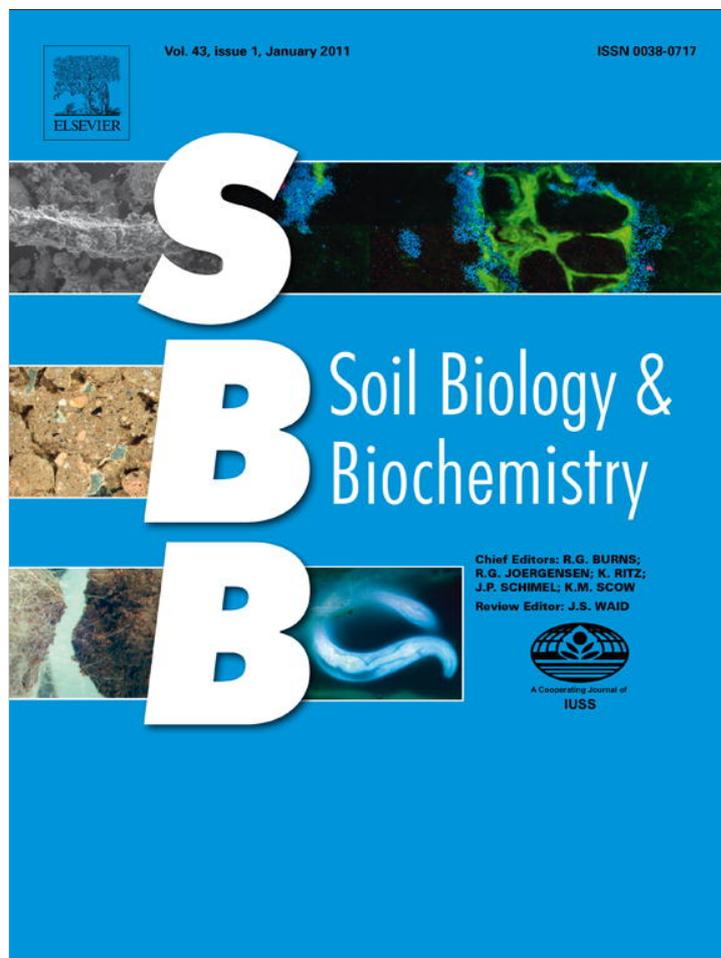


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Elevated CO₂, but not defoliation, enhances N cycling and increases short-term soil N immobilization regardless of N addition in a semiarid grassland

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ABSTRACT

Elevated CO₂ and defoliation effects on nitrogen (N) cycling in rangeland soils remain poorly understood. Here we tested whether effects of elevated CO₂ (720 μL L⁻¹) and defoliation (clipping to 2.5 cm height) on N cycling depended on soil N availability (addition of 1 vs. 11 g N m⁻²) in intact mesocosms extracted from a semiarid grassland. Mesocosms were kept inside growth chambers for one growing season, and the experiment was repeated the next year. We added ¹⁵N (1 g m⁻²) to all mesocosms at the start of the growing season. We measured total N and ¹⁵N in plant, soil inorganic, microbial and soil organic pools at different times of the growing season. We combined the plant, soil inorganic, and microbial N pools into one pool (PIM-N pool) to separate biotic + inorganic from abiotic N residing in soil organic matter (SOM). With the ¹⁵N measurements we were then able to calculate transfer rates of N from the active PIM-N pool into SOM (soil N immobilization) and *vice versa* (soil N mobilization) throughout the growing season. We observed significant interactive effects of elevated CO₂ with N addition and defoliation with N addition on soil N mobilization and immobilization. However, no interactive effects were observed for *net* transfer rates. Net N transfer from the PIM-N pool into SOM increased under elevated CO₂, but was unaffected by defoliation. Elevated CO₂ and defoliation effects on the net transfer of N into SOM may not depend on soil N availability in semiarid grasslands, but may depend on the balance of root litter production affecting soil N immobilization and root exudation affecting soil N mobilization. We observed no interactive effects of elevated CO₂ with defoliation. We conclude that elevated CO₂, but not defoliation, may limit plant productivity in the long-term through increased soil N immobilization.

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1. Introduction

Approximately 40% of the global land surface is under rangelands (13.8% woody savannah and savannah; 12.7% open and closed shrub-lands; 8.3% non-woody grassland; and 5.7% tundra, Suttie et al., 2005), and particularly in semiarid environments, rangelands have shown a large response to a rise in atmospheric CO₂ concentration (hereafter elevated CO₂, Morgan et al., 2004a; Zheng et al., 2010). The productivity and forage quality of semiarid rangelands are to a large degree contingent upon the availability of nitrogen (N) in soil where N is often a limiting nutrient for plant growth (Burke et al., 2008). Both livestock grazing and elevated CO₂ have the potential to alter the availability and cycling of N, but the direction and magnitude of their effects remain unclear.

Domestic livestock grazing affects cycling, loss, and redistribution of N in soil through consumption of N with aboveground plant tissue

and deposition of N in feces and urine (Milchunas et al., 1988; Frank and Evans, 1997; Reeder et al., 2004; Augustine and McNaughton, 2006; Burke et al., 2008; Wolf et al., 2010). Defoliation by livestock also affects the partitioning of photosynthetically fixed C between shoots and roots (Caldwell et al., 1981; Briske et al., 1996; Gao et al., 2008) with, in all likelihood, large consequences for N cycling (Bardgett et al., 2005). For instance, increased root exudation with defoliation (Paterson and Sim, 1999; Paterson et al., 2005; Henry et al., 2008) can stimulate microbial activity in the soil that could eventually result in increased N mineralization and plant N uptake (Hamilton and Frank, 2001; Ayres et al., 2004). However, defoliation can also allocate more C to shoots at the expense of root growth (Holland and Detling, 1990; Fajer et al., 1991; Pietikäinen et al., 2009) that may alter root morphology, root exudation, and the capacity of plants to take up N (Anderson et al., 2007; McInenly et al., 2010).

The increase in productivity of semiarid grasslands in response to elevated CO₂ (Morgan et al., 2004a; Zheng et al., 2010), has been attributed to improved water relations (LeCain et al., 2003; Morgan et al., 2004b), but also to increased mineralization of N (Dijkstra

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et al., 2008). While beneficial soil moisture levels in itself may enhance N mineralization and plant N uptake (Hungate et al., 1997a; Dijkstra et al., 2010a), elevated CO₂ may also stimulate microbial activity through increased root exudation (Hungate et al., 1997b; Cheng, 1999; Pendall et al., 2004). In turn, increased microbial activity under elevated CO₂ has the potential to increase soil N mineralization and plant N uptake (Zak et al., 1993; Dijkstra, 2009), similar to defoliation. In contrast, elevated CO₂ often has no effect on N mineralization (De Graaff et al., 2006) and often reduces soil inorganic N because of increased N immobilization by microbes or plants (Díaz et al., 1993; Hu et al., 2001; Dijkstra et al., 2010b).

Contradictory effects of defoliation and elevated CO₂ on N cycling could occur because defoliation and elevated CO₂ effects may depend on soil N availability. Soil N availability often constrains plant productivity in response to defoliation (Coughenour et al., 1985; Hamilton et al., 1998) and elevated CO₂ (Grünzweig and Körner, 2003; Reich et al., 2006a, 2006b). Defoliation and elevated CO₂ may increase N cycling particularly under low soil N conditions where microbes utilize the increased production of root exudates as an energy source to mine for N locked in soil organic matter (Fontaine et al., 2004; Craine et al., 2007). Thus, these root-microbial interactive effects on N cycling could potentially alleviate N constraints on plant productivity under elevated CO₂ and with defoliation (Hamilton and Frank, 2001; Phillips et al., 2011).

We tested how elevated CO₂ and defoliation, and their interaction, affected N cycling in intact mesocosms extracted from a semiarid grassland, and whether these effects depended on soil N availability. Mesocosms were kept in growth chambers under ambient and elevated CO₂ (720 μL L⁻¹), with and without defoliation (clipping to 2.5 cm height), and with the addition of 1 and 11 g N m⁻², in a full factorial design. We used a ¹⁵N tracer technique to study N dynamics. We followed the ¹⁵N tracer into plant, soil inorganic, microbial and soil organic N pools at multiple times during the experiment. We used a new approach where we combined the Plant, soil Inorganic and Microbial N and ¹⁵N pools into one pool, here defined as the PIM-N pool (Fig. 1). By separating N into these two pools we were then able to calculate rates of N and ¹⁵N flowing from this biotic + inorganic PIM-N pool into the abiotic soil organic N (abiotic SON) pool where N cycling is relatively slow (here defined as soil N immobilization) and *vice versa*, N flowing from the abiotic SON pool into the PIM-N pool (here defined as soil N mobilization). We hypothesized that the rate of soil N mobilization would increase and that the rate of soil N immobilization would decrease under elevated CO₂ and defoliation (and thus result in an increase in the PIM-N pool), but only under the condition of low soil N availability.

2. Materials and methods

2.1. Experimental design

For our growth chamber study we took intact mesocosms from the USDA-ARS High Plains Grassland Research Station in Wyoming, USA (41°11' N latitude, 104°54' W longitude). Mean annual precipitation at this site is 384 mm with most of it occurring in May, June, and July, and mean air temperatures in July and January are 17.5 and -2.5 °C respectively. The vegetation at this site is a northern mixed-grass prairie with the warm-season C₄ grass *Bouteloua gracilis* (H.B.K.) Lag. Ex Steud. and the cool-season C₃ grasses *Pascopyrum smithii* (Rydb.) A. Love and *Hesperostipa comata* Trin & Rupr. as the dominant species. Other species include the subshrub *Artemisia frigida* Willd. and the sedge *Carex eleocharis* L. Bailey. The soil is a fine-loamy, mixed, mesic Aridic Argiustoll.

In the spring of 1997 we pounded 48 PVC cylinders (25 cm diameter) 30 cm into the ground at random locations in a pasture

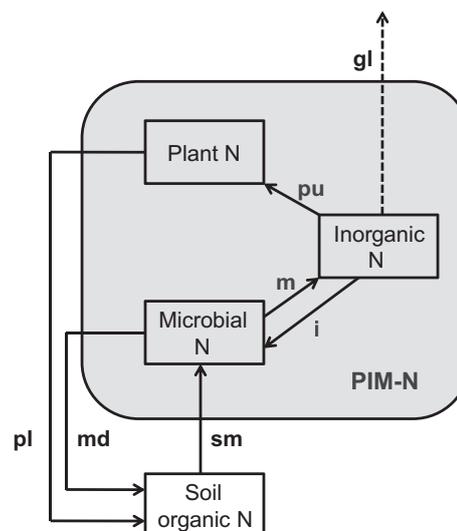


Fig. 1. Simple conceptual diagram of the N pools and flows in our mesocosms. The biotic + inorganic N pools in this system consist of the Plant, soil Inorganic, and Microbial N pools. These relatively active pools combined are here defined as the PIM-N pool (grey box). Exchange of N within the PIM-N pool occurs through plant N uptake (pu), microbial N mineralization (m), and microbial N immobilization (i). Nitrogen leaves the PIM-N pool through gaseous N loss (gl), plant death (pl), and microbial death (md), while N enters the PIM-N pool through soil N mobilization from the abiotic soil organic N pool (sm). If gaseous N loss is assumed to be negligible during the time frame of the experiment, then the size of the PIM-N pool is affected by the net balance of soil N immobilization (si, where $si = pl + md$) and soil N mobilization.

with a long history of light grazing by cattle. We then pulled the cylinders with intact vegetation and soil out of the ground, capped them at the bottom, and transferred them to growth chambers in Fort Collins, CO, USA. On average aboveground biomass in the mesocosms comprised of 23% C₄ grasses, 55% C₃ grasses, and 22% forbs and sedges (LeCain et al., unpublished results). This distribution of functional groups is representative of the site. The mesocosms were randomly placed in 4 growth chambers (EGC Chagrin Falls, OH, USA), 12 mesocosms in each growth chamber. The light level was maintained at 600 μmol m⁻² s⁻¹ photosynthetic photo flux density at canopy height using sodium vapour and metal halide lamps. Photoperiod and day/night temperature were adjusted weekly to mimic the seasonal climate in southeast Wyoming. Weekly water additions to the mesocosms were based on long-term precipitation data (LeCain et al., unpublished results). In two growth chambers we raised the atmospheric CO₂ concentration to 720 ± 50 μL L⁻¹ (mean ± standard deviation, referred to as C+ treatment), while the other two growth chambers were kept under ambient atmospheric CO₂ concentration (370 ± 40 μL L⁻¹, C- treatment). The CO₂ concentration inside the C+ growth chambers was continuously monitored (Vaisala probes, Vantaa, Finland) and computer controlled. These growth chambers have been successfully used in the past to study elevated CO₂ effects on plant growth (Morgan et al., 1998). Before the experiment started (Day 0) we added 10 g N m⁻² as a KNO₃ solution in half of all the mesocosms (6 mesocosms in each growth chamber). We further added 1 g ¹⁵N m⁻² as K¹⁵NO₃ (99 atom% ¹⁵N) in all but two mesocosms in each growth chamber. The N and ¹⁵N were added as solutions by bringing the mesocosms to field capacity with DI water (LeCain et al., unpublished results). The two mesocosms not labelled with ¹⁵N (one with and one without 10 g unlabelled N m⁻²) were harvested after 2 days, and were used to measure background ¹⁵N levels and to calculate recovery of the ¹⁵N label in the different N pools in the ¹⁵N labelled mesocosms (see Equation (2) below). Thus, the N treatment in the remaining 10 mesocosms in each chamber consisted of the addition

of 1 g N m⁻² (N- treatment) to 5 mesocosms and 11 g N m⁻² (N+ treatment) to the other 5 mesocosms. After 35 days, one N- and one N+ mesocosm was harvested from each growth chamber. At the same time, two N- and two N+ mesocosms of the remaining 8 mesocosms in each growth chamber were defoliated at 2.5 cm height (D+ treatment, a one-time defoliation), while the other 4 were not defoliated (D- treatment). This defoliation treatment corresponds with a typical late spring cattle grazing on this rangeland. After 80 days, one replicate of each N and defoliation treatment (a total of 4 mesocosms) in each growth chamber was harvested. The final four mesocosms in each growth chamber were harvested after 125 days. Thus, while the CO₂ and N treatments started on Day 1, the defoliation treatment started on Day 35 and its effect was only measured in the mesocosms harvested on Day 80 and Day 125. The whole experiment was repeated one year later, adopting fresh cores.

2.2. Plant and bulk soil N

At each harvest plant biomass was separated into shoot and root tissue. After breaking up the mesocosm, roots were handpicked and washed. The soil was thoroughly mixed before subsamples were taken. Plant samples and soil subsamples were dried (60 °C), weighed, and ground. The samples were analysed for N and ¹⁵N on an isotope ratio mass spectrometer (IR-MS, Europa Scientific, Cheshire, UK).

2.3. Inorganic N

The soil of each mesocosm was thoroughly mixed. Subsamples were extracted with 1 M KCl and frozen until further analyses. Extracts were analysed for NH₄⁺ and NO₃⁻ on a flow injection analyser (QuikChem FIA+, Lachat Instruments, Milwaukee, WI). Extracts were also analysed for ¹⁵NH₄⁺ and ¹⁵NO₃⁻ using diffusion traps (Stark and Hart, 1996). First NH₄⁺ in the extracts was trapped with acidified filter-paper disks placed inside PTFE tape. Then ¹⁵NO₃⁻ was collected by first converting NO₃⁻ into NH₄⁺ by adding Devarda's Alloy to the extracts, and then trapping the converted NH₄⁺ as above. The filter disks were analysed for ¹⁵N on an IR-MS.

2.4. Microbial N

One soil subsample was immediately extracted with 0.5 M K₂SO₄ while another subsample was extracted with 0.5 M K₂SO₄ after fumigation with chloroform for 2 days. Organic N in the extracts was converted to inorganic N using the persulfate digestion method (Cabrera and Beare, 1993). Analyses for NO₃⁻ and ¹⁵NO₃⁻ in the fumigated and non-fumigated K₂SO₄ extracts were done in the same way as for the KCl extracts.

2.5. Calculations

The plant N pool in each mesocosm was calculated as the sum of N in shoot and root biomass. The soil inorganic N pool was calculated as the sum of NH₄⁺ and NO₃⁻ pools. The microbial N pool was calculated as the difference between the sum of NH₄⁺ and NO₃⁻ in the fumigated sample and the sum of NH₄⁺ and NO₃⁻ in the non-fumigated sample multiplied by 0.54 (Brookes et al., 1985). The PIM-N pool was calculated as the sum of plant, soil inorganic and microbial N. The abiotic SON pool was calculated by subtracting the soil inorganic and microbial N pools from the bulk soil N pool. Thus, the abiotic SON pool included a small fraction of dissolved organic N (DON). Temporal field measurements at the site where our mesocosms were extracted indicated that the DON pool never exceeded 0.2% of the abiotic SON pool (Carrillo et al., unpublished

results). By separating the N of the mesocosms into two distinct pools, the PIM-N pool where N is biotic or directly available to the biota, and the abiotic SON pool where N is not directly available to the biota (with the exception of a small DON pool), we were then able to calculate rates of soil N mobilization and immobilization between these pools (Fig. 1 and see below). All N pools were expressed in g N m⁻².

The amount of ¹⁵N added to the mesocosms recovered in pool *i* (¹⁵N_{rec,i} in g m⁻²) was calculated as:

$$^{15}\text{N}_{\text{rec},i} = N_i \times ^{15}\text{Nfr}_i \quad (1)$$

where *N_i* is the N content of pool *i* (plant, inorganic, or microbial N, in g m⁻²) and ¹⁵Nfr_{*i*} is the applied ¹⁵N label expressed as a fraction of the total N in pool *i* (Dijkstra et al., 2008). This fraction can be calculated as:

$$^{15}\text{Nfr}_i = (\text{At}\%^{15}\text{N}_{\text{post},i} - \text{At}\%^{15}\text{N}_{\text{pre},i}) / (\text{At}\%^{15}\text{N}_{\text{label}} - \text{At}\%^{15}\text{N}_{\text{pre},i}) \quad (2)$$

where At%¹⁵N_{post,*i*}, At%¹⁵N_{pre,*i*} and At%¹⁵N_{label} are the ¹⁵N atom% measured in pool *i* after labelling, the ¹⁵N atom% measured in pool *i* before labelling (i.e., from the mesocosms harvested after 2 days), and the ¹⁵N atom% of the applied label respectively. The ¹⁵N recovery in the bulk soil pool was also calculated with Equation (1) using the N and ¹⁵N values measured in the bulk soil samples. However, since the bulk soil included inorganic and microbial ¹⁵N, we calculated the ¹⁵N recovery in the abiotic SON pool by subtracting the ¹⁵N recovery in the inorganic and microbial biomass from the ¹⁵N recovery in the bulk soil pool. From now on we will refer to the ¹⁵N recovery in the abiotic SON pool as “soil ¹⁵N recovery”. The ¹⁵N recovery in the PIM-N pool was calculated as the sum of the ¹⁵N recoveries in the plant, soil inorganic and microbial pools. Finally, the total ¹⁵N recovery of the whole mesocosm was calculated as the sum of the ¹⁵N recovery in the PIM-N and the abiotic SON pool.

Because we measured N and ¹⁵N pools at different times of the experiment, this allowed us to estimate the rate of N from the PIM-N pool going into the abiotic SON pool (soil N immobilization or *si*, where *si* = *pl* + *md*, Fig. 1) and the rate of N leaving the abiotic SON pool going into the PIM-N pool (soil N mobilization, *sm*). If we assume that gaseous N loss is negligible in this system (Mosier et al., 2002, 2008), then it can be derived that:

$$\Delta\text{PIM-N}/\Delta t = \text{pl} + \text{md} - \text{sm} \quad (3)$$

and:

$$\Delta^{15}\text{N}_{\text{rec,PIM-N}}/\Delta t = \text{pl} \times ^{15}\text{Nfr}_{\text{plant}} + \text{md} \times ^{15}\text{Nfr}_{\text{microbial}} - \text{sm} \times ^{15}\text{Nfr}_{\text{soil}} \quad (4)$$

where ΔPIM-N/Δ*t* and Δ¹⁵N_{rec,PIM-N} (in g N and ¹⁵N m⁻² d⁻¹) are the net changes in N and ¹⁵N recovery in the PIM-N pool between two measuring dates, and ¹⁵Nfr_{plant}, ¹⁵Nfr_{microbial} and ¹⁵Nfr_{soil} are the fractions of labelled N in the plant, microbial and abiotic SON pool respectively. Equation (4) assumes that the fractions of labelled N do not change with time. However, these fractions of labelled N changed somewhat between two measuring dates, but changes were relatively small (Table S2, Supporting Information). We therefore used average values of the two measuring dates (e.g., for the 35–80 day period we averaged the fractions of labelled N measured on Day 35 and Day 80) to calculate soil N immobilization and soil N mobilization. We calculated soil N immobilization and soil N mobilization during the 35–80 and 80–125 day period with two contrasting assumptions. First, we assumed that all N and ¹⁵N loss out of the PIM-N pool occurred through microbial death (*pl* = 0

and $si = md$). Soil N immobilization (si) and soil N mobilization (sm) can now be calculated as:

$$si = \Delta PIM-N/\Delta t + sm \quad (5)$$

$$sm = (\Delta^{15}N_{rec,PIM-N}/\Delta t - \Delta PIM-N/\Delta t \times {}^{15}Nfr_{microbial}) / ({}^{15}Nfr_{microbial} - {}^{15}Nfr_{soil}) \quad (6a)$$

Second, we assumed that all N loss out of the PIM-N pool occurred through plant death ($md = 0$ and $si = pl$). Calculation of si is as in Equation (5) and sm can now be calculated as:

$$sm = (\Delta^{15}N_{rec,PIM-N}/\Delta t - \Delta PIM-N/\Delta t \times {}^{15}Nfr_{plant}) / ({}^{15}Nfr_{plant} - {}^{15}Nfr_{soil}) \quad (6b)$$

In reality N is lost from the PIM-N pool through microbial death and plant death, but our two assumptions provide us with the boundaries of our calculated rates of soil N immobilization and soil N mobilization. Plant death in the mesocosms during the time frame of our experiment mostly happened through root death. For Equation (6b) we therefore used the ${}^{15}Nfr_{plant}$ values measured in roots.

2.6. Statistical analyses

We used ANOVA to test for main effects of CO_2 (C– and C+), defoliation (D– and D+), N addition (N– and N+), time (Days 35, 80, and 125), years (1997 and 1998), and all their interactions on N pools, ${}^{15}N$ recovery, and soil N immobilization and soil N mobilization rates. The mesocosms harvested on Day 2 were included in the ANOVAs testing for effects on N pools. We used a split–split plot design with the CO_2 and year treatments as the between-plots factors, the defoliation, N addition, and time treatments as within-plots factors, and the whole growth chamber as a random factor nested within the CO_2 treatment (Quinn and Keough, 2002). Although there were significant differences between years, there were no significant interactive effects of year \times CO_2 , year \times defoliation, or year \times N addition. Therefore, year effects are not presented and results are presented by combining both years (i.e., the two replicates of each treatment combination in each year were treated as four separate replicates). Whenever there were significant treatment interactions between CO_2 , defoliation, and N addition (i.e., for soil N immobilization and microbial N mobilization rates) we also compared the means of the separate treatment combinations using Tukey's HSD test. In some cases, data were log-transformed to improve assumptions of normality and homoscedasticity. All statistical analyses were performed with JMP (version 8.0.1; SAS Institute, Cary, NC, USA) (Fig. 2).

3. Results

Treatment effects on total shoot and root biomass are summarized in Table S1. In summary, shoot biomass significantly increased under elevated CO_2 in C3 grasses ($P = 0.002$), but not in C4 grasses and forbs, and increased with N addition in C3 grasses ($P = 0.002$) and C4 grasses ($P = 0.0003$), but not in forbs, and was not affected by defoliation. Root biomass significantly increased with N addition, decreased with defoliation, but was not affected by elevated CO_2 .

Total ${}^{15}N$ recovery (in plant, inorganic, microbial and soil N pools) was c. $0.8 \text{ g } {}^{15}N \text{ m}^{-2}$ or 80% of the amount added, and was not affected by elevated CO_2 , defoliation, N addition, and time (Table 1). These results suggest that there was a rapid initial loss of gaseous ${}^{15}N$ during the first 35 days of the experiment, but that after 35 days, N loss was minimal and not affected by the different treatments.

The PIM-N pools (sum of plant, soil inorganic, and microbial pools) increased during the first 35 days of the experiment in most treatments (except for the pots with N addition), but then declined afterwards (Fig. 3). The decline of PIM-N was greater under elevated than ambient CO_2 ($CO_2 \times$ time interaction, $P = 0.02$, Table 1). Defoliation had no effect on PIM-N. Not surprisingly, N addition increased PIM-N. The net addition of 10 g N m^{-2} increased PIM-N by 14 g N m^{-2} after 2 days, but after 35 days the PIM-N increase reduced to 5.4 g N m^{-2} , and remained more or less constant during the remainder of the experiment. There were no significant interactions among treatments ($P > 0.1$).

The decline in PIM-N after 35 days was a result of a decline in the inorganic and microbial N pools, while plant N pools remained more or less constant after 35 days (Fig. 4). Similarly, the greater decline of PIM-N under elevated than ambient CO_2 was caused by a greater decline in the inorganic and microbial N pools under elevated CO_2 ($CO_2 \times$ time interactions, $P = 0.04$ for inorganic N and 0.008 for microbial N, Table 1). Defoliation had no effect on the individual plant, inorganic, and microbial N pools. The N addition initially increased the inorganic and microbial N pools relative to the pools in pots without N addition, but after 35 days these pools returned to levels observed in the pots without N addition. On the other hand, N addition steadily increased the plant N pool relative to plants without N addition. Plants that received 11 g N m^{-2} had on average 5.8 g N m^{-2} more N after 125 days than plants with 1 g N m^{-2} addition. No significant treatment interactive effects on plant, inorganic, or microbial N were observed.

While total ${}^{15}N$ recovery was unaffected by time, ${}^{15}N$ recovery in the PIM-N pool showed a decline with time (Table 1, Fig. 5). This decline was slightly faster under elevated CO_2 ($CO_2 \times$ time interaction, $P = 0.07$) and slower with defoliation ($D \times$ time interaction,

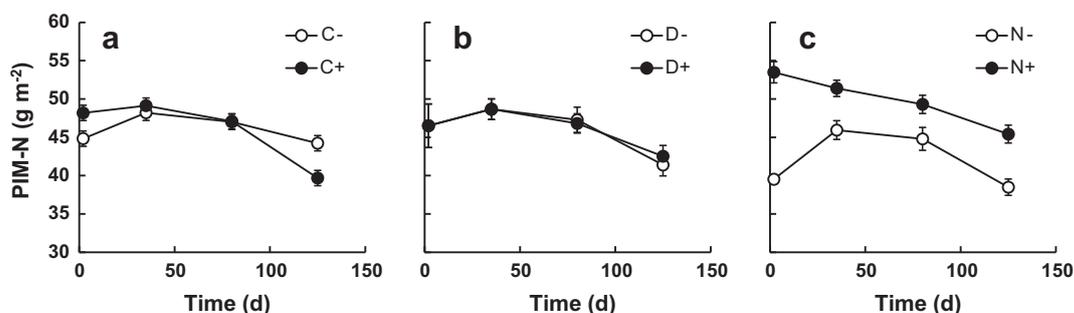


Fig. 2. Main effects of (a) CO_2 (C–: ambient atmospheric $[CO_2]$, C+: $720 \mu\text{L L}^{-1}$ atmospheric $[CO_2]$), averaged across defoliation and N addition treatments, $n = 8$ on Day 2 and Day 35 while $n = 16$ on Day 80 and Day 125); (b) defoliation (D–: no defoliation, D+: with defoliation, averaged across CO_2 and N addition treatments, $n = 16$ on all days, but where defoliation treatment did not start until Day 80); (c) N addition (N– 1 g m^{-2} N added, N+: 11 g m^{-2} N added, averaged across CO_2 and defoliation treatments, $n = 8$ on Day 2 and Day 35 while $n = 16$ on Day 80 and Day 125) on PIM-N pools. Error bars represent 1 standard error.

Table 1

Summary of repeated measures ANOVA results (*P*-values are in bold when *P* < 0.05 and in italics when *P* < 0.1) for the effects of elevated CO₂ (C), defoliation (D), nitrogen (N), and time.

Effect	PIM-N	Plant N	Inorganic N	Microbial N	Total ¹⁵ N recovery	PIM- ¹⁵ N recovery	Soil ¹⁵ N recovery	Soil N immobilization	Microbial N mobilization
	g m ⁻²	g m ⁻²	g m ⁻²	g m ⁻² d ⁻¹	g m ⁻² d ⁻¹				
C	ns	ns	ns	ns	ns	<i>0.08</i>	0.02	ns	ns
D	ns	ns	ns	ns	ns	0.04	ns	0.003	0.05
C × D	ns	ns	ns	ns	ns	ns	ns	ns	ns
N	0.0001	0.0002	<0.0001	ns	ns	0.005	0.003	<i>0.07</i>	ns
C × N	ns	ns	ns	ns	ns	ns	ns	0.003	0.004
D × N	ns	ns	ns	ns	ns	ns	ns	ns	ns
C × D × N	ns	ns	ns	ns	ns	ns	ns	ns	ns
Time	<0.0001	<0.0001	<0.0001	<0.0001	ns	<0.0001	<0.0001	0.04	ns
C × time	0.02	ns	0.04	0.008	ns	<i>0.07</i>	0.02	ns	<i>0.08</i>
D × time	ns	ns	ns	ns	ns	0.02	<i>0.10</i>	ns	ns
C × D × time	ns	ns	ns	ns	ns	ns	ns	ns	ns
N × time	<i>0.10</i>	0.04	<0.0001	<i>0.09</i>	ns	ns	ns	ns	ns
C × N × time	ns	ns	ns	ns	ns	ns	ns	0.008	0.03
D × N × time	ns	ns	ns	ns	ns	ns	ns	0.002	0.0009
C × D × N × time	ns	ns	ns	ns	ns	ns	ns	ns	ns

P = 0.02). Because total ¹⁵N recovery, and thus gaseous ¹⁵N loss, was not affected by the treatments, the faster decline of ¹⁵N in the PIM-N pool under elevated CO₂ and without defoliation was most likely caused by increased transfer of N from the PIM-N pool into the abiotic SON pool through plant and/or microbial death (Fig. 1). Indeed, the ¹⁵N recovery in the abiotic SON pool mirrored the ¹⁵N recovery in the PIM-N pool, where ¹⁵N recovery increased with time, and where the increase was faster under elevated CO₂ (CO₂ × time interaction, *P* = 0.02) and slower with defoliation

(although this time not significantly, Table 1, Fig. 5). These results indicate 1) that the ¹⁵N was transferred from the PIM-N pool into the abiotic SON pool during the experiment through plant and/or microbial death, and 2) that this transfer increased under elevated CO₂ and decreased with defoliation. The ¹⁵N recovery in the PIM-N pool was greater with N addition (Table 1, Fig. 5), most likely because N addition diluted the ¹⁵N in the PIM-N pool, which resulted in a decreased loss of ¹⁵N per unit of N transferred from the PIM-N pool into the abiotic SON pool. Of the two N loss pathways

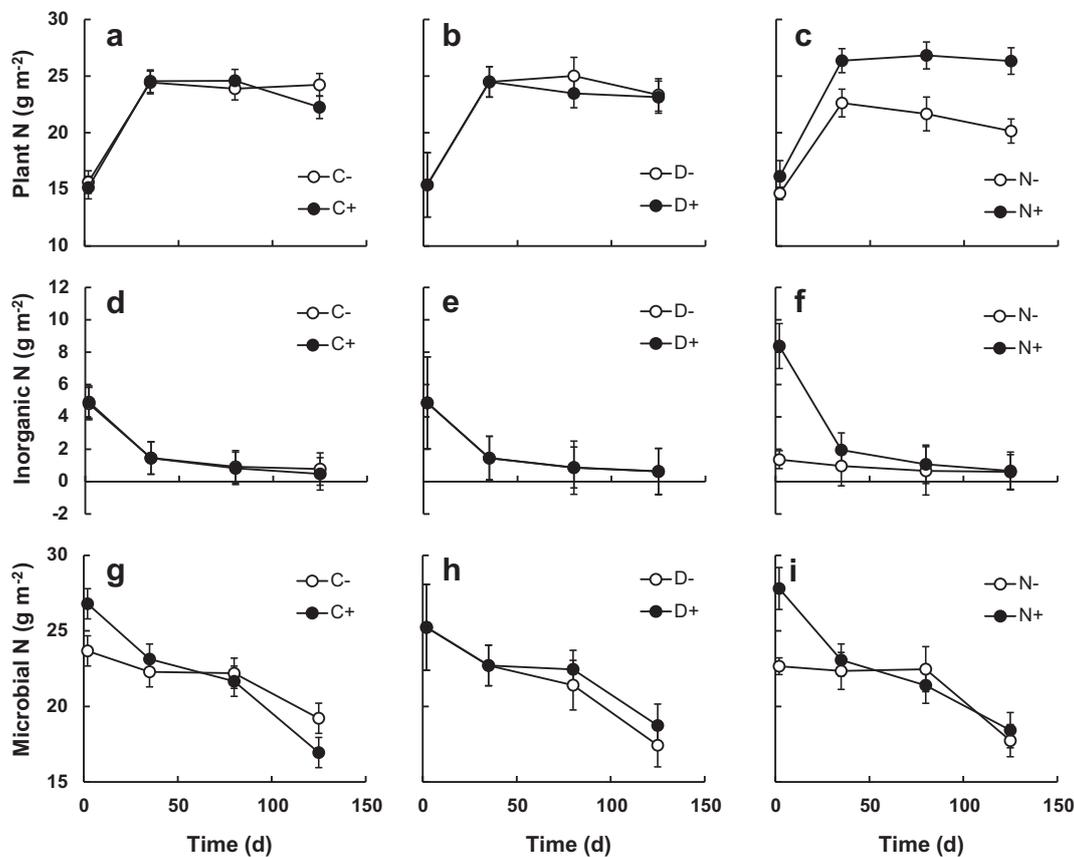


Fig. 3. Main effects of CO₂ (Panels a, d, and g), defoliation (Panels b, e, and h), and N addition (Panels c, f, and i) on total Plant N (Panels a, b, and c), Inorganic N (Panels d, e, and f), and Microbial N (Panels g, h, and i). See Fig. 2 for treatment abbreviations. Error bars represent 1 standard error.

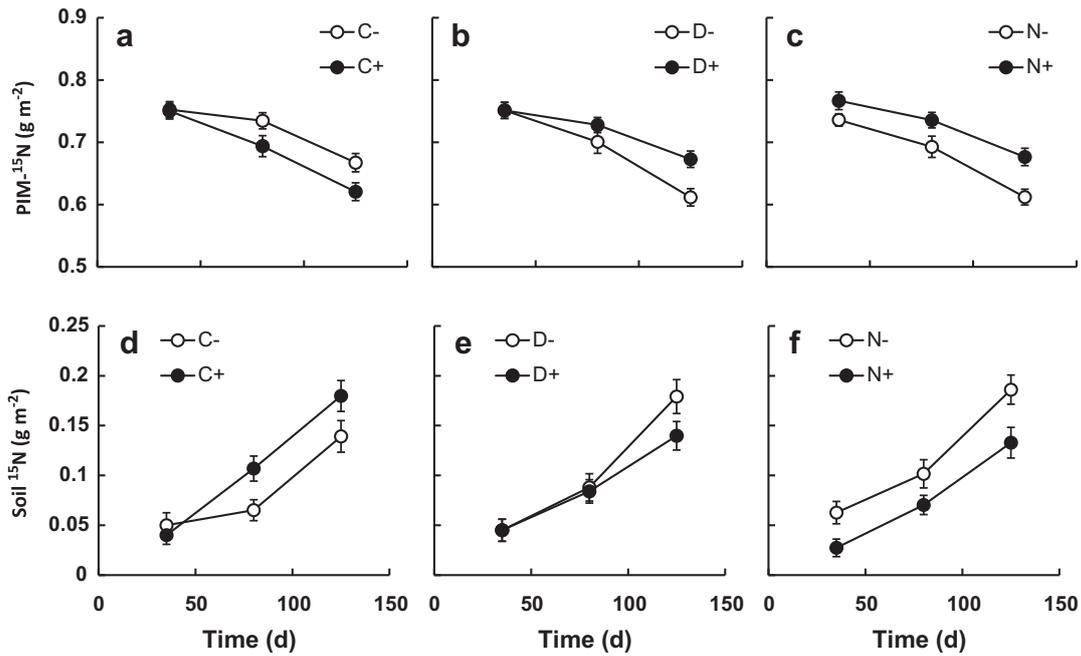


Fig. 4. Main effects of CO₂ (Panels a and d), defoliation (Panels b and e), and N addition (Panels c and f) on ¹⁵N recovery in the PIM-N pool (Panels a, b, and c, PIM-¹⁵N) and in the abiotic soil organic N pool (Panels d, e, and f, soil ¹⁵N). See Fig. 2 for treatment abbreviations). Error bars represent 1 standard error.

(root and microbial death) N addition diluted the ¹⁵N particularly in microbes (Supporting Information, Table S2), suggesting that N and ¹⁵N transfer by microbes rather than by plants caused the increased ¹⁵N recovery in the PIM-N pool with N addition.

When we calculated the rate of N transferred from the PIM-N pool into the abiotic SON pool (soil N immobilization) and the rate of N transferred from the abiotic SON pool into the PIM-N pool (soil N mobilization) based on the assumption that all N leaving the

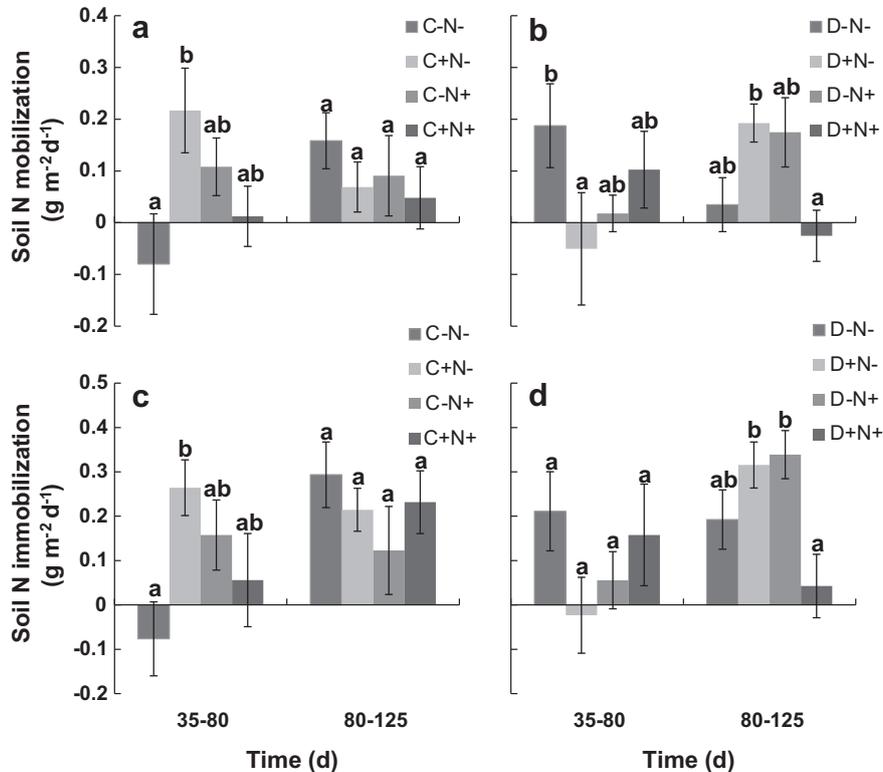


Fig. 5. Calculated rates of soil N immobilization (Panels a and b) and soil N mobilization (Panel c and d) during the 35–80 and 80–125 day period assuming that all N loss out of the PIM-N pool occurred through microbial death. In Panel (a) and (c) the interactive effects of the CO₂ and N treatments are shown (averaged across defoliation treatment, n = 8), in Panel (b) and (d) the interactive effects of the defoliation and N treatments are shown (averaged across CO₂ treatment, n = 8). Error bars represent 1 standard error. Different letters above bars indicate significant differences for each time separately (P < 0.05, Tukey's HSD test).

PIM-N pool occurred through microbial death (Equations (5) and (6a)), then for the first time we observed significant treatment interactions. For both for soil N immobilization and microbial N mobilization we observed significant $\text{CO}_2 \times \text{N} \times \text{time}$ and $\text{D} \times \text{N} \times \text{time}$ interaction effects (Table 1). The $\text{CO}_2 \times \text{N} \times \text{time}$ interactions occurred because both N immobilization and mobilization fluxes increased under elevated CO_2 , but only without N addition, and only during the 35–80 day period of the experiment (Fig. 5). Similarly, the $\text{D} \times \text{N} \times \text{time}$ interactions occurred because both N immobilization and mobilization fluxes decreased with N addition, but only when plants were defoliated, and only during the last 80–125 days of the experiment. During the 35–80 day period microbial N mobilization also decreased with defoliation, but only without N addition. On average defoliation decreased soil N immobilization by 37% and microbial N mobilization by 46%. When we calculated soil N immobilization and microbial N mobilization based on the assumption that all N leaving the PIM pool occurred through plant death (Equations (5) and (6b)), then we observed in general smaller fluxes but similar treatment effects (Supporting Information, Table S3 and Fig. S1).

4. Discussion

Because of the complex nature of the N cycle involving plants, microbes and environmental factors, studying N dynamics in intact plant-soil systems has been notoriously difficult (Schimel and Bennett, 2004; Frank and Groffman, 2009). We used a novel approach where we estimated N transfer between the biotic + inorganic PIM-N pool (sum of plant N, soil inorganic N, and microbial N), and the abiotic SON pool. If gaseous N loss is negligible, the temporal change in size of the PIM-N pool reflects the net change in N flowing from the abiotic SON pool into the PIM-N pool (soil N mobilization) and N flowing out of the PIM-N pool into the abiotic SON pool (soil N immobilization associated with plant and microbial death, Fig. 1). We used a ^{15}N tracer technique where we successfully separated these two N fluxes.

4.1. Testing of assumptions

Our assumption that gaseous N loss during the experiment was negligible appears to be valid during the 35–125 day period. Moreover, total ^{15}N recovery was not affected by the CO_2 , defoliation, or N addition treatments. Total ^{15}N recovery in our mesocosms remained flat at 80% between Day 35 and Day 125, suggesting that during this time very little ^{15}N was lost as gaseous N. Rates of N_2O and NO_x emissions under field conditions in a similar semiarid grassland were also small because of high plant N demand (Mosier et al., 2002). However, denitrification may have caused the 20% loss of ^{15}N during the first 35 days of the experiment when soil moisture was still near field capacity (LeCain et al., unpublished results), and NO_3^- concentrations were relatively high, conditions that are favourable for denitrification.

Because we did not know what the relative contribution of microbes and plants was to the transfer of N into the abiotic SON pool, we calculated soil N mobilization and immobilization rates based on two contrasting assumptions. First, we calculated rates based on the assumption that all N transfer into the abiotic SON pool occurred through microbial death, and second, based on the assumption that all N transfer into the abiotic SON pool occurred through plant death (Fig. 1). Although N rates calculated with the second assumption were slightly lower, very similar patterns were observed in terms of treatment effects (Supporting Information, Table S3 and Fig. S1). In other words, soil N mobilization and immobilization rates were rather insensitive to the two contrasting methods used to calculate these fluxes.

4.2. Main effects of elevated CO_2 and interactive effects of elevated CO_2 with soil N addition

Our hypothesis that soil N mobilization would increase under elevated CO_2 only under the condition of low soil N addition was confirmed during the 35–80 day period of our experiment (Fig. 5, Table 1). During this period, soil microbes grown in conditions of low N availability may have used an increased availability of labile C (including root exudates) under elevated CO_2 as an energy source to mine for N locked in soil organic matter (Phillips, 2007; Rütting et al., 2010). Increased microbial mining of N from soil organic matter under elevated CO_2 could benefit plant growth if the mined N is eventually transferred to the plants after microbial turnover (Cheng and Kuzyakov, 2005). However, we found no evidence that this transfer to plants happened in our mesocosms. The plant N pool was unaffected by elevated CO_2 (Fig. 4). Further, while soil N mobilization increased during the 35–80 day period in the low N treatment, so did soil N immobilization (Fig. 5). Our results suggest that most of the extra N mined by microbes from soil organic matter under elevated CO_2 was returned to the soil. Thus, elevated CO_2 enhanced cycling of N between the PIM-N and abiotic SON pool during the 35–80 day period independent of soil N availability and without having an impact on N in plants. Possibly, enhanced N cycling did not increase plant N uptake under elevated CO_2 because of intensified plant–microbial competition for N (Díaz et al., 1993). The abiotic SON pool included dissolved organic N (DON), and much of the transfer of N between the PIM-N and abiotic SON pool most likely occurred through DON. Indeed, all N in soil organic matter first has to depolymerise where much of the N enters the DON pool before it is available to plants and microbes (Schimel and Bennett, 2004).

The $\text{CO}_2 \times \text{N}$ interactive effect on soil N immobilization and mobilization disappeared during the 80–125 day period, suggesting that the $\text{CO}_2 \times \text{N}$ interactive effect was transient. The $\text{CO}_2 \times \text{N}$ interactive effect on soil N mobilization may have disappeared because soil inorganic N and microbial N pools were low in all mesocosms by the end of the experiment (Fig. 4). As such, microbial demand for N and the potential for microbial mining for N from SOM may have been high in all mesocosms, including mesocosms with N addition, by the end of the experiment. At the same time, translocation of C to root exudates may have been low in all mesocosms. It has been suggested that root exudation declines as plants mature (Meharg and Killham, 1990; Warembourg and Estelrich, 2001), resulting in reduced rhizosphere effects on soil organic matter decomposition (Cheng et al., 2003; Dijkstra et al., 2010c). This may then have subdued any elevated CO_2 effects on soil N mobilization at the end of the experiment. Soil N immobilization, on the other hand, remained high in all treatments (Fig. 5). A prolonged low soil moisture content during the last 50 days of the experiment when no water was added to the mesocosms may have caused water stress to microbes and plants inducing microbial and plant death in all treatments where the associated N returned to the soil pool.

While elevated CO_2 and $\text{CO}_2 \times \text{N}$ interactive effects on soil N mobilization and immobilization were transient, the effect of elevated CO_2 on the PIM-N pool, which reflects the net difference in soil N mobilization and immobilization, increased with time. But in contrast to our hypothesis, the PIM-N pool declined more under elevated than ambient CO_2 (Fig. 3), indicating that the net N transfer from the PIM-N pool to the abiotic SON pool was higher under elevated CO_2 , as is also illustrated by the increased transfer of ^{15}N into the abiotic SON pool under elevated CO_2 (Fig. 5). Moreover, this transfer was not affected by initial differences in soil N availability. Despite a greater transfer of N to the abiotic SON pool and a greater decrease in soil inorganic N under elevated CO_2 , the plant

N pool was not affected by elevated CO₂. Likewise, the plant N pool was not affected after three years of elevated CO₂ in a field experiment (the same site where our mesocosms were extracted from), indicating that during the first three years plants under elevated CO₂ were able to take up the same amount of N despite higher soil N immobilization (Dijkstra et al., 2010b). However, increased immobilization of N into the soil could constrain plant N uptake in the long-term (Díaz et al., 1993; Luo et al., 2004). In contrast, plant N pools remained significantly higher after five years of elevated CO₂ in a semiarid grassland in Colorado with similar plant species (Dijkstra et al., 2008).

4.3. Main effects of defoliation and interactive effects of defoliation with soil N addition

We found no evidence for an increase in soil N mobilization after defoliation under low soil N. We expected that soil N mobilization would increase after defoliation, but only in the low N treatment, where microbes would utilize the increased supply of root exudates as an energy source to mine for N locked in SOM (Fontaine et al., 2004; Craine et al., 2007). Instead, soil N mobilization decreased directly after defoliation in the low N treatment, while soil N mobilization was unaffected by defoliation in the high N treatment (Fig. 5). In contrast, defoliation of *Poa pratensis* increased rhizodeposition and caused a short-lived increase in potential N mineralization, which resulted in increased plant N uptake (Hamilton and Frank, 2001). Our results are more in line with a recent study by Augustine et al. (2011) where rhizodeposition was significantly reduced directly after defoliation of *B. gracilis* and *P. smithii*. Reduced rhizodeposition may then also have caused the temporary reduction in soil N mobilization in the low N treatment after defoliation in our mesocosms.

Defoliation reduced soil N mobilization and soil N immobilization in a similar way, and there was no defoliation effect on the net transfer between the PIM-N pool and passive soil organic N pool (i.e., no effect on the size of the PIM-N pool). The plant N pool was also not affected by defoliation (although shifts in N occurred between roots and shoots, LeCain et al., unpublished results), suggesting that despite changes in absolute rates of soil N mobilization and immobilization, defoliation may not improve or worsen the N status in soil for plant N uptake in this system. Indeed, Burke et al. (2008) concluded that the overall effect of cattle grazing on the N budget in a semiarid grassland, similar to where our mesocosms were from, is small. On the other hand, defoliation or grazing often increases net N mineralization in the soil (Frank and Groffman, 1998; Stark et al., 2002; Ayres et al., 2004).

4.4. Contrasting elevated CO₂ and defoliation effects on N cycling caused by a shift in the balance between root exudation and root litter inputs?

Not only changes in root exudation, but also changes in root litter inputs could influence the net N transfer between active and passive pools, which could then also help explain some of the contrasting effects of elevated CO₂ and defoliation on N cycling observed in different field experiments. For instance, while the reduction in soil N mobilization with defoliation (on average by 46%) may have been a result of lower root exudation rates, the reduction in soil N immobilization (on average by 37%) may have been a result of reduced root C inputs as root litter after defoliation (Holland and Detling, 1990; Frank and Groffman, 1998). Similarly, the increase in the net transfer of N from the PIM-N pool into SOM under elevated CO₂ may have been a result of a larger increase in root litter inputs increasing soil N immobilization (Jastrow et al., 2000; Dilustro et al., 2001). We have no information about root

litter inputs in our mesocosms. Root biomass no longer increased after 35 days in defoliated mesocosms (in fact, there was a slight decrease, on average by 2%) while root biomass increased on average by 10% in non-defoliated mesocosms (Table S1). A relative decrease in root biomass with defoliation may also have decreased root turnover, but direct evidence for this is lacking. Root biomass was not affected by elevated CO₂, but root turnover has been found to increase under elevated CO₂ in the field (Milchunas et al., 2005), suggesting that root litter inputs increased. Thus, relative changes in allocation of C between root litter and root exudation caused by elevated CO₂ and defoliation could affect soil N mobilization and immobilization in opposite ways, where it is the balance between the two C allocation pathways that determines long-term N availability to plant growth.

5. Conclusions

While we observed significant interactive effects of elevated CO₂ with N addition (CO₂ × N) and defoliation with N addition (D × N) on rates of transfer between the PIM-N pool (Plant, soil Inorganic, and Microbial pool) and the abiotic SON pool, net transfer of N between these pools was independent of soil N availability. This is surprising, because many studies have suggested that ecosystem responses to CO₂ and defoliation should depend on N level (e.g., Hamilton et al., 1998; Grünzweig and Körner, 2003; Reich et al., 2006a). We may not have observed CO₂ × N and D × N interactive effects on net N transfer because the N addition only had a transient effect on soil inorganic N and microbial N pools, and thus CO₂ × N and D × N interactive effects on soil N mobilization and immobilization may have been also transient. However, integrative measures of soil N mobilization and immobilization have often been measured in disturbed soils incubated in the laboratory (e.g., Frank and Groffman, 1998; Rütting et al., 2010), and not in undisturbed soils in the presence of plants. Plant presence however, can have a significant influence on N cycling in soils (Dijkstra et al., 2009). Here we used a new and robust method that provides an aggregative measure of N transfer between biotic (including directly available inorganic) N and abiotic N. Using our new approach, we showed that the net transfer from the biotic + inorganic PIM-N pool into the abiotic SON pool increased under elevated CO₂, which could eventually cause a negative feedback mechanism for the enhanced plant growth under elevated CO₂ (Luo et al., 2004; Reich et al., 2006b). On the other hand, defoliation slowed down both soil N mobilization and immobilization, and had no effect on the net transfer between the PIM-N and abiotic SON pool. Therefore, defoliation may only have limited effects on the N budget in semiarid grasslands in the long-term.

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Appendix. Supporting information

The supplementary information associated with this article can be found in the online version, at doi:10.1016/j.soilbio.2011.07.017.

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