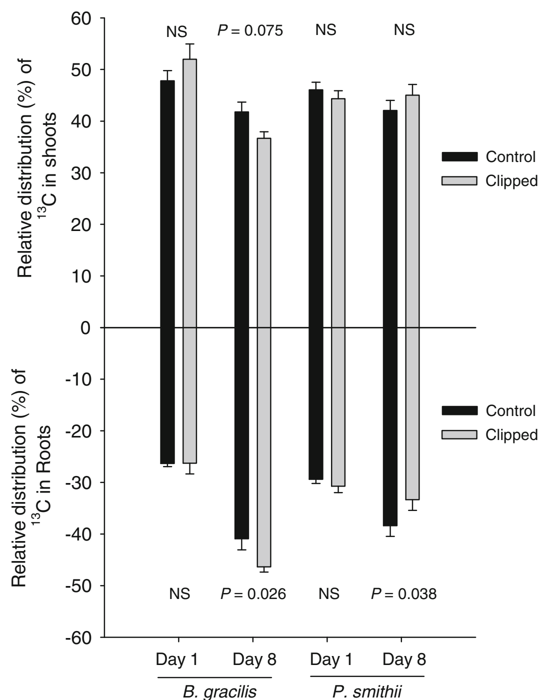

Fig. 6 The relative distribution of ^{13}C label in shoots (positive values) and roots (negative values) of *B. gracilis* and *P. smithii* measured 1 and 8 days after defoliation and labeling of plants with ^{13}C. No significant effects of defoliation were detected for ^{13}C in plant crowns (data not shown). *NS* indicates contrasts with $P > 0.10$

CO_2 and clipping ($F_{3,24} = 14.15$, $P < 0.0001$) because rates were enhanced for clipped plants growing at elevated CO_2, but not for clipped plants at ambient CO_2 (Fig. 9b).

Discussion

Plant production responses in our study were consistent with multiple field and laboratory studies indicating that *B. gracilis* is highly grazing resistant, while *P. smithii* can exhibit some negative responses (Menke and Trlica 1981; Polley and Detling 1988; Eneboe et al. 2002; Milchunas et al. 2008). Also consistent with previous field studies, we found elevated CO_2 influenced both species through water relations (reduced water stress; LeCain et al. 2003), enhanced production of the C_3 grass (Read and Morgan 1996; Morgan et al. 2004b), and reduced shoot [N] for the C_3 grass (Read and Morgan 1996; King et al. 2004).

Rhizosphere responses to defoliation

Contrary to our original hypothesis, we found no support for the stimulation of rhizodeposition following defoliation in either species. Instead, all measures of soil C components (total rhizosphere soil C, microbial biomass C, ^{13}C inputs to the rhizosphere soil and ^{13}C on root surfaces) indicated that defoliation reduced short-term C inputs to the rhizosphere. Overall, these findings are consistent with the traditional view that defoliation-induced reductions in whole-plant photosynthesis often have immediate negative consequences for the maintenance of root activity (Briske and Richards 1995).

Reduced rhizodeposition following defoliation could potentially result if soil N supply was sufficiently high to alleviate N limitation to regrowth. However, no external N was supplied during our experiment, and native shortgrass soil comprised only one-third of the soil volume. Inorganic N in the rhizospherein relation to date (Fig. 7) was similar to levels in other studies where grasses did increase rhizodeposition following defoliation (Hamilton and Frank 2001; Hamilton et al. 2008). Furthermore, extractable N was lower in our study (~2–4 μN g dry soil^{-1}) than in shortgrass steppe during the growing season (~10 μN g dry soil^{-1}; McCulley et al. 2009), suggesting low soil N availability in our study. Also, shoot [N] in our study was similar to field measurements for *B. gracilis* (King et al. 2004; McCulley et al. 2009) and *P. smithii* (Karn and Ries 2002), and root [N] ($\bar{X} = \pm 1SE = 1.0 \pm 0.02$ for *B. gracilis* and 1.2 ± 0.03 for *P smithii*) was similar to the lowest values measured during a 4-year study at a site with a mixture of the two species (Milchunas et al. 2005b). Thus, our results suggest that in spite of relatively low soil N availability, and plant N status similar to field conditions, neither species increased rhizodeposition following defoliation.

One limitation of the ^{13}C pulse-labelling method we used is that it only measures allocation of post-defoliation assimilate. Under some circumstances, defoliation can stimulate exudation of pre-defoliation assimilate, while post-defoliation assimilate is allocated elsewhere (Paterson et al. 2005). In our study, defoliation effects on soil C and MBC were similar in direction to effects on soil ^{13}C and $MB^{13}C$. In particular, the significant negative effect of defoliation on MBC both 1 and 8 days after defoliation (Fig. 4) is a strong indicator that defoliation suppressed rather than enhanced short-term rhizodeposition.

Our findings are in contrast to the increase in rhizodeposition following defoliation documented for several grazing-adapted plant species (Hamilton and Frank 2001; Paterson et al. 2003, 2005; Hamilton et al. 2008; Henry et al. 2008). While it has been hypothesized that increased rhizodeposition following defoliation may simply be a plant response to wounding (Henry et al. 2008), our finding of reduced post-defoliation rhizodeposition suggests the response may be under plant control for some species. Thus, the direction and magnitude of defoliation effects on rhizodeposition appears to vary widely depending on the

Table 3 Results of analyses of variance for the influence of defoliation, elevated CO_2, species, and in some cases date of measurement (0, 1 and 8 days after clipping) on plant and rhizosphere nitrogen dynamics

Variable	Term	*df*	*F*	*P*
Total dissolved soil N	CO_2 × species	1, 60	4.50	0.038
	Species × date	1, 60	3.00	0.058
	Clipping	1, 60	0.76	0.388
Total dissolved soil ^{15}N	CO_2 × species	1, 59	5.00	0.029
	CO_2 × date	1, 59	2.96	0.059
	Species × date	1, 59	21.62	0.0001
	Clipping	1, 59	0.02	0.8928
Extractable soil NO_3^-	Species × date	1, 60	6.78	0.002
	CO_2	1, 60	13.71	0.0005
	Clipping	1, 60	2.31	0.134
Microbial biomass N	Clipping	1, 59	1.25	0.2676
	CO_2	1, 59	12.61	0.0008
	Species	1, 59	0.24	0.6258
	Date	1, 59	2.33	0.1064
Microbial biomass ^{15}N	CO_2 × species	1, 59	5.00	0.029
	Clipping	1, 59	0.16	0.692
	Date	1, 59	12.10	<0.0001
Change in shoot N, day 0–8	CO_2 × species	1, 24	3.67	0.0684
	Clipping	1, 24	4.04	0.0568
	Species	1, 24	7.34	0.0122
	CO_2	1, 24	0.65	0.4286
Change in crown N, day 0–8	CO_2 × species	1, 24	6.18	0.0203
	Clipping	1, 24	0.20	0.6626
Change in root N, day 0–8	Clipping × species	1, 24	6.08	0.02
	CO_2	1, 24	16.45	0.0005
Change in shoot ^{15}N, day 0–8	Clipping	1, 24	7.19	0.014
	Species	1, 24	2.75	0.1117
	CO_2	1, 24	2.18	0.1542
Change in crown ^{15}N, day 0–8	CO_2 × species	1, 24	9.15	0.0059
	Clipping	1, 24	0.29	0.594
Change in root ^{15}N, day 0–8	Clipping × species	1, 24	2.93	0.1
	CO_2	1, 24	9.22	0.0057
Shoot [N], day 8	CO_2 × species	1, 24	9.75	0.0046
	Clipping x species	1, 24	9.73	0.0047
Net photosynthesis rate, day 8	Clipping × CO_2 × species	1, 24	3.13	0.089

For each response variable, we report *F* and *P* statistics for those higher-order interactions for which $P \leq 0.10$, and for those main factors for which $P > 0.10$ for all higher-order interactions. Degrees of freedom (*df*) are given as (numerator, denominator) for each *F* statistic

plant species and intensity of defoliation (Dilkes et al. 2004; Mikola and Kytoviita 2002; Fu and Cheng 2004; Ilmarinen et al. 2008). Although our measures of total microbial biomass do not address potential shifts in the abundance or activity of specific microbial functional groups in response to defoliation, our findings suggest that increased shoot N concentration and yield in *B. gracilis* and *P. smithii* following defoliation are not necessarily mediated by rhizodeposition. Instead, responses by some plant species may be better explained by enhanced N uptake efficiency by roots and increased allocation of N to shoots in recently defoliated plants (Ilmarinen et al. 2008).

Grazing tolerance and avoidance strategies

Our original motivation was to examine how rhizosphere processes contribute to grazing tolerance of *B. gracilis* and *P. smithii*, but the C allocation patterns we documented also provide important insights concerning grazing avoidance. Both species increased allocation of N and ^{15}N to regrowing shoots during the first 8 days after defoliation. However, the large allocation to shoot regrowth in *P. smithii* occurred at the expense of roots, while the smaller amount of N allocated to shoot regrowth in *B. gracilis* was not associated with a concomitant decline in root N. Over

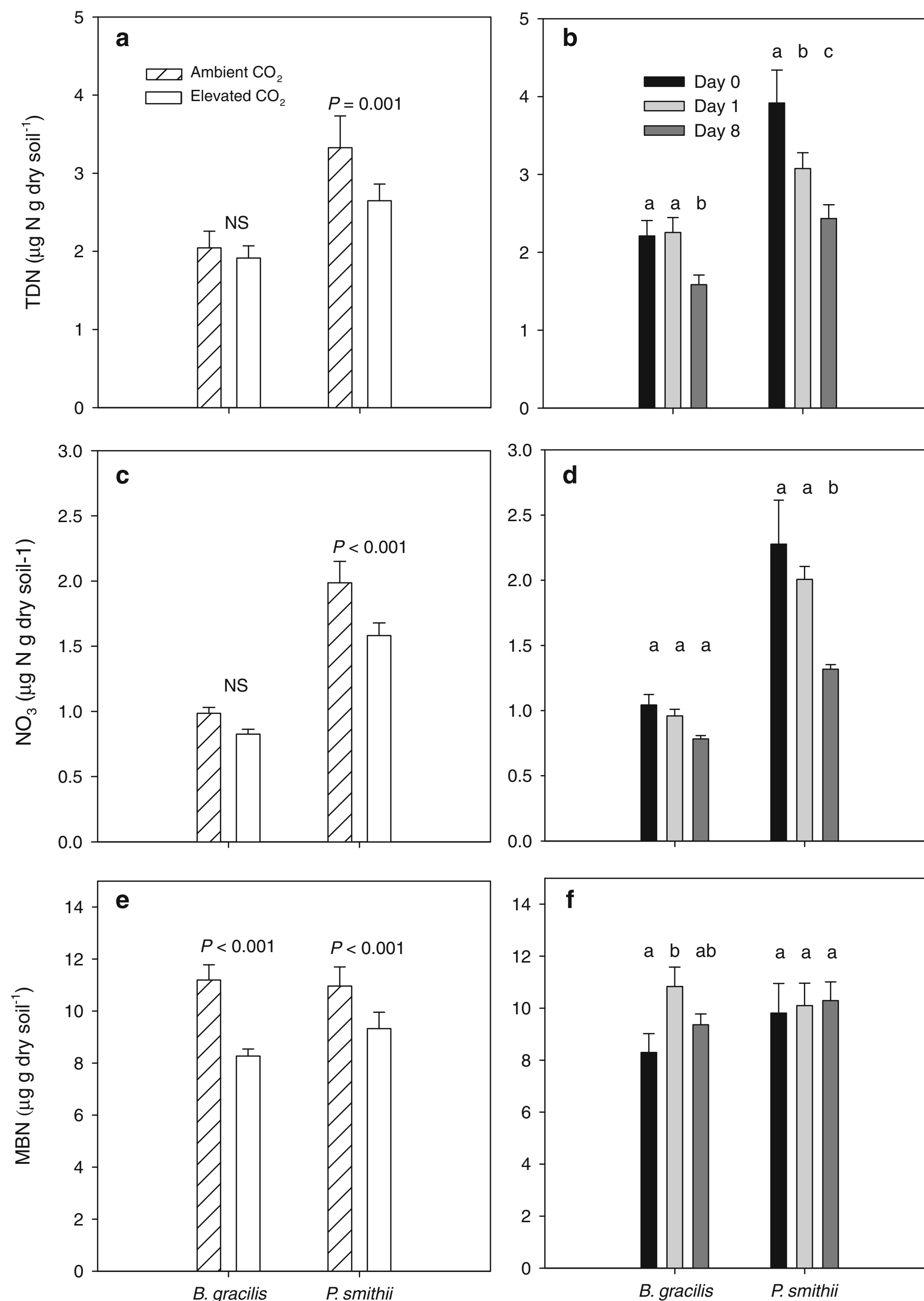

Fig. 7 Variation in total dissolved nitrogen (*TDN*; **a**, **b**), NO_3^- extracted with 0.5 M K_2SO_4 (**c**, **d**) and microbial biomass nitrogen (*MBN*; **e**, **f**) in rhizosphere soil in relation to elevated CO_2 and date of measurement. *Bars* with different *letters* indicate a significant difference ($P < 0.05$) between sampling dates for each grass species. Extractable soil N pools and MBN were not affected by the clipping treatment

the course of a growing season, *B. gracilis* maintains more consistent levels of root and crown carbohydrate reserves compared to other co-occuring grasses and forb species, while *P. smithii* can show substantially depleted root and crown carbohydrate reserves during periods of aboveground growth (Menke and Trlica 1981). This pattern reflects the relatively high and consistent allocation of photosynthate to roots and crowns by *B. gracilis*, and the substantially higher root:shoot ratios found in shortgrass steppe dominated by *B. gracilis*, as compared with other grassland ecosystems (Milchunas et al. 2008). Furthermore, *B. gracilis* increased allocation of recently

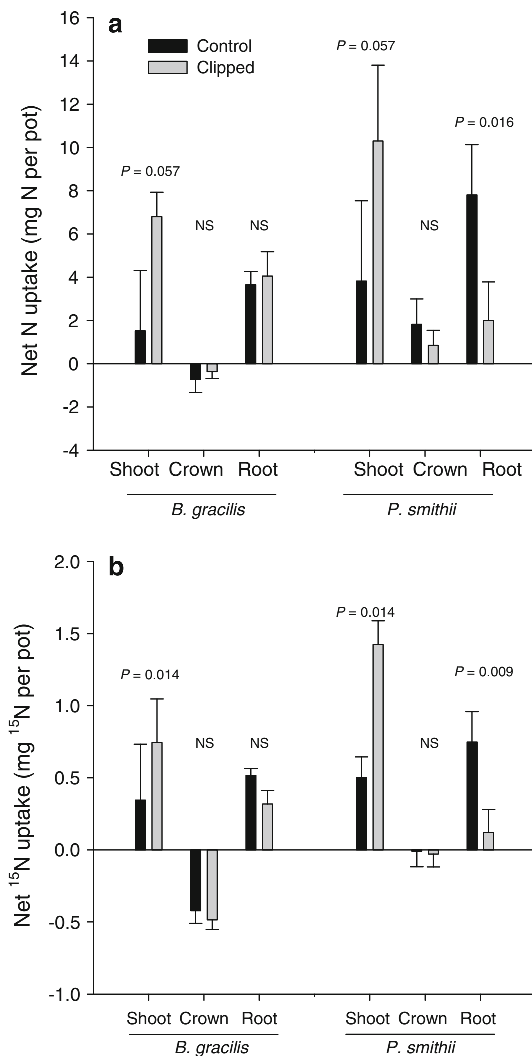

Fig. 8 Net change in N (**a**) and ^{15}N (**b**) in plant shoots, crowns and roots during the first 8 days after a defoliation event (day 0) for *B. gracilis* and *P. smithii*. Effects of clipping did not interact with the CO_2 treatment, so main effects of clipping are presented for each species

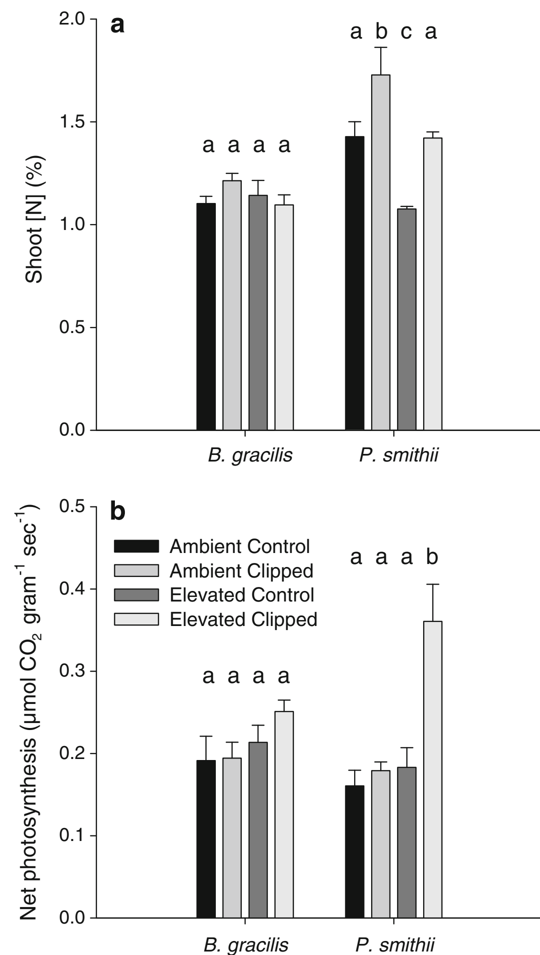

Fig. 9 Effects of defoliation and elevated atmospheric CO_2 on shoot [N] (**a**) and net photosynthesis rates (**b**) 8 days after a defoliation treatment applied to *B. gracilis* and *P. smithii*. *Bars* with different *letters* indicate a significant difference ($P < 0.05$) between sampling dates for each grass species

assimilated C to roots rather than shoots during the first 8 days after defoliation, consistent with the finding that defoliation did not reduce *B. gracilis* root production.

Roots of *B. gracilis* can respond rapidly to moisture pulses following dry periods, indicating an ability to survive drought and minimize the need for root regrowth before water and nutrient uptake can occur (Lauenroth et al. 1987). Post-defoliation recovery rates of many grass species are strongly dependent upon soil N supply rates (Hamilton et al. 1998; Morgan et al. 2001). We found that defoliation reduced soil C inputs and microbial biomass C in the *B. gracilis* rhizosphere, and had no effect on inorganic N pools or changes in ^{15}N within the microbial biomass or the inorganic N pool during the first 8 days after defoliation. Despite the lack of effect on soil N availability, defoliation increased N uptake into *B. gracilis* shoots without reducing N in roots and crowns. These results suggest that the maintenance of root growth in *B. gracilis* after defoliation allowed plants to maintain or increase N uptake from the soil in response to increased shoot demands.

Our combined results also suggest that (1) grazing avoidance is an important component of grazing resistance in *B. gracilis*, and (2) resource allocation patterns underlying avoidance also contribute to the potential for regrowth and hence grazing tolerance. High belowground biomass allocation and the maintenance of a prostrate canopy both minimize tissue loss to herbivores (e.g., defoliation to constant height resulted in significantly lower tissue loss for *B. gracilis* compared to *P. smithii*). When combined with increased allocation of recently assimilated C belowground to maintain the root system after

defoliation, these traits appear to allow *B. gracilis* to acquire sufficient N for leaf regrowth.

Grazing tolerance in *P. smithii* was reflected in its ability to maintain aboveground plant production and replace shoot N lost to defoliation (Figs. 2 and 9). Compared with *B. gracilis*, however, these responses were affected by atmospheric CO_2 and involved tradeoffs with root growth and N content. The highest net rates of N and ^{15}N uptake into shoots that we measured were for defoliated *P. smithii*. High rates of N transfer into shoots were associated with (1) a large decline in the extractable inorganic N and ^{15}N soil pools between day 0 and 8, (2) no increase in N and ^{15}N in microbial biomass, and (3) no net change in N or ^{15}N in roots of defoliated *P. smithii* as compared with a large increase in root N in non-defoliated plants. The lack of clipping effects on extractable N and ^{15}N pools in the soil on both days 1 and 8 suggests that N uptake continued at similar rates in both defoliated and non-defoliated *P. smithii*, but that defoliated plants allocated more N from roots to shoots than did non-defoliated plants. Grazing tolerance via reallocation of C and N from roots to shoots therefore appears to be an important component of grazing resistance in *P. smithii*, but also makes the species more vulnerable to frequent, repeated grazing events.

Rhizosphere responses to elevated CO_2

In many studies, elevated CO_2 has been found to increase C inputs to the rhizosphere (reviewed by Cheng 1999; see also Allard et al. 2006; Johansson et al. 2009). However, such studies are typically conducted with plants grown with non-limiting water supply. In contrast, we found that elevated CO_2 reduced both total inputs of C to the rhizosphere over the course of our experiment (Figs. 3 and 4), and reduced short-term inputs of assimilate (pulse-labeled ^{13}C) to the rhizosphere (Fig. 5). We suggest that these findings are related to the strong, positive effects of elevated CO_2 on plant–water relations in semi-arid ecosystems. Under water-limiting growth conditions in both our experiment (Fig. 1) and in field studies in the semi-arid shortgrass steppe (LeCain et al. 2003; Morgan et al. 2004b), elevated CO_2 results in water savings that enhance soil moisture. Consistent with our findings, studies of a calcareous grassland in Switzerland that incorporated CO_2 effects on soil–water relations found that elevated CO_2 did not increase rhizodeposition, even though elevated CO_2 did increase C allocation to roots (Niklaus et al. 2001). One potential consequence of reduced water stress may be a reduction in fine root mortality, which in turn could reduce C inputs to the rhizosphere. Another possibility is that alleviation of moisture stress alters root physiology in a manner that also reduces C loss through exudation, but we are unaware of studies addressing this issue. The lack of a CO_2 effect on relative allocation to above versus belowground production in our study is similar to field results, as is the average aboveground:belowground production ratio for plants in our study (1.2) versus measurements under field conditions (0.9–1.3; Milchunas et al. 2005b). However, our study was conducted with young plants that did not have older, more suberized roots and well-developed crowns, which must also be considered when comparing with field studies.

Elevated CO_2 and plant recovery from defoliation

One of the few clear interactive effects of defoliation and elevated CO_2 was a substantial increase in the photosynthesis rate of defoliated versus non-defoliated *P. smithii*, but only under elevated CO_2 (Fig. 9). Build-up of leaf carbohydrates has long been understood to reduce photosynthetic capacity in plants grown in CO_2-enriched atmospheres (Stitt 1991; Jacob et al. 1995; Ziska et al. 1995). We suggest that defoliation likely created new and/or stronger carbohydrate sinks, thereby enhancing the transport of carbohydrate away from the chloroplast and enhancing the plants' ability to respond to higher CO_2. Such a mechanism is consistent with findings for photosynthesis and leaf carbohydrates of grazed *Lolium perenne* under elevated CO_2 in New Zealand (Guo et al. 2006). In addition, the reduction in leaf [N] under elevated CO_2 was offset by increased shoot [N] in defoliated *P. smithii*, which may have contributed to the strong photosynthetic response to CO_2. Our finding of enhanced photosynthesis for defoliated *P. smithii* under elevated CO_2 suggests that shoot growth beyond the first week after defoliation could have continued at higher rates for defoliated plants under elevated CO_2, and hence that elevated CO_2 may enhance grazing tolerance of *P. smithii*. However, this finding appears to be related to physiological responses of *P. smithii* to defoliation and elevated CO_2, rather than interactions with the soil microbial community.

Reduced leaf [N] under elevated atmospheric CO_2 can also result in critical reductions in forage quality for grazing ruminants (Milchunas et al. 2005a). We found that defoliation of *P. smithii* increased leaf [N] to the same extent that elevated CO_2 reduced it, suggesting that grazing management could potentially play a role in mitigating CO_2 effects on forage quality. Improved understanding of how the timing and intensity of defoliation influence temporal patterns of plant N uptake and regrowth under elevated CO_2 could improve our ability to develop and test effective strategies to minimize the influence of rising atmospheric CO_2 on forage quality.

Acknowledgments We thank Dan LeCain for assistance with leaf water potential measurements, experimental set-up and greenhouse

maintenance, and Caitlin Brooks, Ally Eden, Erik Hardy, Pat McCusker and David Smith for assistance with plant harvests and laboratory analyses. We thank Daniel Milchunas, Shenglei Fu, Christian Korner, and three anonymous reviewers for thoughtful reviews that improved the manuscript.

References

Allard V, Robin C, Newton PCD, Lieffering M, Soussana JF (2006) Short and long-term effects of elevated CO_2 on *Lolium perenne* rhizodeposition and its consequences on soil organic matter turnover and plant N yield. Soil Biol Biochem 38:1178–1187

Bardgett RD, Wardle DA (2003) Herbivore-mediated linkages between aboveground and belowground communities. Ecology 84:2258–2268

Bardgett RD, Wardle DA, Yeates GW (1998) Linking above-ground and below-ground interactions: how plant responses to foliar herbivory influence soil organisms. Soil Biol Biochem 30:1867–1878

Bement RE (1969) A stocking-rate guide for beef production on blue-grama range. J Range Manage 22:83–86

Biondini ME, Klein DA, Redente EF (1988) Carbon and nitrogen losses through root exudation by *Agropyron cristatum, A. smithii* and *Bouteloua gracilis*. Soil Biol Biochem 20:477–482

Briske DD, Richards JH (1995) Plant responses to defoliation: a physiological, morphological and demographic evaluation. In: Bedunah D, Sosebee R (eds) Wildland plants: physiological ecology and developmental morphology. Society for Range Management, Denver, pp 635–710

Briske DD, Boutton TW, Wang Z (1996) Contribution of flexible allocation priorities to herbivory tolerance in C_4 perennial grasses: an evaluation with ^{13}C lablelling. Oecologia 105:151–159

Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol Biochem 17:837–842

Bruulsema TW, Duxbury JM (1996) Simultaneous measurement of soil microbial nitrogen, carbon, and carbon isotope ratio. Soil Sci Soc Am J 60:1787–1791

Burke IC, Lauenroth WK, Riggle R, Brannen P, Madigan B, Beard S (1999) Spatial variability of soil properties in the shortgrass steppe: the relative importance of topography, grazing, microclimate, and plant species in controlling spatial patterns. Ecosystems 2:422–438

Caldwell MM, Richards JH, Johnson DA, Nowak RS, Dzurec RS (1981) Coping with herbivory: photosynthetic capacity and resource allocation in two semiarid *Agropyron* bunchgrasses. Oecologia 50:14–24

Chapin F, McNaughton S (1989) Lack of compensatory growth under phosphorus deficiency in grazing-adapted grasses from the Serengeti Plains. Oecologia 79:551–557

Cheng W (1999) Rhizosphere feedbacks in elevated CO_2. Tree Physiol 19:313–320

Clark NM, Apple ME, Nowak RS (2010) The effects of elevated CO_2 on root respiration rates of two Mojave Desert shrubs. Glob Chang Biol 16:1566–1575

Detling J, Painter E (1983) Defoliation responses of western wheatgrass populations with diverse histories of prairie dog grazing. Oecologia 57:65–71

Detling JK, Dyer MI, Winn DT (1979) Net photosynthesis, root respiration, and regrowth of *Bouteloua gracilis* following simulated grazing. Oecologia 41:127–134

Diaz S, Grime JP, Harris J, McPherson E (1993) Evidence of a feedback mechanism limiting plant response to elevated carbon dioxide. Nature 364:616–617

Dijkstra FA, Pendall E, Mosier AR, King JY, Milchunas DG, Morgan JA (2008) Long-term enhancement of N availability and plant growth under elevated CO_2 in a semi-arid grassland. Funct Ecol 22:975–982

Dijkstra FA, Blumenthal DM, Morgan JA, Pendall E, Carrillo Y, Follett R (2010) Contrasting effects of elevated CO_2 and warming on nitrogen cycling in a semiarid grassland. New Phytol 187:426–437

Dilkes NB, Jones DL, Farrar J (2004) Temporal dynamics of carbon partitioning and rhizodeposition in wheat. Plant Physiol 134:706–715

Eneboe EJ, Sowell BF, Heitschmidt RK, Karl MG, Haferkamp MR (2002) Drought and grazing IV: blue grama and western wheatgrass. J Range Manage 55:73–79

Fu S, Cheng W (2004) Defoliation affects rhizosphere respiration and rhizosphere priming effect on decomposition of soil organic matter under a sunflower species: *Helianthus annuus*. Plant Soil 263:345–352

Goverde M, Erhardt A (2003) Effects of elevated CO_2 on development and larval food-plant preference in the butterfly *Coenonympha pamphilus* (Lepidoptera, Satyridae). Glob Chang Biol 9:74–83

Guitian R, Bardgett RD (2000) Plant and soil microbial responses to defoliation in temperate semi-natural grassland. Plant Soil 220:271–277

Guo J, Trotter CM, Newton PCD (2006) Initial observations of increased requirements for light-energy dissipation in ryegrass (*Lolium perenne*) when source/sink ratios become high at a naturally grazed free air CO_2 enrichment (FACE) site. Funct Plant Biol 33:1045–1053

Hamilton EW, Frank DA (2001) Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. Ecology 82:2397–2402

Hamilton EW, Giovannini MS, Moses SJ, Coleman JS, McNaughton SJ (1998) Biomass and mineral element responses of a Serengeti short grass species to nitrogen supply and defoliation: compensation requires a critical [N]. Oecologia 114:407–418

Hamilton EW, Frank DA, Hinchey P, Murray T (2008) Defoliation induces root exudation and triggers positive rhizospheric feedbacks in a temperate grassland. Soil Biol Biochem 40:2865–2873

Henry F, Vestberg M, Christensen S (2008) Evidence for a transient increase of rhizodeposition within one and a half day after a severe defoliation of *Plantago arenaria* grown in soil. Soil Biol Biochem 40:1264–1267

Hu S, Chapin FS, Firestone MK, Field CB, Chiariello NR (2001) Nitrogen limitation of microbial decomposition in a grassland under elevated CO_2. Nature 409:188–191

Hungate BA, Lund CP, Pearson HL, Chapin FS (1997) Elevated CO_2 and nutrient addition alter soil N cycling and N trace gas fluxes with early season wet-up in a California annual grassland. Biogeochemistry 37:89–109

Ilmarinen K, Mikola J, Vestberg M (2008) Do interactions with soil organisms mediate grass responses to defoliation? Soil Biol Biochem 40:894–905

Jacob J, Greinter C, Drake BG (1995) Acclimation of photosynthesis in relation to Rubisco and non-structural carbohydrate contents and in situ carboxylase activity in *Scirpus olneyi* grown at elevated CO_2 in the field plant. Cell Environ 18:875–884

Johansson EM, Fransson PMA, Finlay RD, van Hees PAW (2009) Quantitative analysis of soluble exudates produced by ectomycorrhizal roots as a response to ambient and elevated CO_2. Soil Biol Biochem 41:1111–1116

Karn JF, Ries RE (2002) Free-choice grazing of native range and cool-season grasses. J Range Manage 55:469–473

King JY, Mosier AR, Morgan JA, LeCain DR, Milchunas DG, Parton WJ (2004) Plant nitrogen dynamics in shortgrass steppe under elevated atmospheric carbon dioxide. Ecosystems 7:147–160

Lauenroth WK, Sala OE, Milchunas DG, Lathrop RW (1987) Root dynamics of *Bouteloua gracilis* during short-term recovery from drought. Funct Ecol 1:117–124

Lauenroth WK, Burke IC, Gutmann MP (1999) The structure and function of ecosystems in the central North American grassland region. Gt Plains Res 9:223–260

LeCain DR, Morgan JA, Mosier AR, Nelson JA (2003) Soil and plant water relations determine photosynthetic responses of C_3 and C_4 grasses in a semi-arid ecosystem under elevated CO_2. Ann Bot 92:41–52

Luo Y et al (2004) Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. Bioscience 54:731–739

Mawdsley JL, Bardgett RD (1997) Continuous defoliation of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) and associated changes in the composition and activity of the microbial population of an upland grassland soil. Biol Fertil Soils 24:52–58

McCulley RL, Burke IC, Lauenroth WK (2009) Conservation of nitrogen increases with precipitation across a major grassland gradient in the Central Great Plains of North America. Ecosyst Ecol 159:571–581

Menke JW, Trlica MJ (1981) Carbohydrate reserve, phenology, and growth cycles of nine Colorado range species. J Range Manage 34:269–277

Mikola J, Kytoviita MM (2002) Defoliation and the availability of currently assimilated carbon in the *Phleum pratense* rhizosphere. Soil Biol Biochem 34:1869–1874

Mikola J, Yeates GW, Barker GM, Wardle DA, Bonner KI (2001) Effects of defoliation intensity on soil food-web properties in an experimental grassland community. Oikos 92:333–343

Milchunas DG, Lauenroth WK (1992) Carbon dynamics and estimates of primary production by harvest, ^{14}C dilution, and ^{14}C turnover. Ecology 73:593–607

Milchunas DG, Mosier AR, Morgan JA, LeCain DR, King JY, Nelson JA (2005a) Elevated CO_2 and defoliation effects on a shortgrass steppe: forage quality versus quantity for ruminants. Agric Ecosyst Environ 111:166–184

Milchunas DG, Mosier AR, Morgan JA, LeCain DR, King JY, Nelson JA (2005b) Root production and tissue quality in a shortgrass steppe exposed to elevated CO_2: using a new ingrowth method. Plant Soil 268:111–122

Milchunas D, Lauenroth W, Burke I, Detling JK (2008) Effects of grazing on vegetation. In: Lauenroth W, Burke IC (eds) Ecology of the shortgrass steppe: a long-term perspective. Oxford University Press, New York, pp 389–446

Morgan JA, LeCain DR, Read JJ, Hunt HW, Knight WG (1998) Photosynthetic pathway and ontogeny affect water relations and the impact of CO_2 on *Bouteloua gracilis* (C_4) and *Pascopyrum smithii* (C_3). Oecologia 114:483–493

Morgan JA, Skinner RH, Hanson JD (2001) Nitrogen and CO_2 affect regrowth and biomass partitioning differently in forages of three functional groups. Crop Sci 41:78–86

Morgan JA et al (2004a) Water relations in grassland and desert ecosystems exposed to elevated atmospheric CO_2. Oecologia 140:11–25

Morgan JA, Mosier AR, Milchunas DG, LeCain DR, Nelson JA, Parton WJ (2004b) CO_2 enhances productivity, alters species composition, and reduces digestibility of shortgrass steppe vegetation. Ecol Appl 14:208–219

Niklaus P, Glockler E, Siegwolf R, Korner C (2001) Carbon allocation in calcareous grassland under elevated CO_2: a combined ^{13}C pulse-labelling/soil physical fractionation study. Funct Ecol 15:43–50

Paterson E, Thornton B, Sim A, Pratt S (2003) Effects of defoliation and atmospheric CO_2 depletion on nitrate acquisition, and exudation of organic compounds by roots of *Festuca rubra*. Plant Soil 250:293–305

Paterson E, Thornton B, Midwood AJ, Sim A (2005) Defoliation alters the relative contributions of recent and non-recent assimilate to root exudation from *Festuca rubra*. Plant Cell Environ 28:1525–1533

Polley H, Detling J (1988) Herbivory tolerance of *Agropyron smithii* populations with different grazing histories. Oecologia 77:261–267

Read JJ, Morgan JA (1996) Growth and partitioning in *Pascopyrum smithii* (C3) and *Bouteloua gracilis* (C4) as influenced by carbon dioxide and temperature. Ann Bot 77:487–496

Sala OE, Lauenroth WK, Parton WJ (1992) Long-term soil water dynamics in the shortgrass steppe. Ecology 73:1175–1181

Skinner RH, Morgan JA, Hanson JD (1999) Carbon and nitrogen reserve remobilization following defoliation: nitrogen and elevated CO_2 effects. Crop Sci 39:1749–1756

Stitt M (1991) Rising CO_2 levels and their potential significance for carbon flow in photosynthetic cells. Plant Cell Environ 14:741–762

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707

Wilsey BJ, Coleman JS, McNaughton SJ (1997) Effects of elevated CO_2 and defoliation on grasses: a comparative ecosystem approach. Ecol Appl 7:844–853

Ziska LH, Sicher RC, Kremer DF (1995) Reversibility of photosynthetic acclimation of swiss chard and sugarbeet grown at elevated concentrations of CO_2. Physiol Plant 95:355–364