Soil microbial activity and N availability with elevated CO_2 in Mojave Desert soils

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[1] We examined the effects of elevated CO_2 on soil nitrogen (N) dynamics in the Mojave Desert by measuring plant N isotope composition ($\delta^{15}N$), soil microbial biomass N, soil respiration, resin-available N, and C and N dynamics during soil incubations. With elevated CO_2, foliage of *Larrea tridentata* and *Krameria erecta* had mean $\delta^{15}N$ 2.1 and 1.1‰ higher with elevated CO_2, respectively, and elevated CO_2 increased microbial biomass N in dry soils under a perennial grass (6.8 ± 1.4 versus 3.7 ± 0.3 μg/g). Elevated CO_2 significantly increased cumulative resin-available N in the field by 12%, driven by available soil moisture. Rates of soil respiration with elevated CO_2 were sporadically higher under *Pleuraphis* and *Larrea*. Soils under shrubs had greater potential net N mineralization (102.6 ± 24.2 μg/g) than soils under grasses and in plant interspaces (40.0 ± 9.69 μg/g). Rates of recalcitrant N turnover in soil incubations were related to soil substrate availability. Results indicate that shifts in soil microbial structure and/or activity may occur with elevated CO_2 and may result in increases in plant-available N when soil moisture is available. *INDEX TERMS:* 1615 Global Change: Biogeochemical processes (4805); *KEYWORDS:* elevated CO_2, soil microorganisms, soil carbon, nitrogen cycling, Mojave Desert, nutrient availability

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1. Introduction

[2] Ecosystem productivity is governed in part by the spatial and temporal patterns of plant-available nitrogen (N) [*Vitousek and Howarth*, 1991; *Vitousek et al.*, 1997]. Sustained increases in plant productivity that are predicted with elevated CO_2 [*Curtis and Wang*, 1998; *Smith et al.*, 2000] will depend on continued availability of N and the biogeochemical cycles that govern it [*Curtis and Wang*, 1998; *Pregitzer et al.*, 2000; *Zak et al.*, 2000a; *Schaeffer et al.*, 2003]. Pools and fluxes of N are influenced by soil carbon (C) availability, which likely will be altered under elevated CO_2 [*Zak et al.*, 1993, 2000a].

[3] Numerous studies show increases in plant C allocation to roots, rhizosphere respiration, and rhizodeposition with elevated CO_2 [*Cheng and Johnson*, 1998], but an emergent pattern describing the effects of these processes on soil N availability remains elusive. Increases [*Zak et al.*, 1993; *Hungate et al.*, 1996, 1997a, 1997b] or decreases [*Diaz et al.*, 1993; *Hungate et al.*, 1996; *Berntson and Bazzaz*, 1997, 1998] in rates of N cycling can occur with elevated CO_2. *Zak et al.* [2000b] recently concluded that few studies have found significant increases in microbial respiration with elevated CO_2, and that there is high variation in microbial response to elevated CO_2. This implies that the microbes responsible for soil N transformations have varied responses to elevated CO_2.

[4] The response of N cycling to rising atmospheric CO_2 concentrations in arid ecosystems is equally unclear, even though arid ecosystems are expected to experience large increases in productivity with elevated CO_2 [*Strain and Bazzaz*, 1983; *Melillo et al.*, 1993; *Smith et al.*, 1997]. Such increases will result in deserts playing an increased role in global biogeochemical cycling. *Billings et al.* [2002] suggest that elevated CO_2 in the Mojave Desert may result in increased soil microbial activity with increases in soil microbial substrate. Nitrogen may become more limiting to plants if elevated CO_2 increases soil C; increases in labile soil C result in increased soil microbial biomass and reduced N availability in the Chihuahuan [*Gallardo and Schlesinger*, 1995] and Mojave Deserts [*Schaeffer et al.*, 2003]. An increase in the amount of plant litter and changes in litter chemistry have reduced plant-available N in an arid grassland [*Evans et al.*, 2001]. Such reductions in plant-available N suggest that increases in arid ecosystem productivity with elevated CO_2 may be limited by N availability. Alternatively, these effects could be mitigated by increases in net N

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mineralization [*Klironomos et al.*, 1996; *Jones et al.*, 1998; *Kampichler et al.*, 1998; *Lussenhop et al.*, 1998; *Zak et al.*, 2000a].

[5] This study had three primary objectives. First, we assessed perturbations in the N cycle with elevated CO_2 using a stable isotope approach similar to that of *Billings et al.* [2002]. Second, we quantified changes in microbial activity and N availability with elevated CO_2 by monitoring resin-available N and rates of soil respiration in the field and measuring potential C evolution and net N mineralization in long-term soil incubations. Third, we applied a model to net N mineralization data from the incubations to estimate pool sizes of labile organic N, labile pool rate constants, and mineralization rates of recalcitrant N [*Wedin and Pastor*, 1993]. We hypothesized that microbes in elevated CO_2 soils would be relatively less C limited than their ambient counterparts; this would be reflected in higher vegetation $\delta^{15}N$ values, lower N availability in the field, higher C evolution in laboratory incubations and in the field, and smaller pool size estimates of labile organic N.

2. Methods

2.1. Study Site

[6] The study took place at the Nevada Desert Free-Air Carbon Enrichment (FACE) facility, 15 km north of Mercury, Nevada (36°49′N, 115°55′W; elevation 965 to 970 m) [*Jordan et al.*, 1999]. *Larrea tridentata* (DC.) Cov., *Ambrosia dumosa* (A. Gray) Payne, *Lycium andersonii* (A. Gray), *Lycium pallidum* (Miers var. *oligospermum* C. Hitchc.), and *Krameria erecta* Schultes are the dominant shrubs. Dominant perennial grasses are *Pleuraphis rigida* Thurber and *Achnatherum hymenoides* (Roemer and Schultes) Barkworth. Temperatures range from −19°C to 48°C. Rainfall occurs primarily in the winter months, averaging 134.33 ± 2.53 mm yr^{-1} from 1996 to 2002. Winter and summer annuals occur when rainfall is sufficient, including the exotic *Bromus madritensis ssp. rubens* (L.) Husnot. Biological soil crusts, some of which fix atmospheric N_2, can dominate plant interspaces [*Jordan et al.*, 1999].

[7] The facility has nine, 23-m diameter experimental plots following the design of *Hendrey and Kimball* [1994] and has been operational since April 1997. Atmospheric $[CO_2]$ is maintained at 550 μL/L in three plots, except when air temperatures are below freezing or when average wind speeds are greater than 6 m/s. A second set of three plots is fumigated with ambient air. Three plots serve as controls with no fumigation to test for effects of fan disturbance. This study focuses on the elevated CO_2 and ambient plots. Investigators access the site from a suspended platform [*Jordan et al.*, 1999] to retain the integrity of biological soil crusts that are an important source of plant-available N [*Evans and Ehleringer*, 1993; *Billings et al.*, 2003a].

2.2. Carbon and Nitrogen Content and Isotope Composition

[8] We sampled foliage for $\delta^{15}N$ from seven dominant perennial shrubs and grasses. *Larrea tridentata* foliage was sampled approximately every 5 weeks from March through August of 1999 and 2000, and March through June 2001. *Krameria erecta* foliage was sampled from May through August 1999, in August of 2000, and from May through June of 2001. Foliage of five other perennial species (*Ambrosia*, *L. andersonii*, *L. pallidum*, *Achnatherum*, *Pleuraphis*) was sampled in May and June 1999, and May 2000. In addition to these sampling dates, *L. andersonii* and *Achnatherum* foliage was sampled in March and April of 2000, *L. pallidum* foliage was sampled in April 2000, and *Ambrosia* foliage was sampled in April and May 2001. In 2001, between four and nine plants of each species were sampled in each plot. In 1999 and 2000, between two and four *Larrea* plants and one plant of all other species were sampled in each plot. Foliar samples were dried for 48 hours at 60°C and ground to a fine powder for analysis.

[9] Soils (0 to 5 cm) underneath *Larrea*, *Lycium* spp., and *Pleuraphis* and in the plant interspaces were sampled from 27 February to 1 March 2001. Soil was collected at three locations for each cover type in each of the six plots, and pooled according to cover type to generate four composited soil samples per plot. Soils were air-dried and sieved (2 mm). We ground samples to a fine powder and acid washed them three times with 3N H_3PO_4 to remove carbonates. To ensure that organic matter was not lost during acid washing, we compared total organic N and soil $\delta^{15}N$ in acid washed and non-acid washed soils. No differences were found. Both vegetation and soil samples were analyzed for $\delta^{15}N$ on a Carlo Erba elemental analyzer (NA1500 CHN Combustion Analyzer, Carlo Erba Strumentazione, Milan) coupled to a Finnigan Delta$^+$ mass spectrometer (Finnigan MAT, Bremen, Germany) via a Finnigan Conflo II Interface, at the University of Arkansas Stable Isotope Facility. All isotopic data reflect natural abundances.

2.3. Microbial Biomass

[10] Soils were collected in March 2001 and April 2002 following the sampling protocol described above. Soils collected in 2001 were incubated at 30°C for 24 hours at field water content (between 1 and 2% gravimetric water content for all soils). We extracted 8 g of each soil type in 40 mL 0.5M K_2SO_4 after the incubation period. A second replicate set was fumigated with $ClCH_4$ for 5 days in a dessicator before extraction. Extracts were subjected to a Kjeldahl digest and analyzed for total ammonium-N (NH_4^+-N) on an Alpkem autoanalyzer (OI Analytical, College Station, Texas). Using bulk density data obtained from previous experiments and gravimetric water content data, soils collected in 2002 were moistened to approximately 60% water-filled pore space with a pipette and mixed before being subjected to the $ClCH_4$ fumigation and extraction. Adding water was intended to give an indication of microbial biomass after rainfall. These extracts were subjected to a persulfate digest [*D'Elia et al.*, 1977] and analyzed for total nitrate-N (NO_3-N) on an Alpkem autoanalyzer. Microbial biomass N was calculated as the difference in NH_4^+-N or NO_3-N between fumigated and unfumigated samples, divided by the constant 0.69 [*Brookes et al.*, 1985; *Gallardo*

and Schlesinger, 1992] to account for estimated extraction efficiency. Previous experiments comparing the Kjeldahl and persulfate methods revealed no significant differences in microbial biomass N.

2.4. Resin-Available N

[11] Cation-anion exchange resin (10 g) (Dowex MR-3, Dow Chemical) was placed in nylon pouches [*Binkley and Matson*, 1983; *Binkley*, 1984] in the top 5 cm of the soil surface under five cover types (*Larrea*, *Ambrosia*, *Pleuraphis*, *Achnatherum*, and in plant interspaces). One bag was placed under each cover type in six plots. Bags were replaced approximately every 6 weeks from 21 December 1998 through April 2001. Upon removal, bags were transported to the University of Arkansas for analysis. Each bag was extracted in 50 mL 2M KCl, and extracts were analyzed colorimetrically for NH_4^+ and NO_3^-.

2.5. Soil Respiration

[12] In February 1999 we inserted two beveled, 25.5-cm-diameter PVC chambers approximately 7.5 cm into the soil under each of the four different cover types, *Larrea tridentata*, *Pleuraphis rigida*, *Lycium spp.*, and in plant interspaces, for a total of eight chambers per plot. Chamber tops of the same material and diameter, fitted with airtight plexiglas lids and a strip of closed cell foam on the bottom rim, were placed on top of the installed chambers in the experimental plots during measurements. A snug-fitting rubber strap was fastened around the seal to maintain air tightness. Chamber volumes were determined in the field by recording the amount of water needed to fill a plastic bag within the chambers.

[13] Soil respiration was determined by taking three, 9-mL gas samples sequentially from the closed chambers over 2 hours. Samples were injected into previously evacuated gas-tight vials, transported to the University of Arkansas, and analyzed for CO_2 by gas chromatography. Sampling occurred in May, July, and October 1999, March, May, and October 2000, and March 2001. Fluxes of CO_2 were calculated as the slope of the line that best fit any observed increase in concentration over time, accounting for chamber volume and area. When concentration increases were best described by a curvilinear relationship, rates of CO_2 flux were determined using the slope of the curve at time zero [*Billings et al.*, 1998]. This method helps to account for the potential underestimation of soil respiration resulting from CO_2 accumulation in the chambers, and the accompanying decreasing concentration gradient across the soil surface over time.

2.6. Long-Term Soil Incubations

[14] Soils were collected following the sampling protocol described above, from 27 February to 1 March 2001. Fifty grams of each of the 24 soil types (four cover types in six plots) were placed in a 5.3 cm diameter $\times$ 5.0 cm tall polyvinyl chloride core held by glass fiber filter paper taped to the bottom. We vacuum extracted inorganic N from each soil sample (40 kPa) with N-free nutrient solution [*Nadelhoffer*, 1990] at the beginning of the incubation and placed each soil sample in a 1-L gas-tight jar equipped with a gas sampling port. Samples rested on glass marbles to allow air flow across the bottom of the cores. We extracted 9 mL of gas from each jar on days 8, 21, 41, 71, 136, 198, and 280. Samples were stored in 12-mL pre-evacuated, gas-tight vials (Tekmar-Dohrman, Cincinnati, Ohio). Headspace was calculated by subtracting the volume of the soil core and marbles from the jar volume. After gas sampling, we vacuum-extracted inorganic N from each soil sample with N-free nutrient solution, placed the samples back in the jars, and took another gas sample before sealing the jars. Samples were stored in the dark at 30°C between sampling dates. At the end of the incubation, we took subsamples of incubated soils and processed them for isotope sampling as described above. More frequent sampling early in the incubation ensured relatively frequent flushing of the jar environment, to ensure incubations remained aerobic. This was less critical near the end of the incubation, when microbial activity was diminished.

[15] Extracts were analyzed colorimetrically for NH_4^+ and NO_3^-. Potential net N mineralization was calculated as the difference in inorganic N between extraction dates. Gas samples were analyzed for CO_2 on a Shimadzu 14A gas chromatograph equipped with an electron capture detector (Dallas, Texas). Rates of C evolution were calculated as the increase in CO_2 concentration over time.

[16] The size of the labile N pool and rate constants for mineralization of labile and recalcitrant pools were estimated as

$$N_t = N_l\left(1 - e^{-h_l t}\right) + c_r t,$$

where N_t is the cumulative amount of N mineralized at time t, N_l is the pool size of the labile pool of N relative to total soil N, h_l is the rate constant for the labile N pool, and c_r is the mineralization rate of the recalcitrant pool of N [*Bonde and Rosswall*, 1987; *Wedin and Pastor*, 1993]. The model assumes that mineralization of recalcitrant N is constant. We estimated parameter values using a nonlinear curve fitting procedure (PROC NLIN, SAS 8.01) on cumulative net N mineralization data. This procedure finds the best fitting equation by minimizing the sum of squares of the residuals. We tested the robustness of parameter estimates by changing the starting values of the iterative procedure to values within the 95% confidence intervals; no change in parameter estimates resulted.

2.7. Statistical Analyses

[17] We used a repeated measures, mixed random and fixed effects analysis (PROC MIXED, SAS 8.01) to determine effect of atmospheric CO_2, date of sampling, and their interaction on each plant species' $\delta^{15}N$. This test allows modeling of the data's covariance structure to account for unevenly spaced sampling dates [*Littell et al.*, 1996]. The same analysis was used to test the effect of CO_2 treatment, date, cover type, and their interactions on resin-available N, field rates of soil respiration, and C evolution and net N mineralization in laboratory incubations. An analysis of variance (PROC GLM, SAS 8.01) was used for determining the effect of CO_2 treatment, plant cover type, and their interaction on soil $\delta^{15}N$ before and after the incubation. An

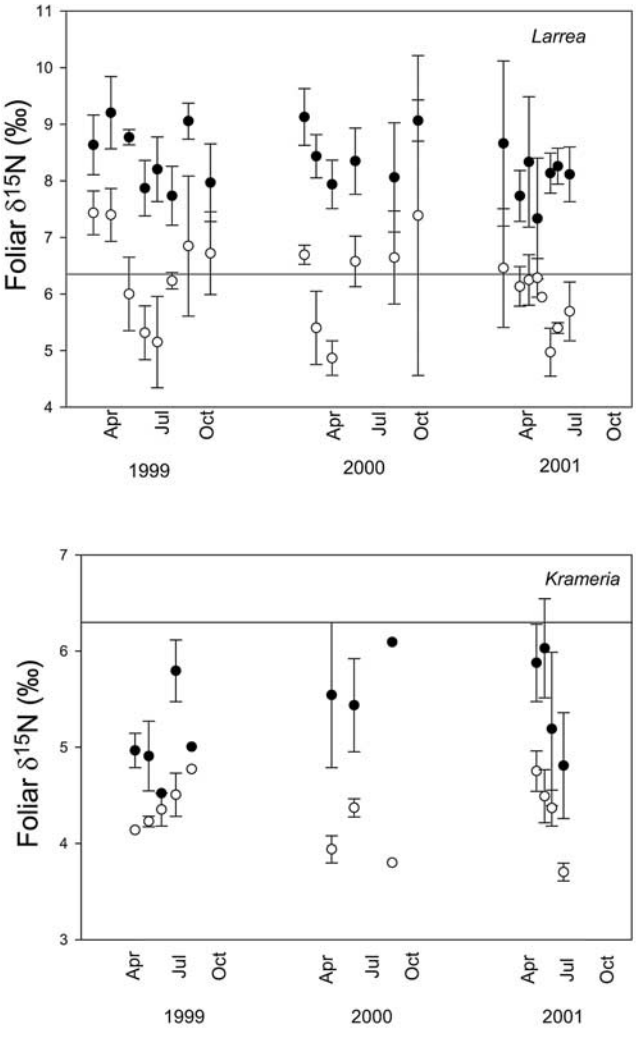

Figure 1. Mean $\delta^{15}N$ values of (top) *Larrea tridentata* and (bottom) *Krameria erecta* under elevated (solid circles) and ambient (open circles) CO_2. Reference line is the mean value for soil $\delta^{15}N$. Vertical lines are 1 standard error of the mean.

analysis of variance was also used to determine the effect of CO_2 treatment, plant cover type, and their interaction on soil microbial biomass N. Data were log transformed to account for non-normal distributions when necessary. All analyses were performed using SAS statistical software (Cary, N. C.). Statistical significance was determined at $\alpha = 0.10$. Errors are presented as 1 standard error of the mean.

3. Results

3.1. Vegetation Isotope Composition

[18] Foliage of *Larrea* shrubs with elevated CO_2 had significantly higher $\delta^{15}N$ values than ambient plants and reflected a seasonal pattern, with the lowest points shifting from July (1999) to April (2000) or May (2001) (Figure 1). The mean difference between elevated CO_2 and ambient shrub $\delta^{15}N$ values was 2.1 ± 0.1‰. Foliage of *Krameria* showed a significant CO_2 effect, with a mean difference between elevated CO_2 and ambient shrub $\delta^{15}N$ values of 1.1 ± 0.2‰. Foliar $\delta^{15}N$ did not change with elevated CO_2 for other species.

3.2. Microbial Biomass N

[19] Soils under *Pleuraphis* had higher microbial biomass N with elevated CO_2 when analyzed in field-dry conditions (6.8 ± 1.4 versus 3.7 ± 0.3 μg/g, Figure 2). For soils collected in 2002 that were wetted prior to measurement, there was no effect of CO_2 treatment and no interaction between cover type and CO_2 treatment. There was an effect of cover type on microbial biomass, with soils under shrubs having higher microbial biomass N (52.9 ± 4.8 μg/g) than soils from plant interspaces (27.5 ± 5.2 μg/g).

3.3. Resin-Available N

[20] Soils under *Larrea* experienced higher resin-available N than soils under *Pleuraphis* and *Achnatherum* (Figure 3). Analysis of cumulative resin-available N revealed a significant interaction between CO_2 treatment

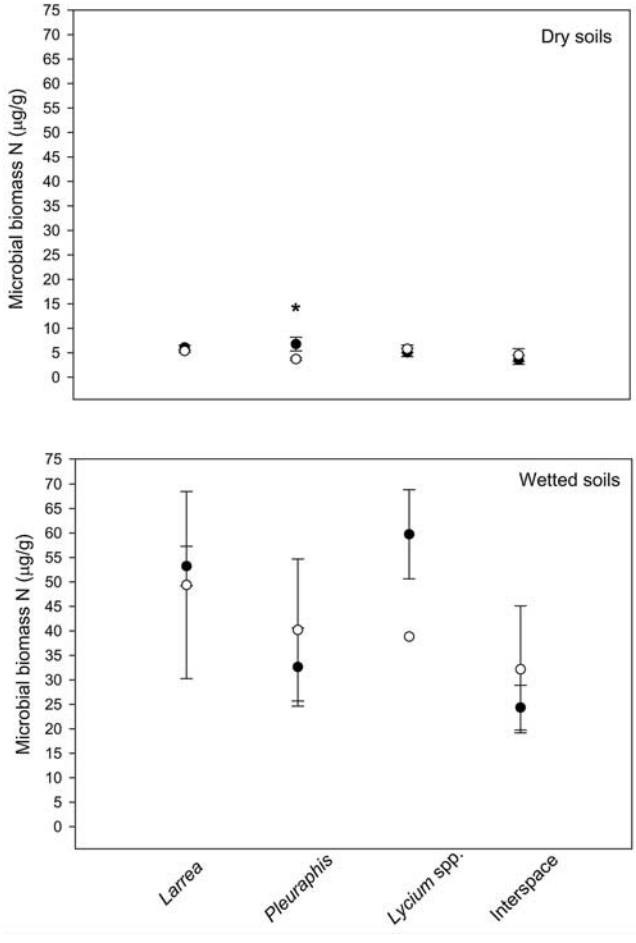

Figure 2. Mean values of microbial biomass N in soils under elevated (solid circles) and ambient (open circles) CO_2, for both dry and wetted soils. Asterisk indicates statistical significance. Lack of error bars for wetted, *Lycium* spp. ambient soils reflects loss of two samples during analysis. Vertical lines are 1 standard error of the mean.

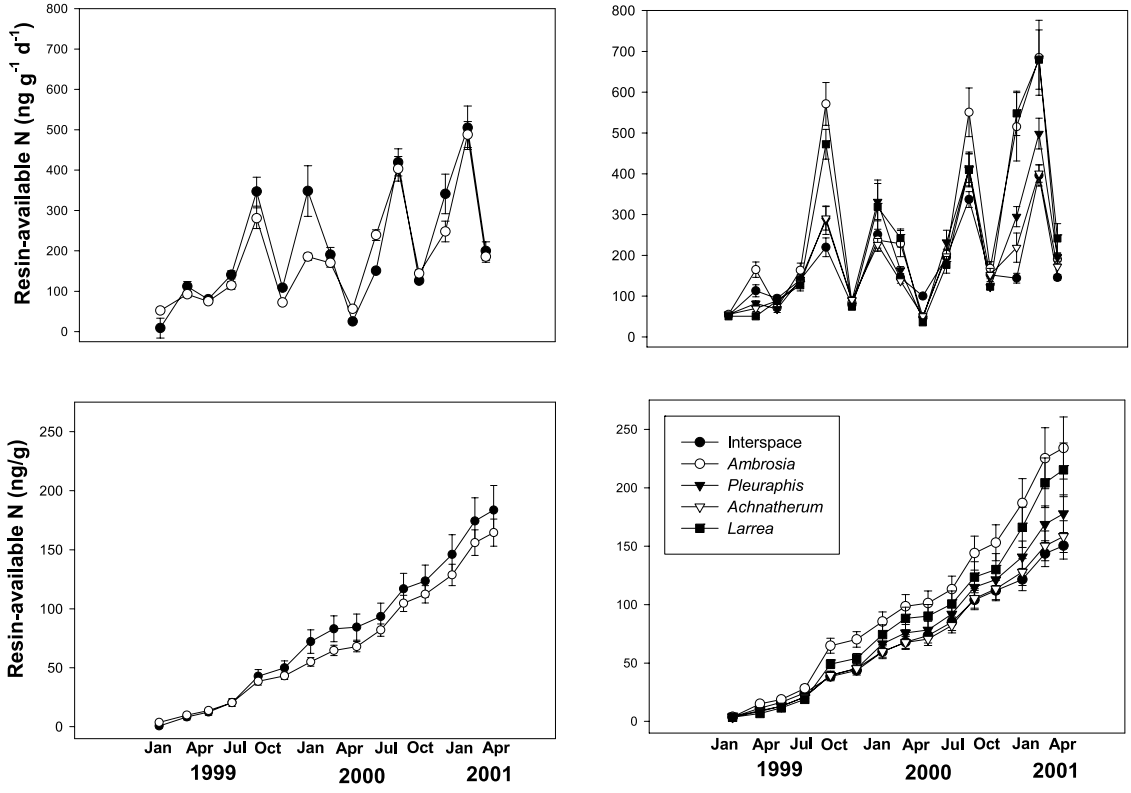

Figure 3. Soil inorganic nitrogen availability from January 1999 through April 2001. (top) Rates and (bottom) cumulative amounts are presented by CO_2 treatment (left panels: Elevated CO_2, solid circles; ambient CO_2, open circles) and cover type (see legend). Vertical lines are 1 standard error of the mean.

and date. From January 2000 through May 2001, elevated CO_2 had a positive effect on cumulative resin-available N. At the end of the final collection period, soils with elevated CO_2 had experienced a 12% increase in resin-available N, largely driven by a shift with elevated CO_2 in January 2000 when soil moisture was available. There was a significant interaction between date and plant cover on cumulative resin-available N. For October 2000 through February 2001, *Larrea* soils had more resin-available N than all cover types except *Ambrosia*. In March 2001, interspace soils had less resin-available N than soils under *Larrea* and *Ambrosia*. In April and May 2001, *Larrea* soils had more resin-available N than all cover types except *Ambrosia*, and soils under *Ambrosia* had more resin-available N than interspace soils.

3.4. Soil Respiration

[21] There was a significant interaction between CO_2 treatment, soil cover type, and measurement date on field rates of soil respiration (Figure 4). Soil respiration was significantly higher with elevated CO_2 on three occasions of 28 sampling units (four cover types × seven sampling dates): Under *Pleuraphis* in July 1999 (5.20 ± 1.86 versus 0.68 ± 0.68 μg C m^{-2} s^{-1}) and March 2000 (7.76 ± 5.57 versus 0.00 ± 0.00 μg C m^{-2} s^{-1}); and under *Larrea* (10.57 ± 1.99 versus 0.00 ± 0.00 μg C m^{-2} s^{-1}) in March 2000. In October 1999 we observed a negative effect of elevated CO_2 compared to ambient soils under *Pleuraphis* (1.63 ± 0.83 versus 7.49 ± 6.23 μg C m^{-2} s^{-1}). It does not appear that significant differences in soil respiration between CO_2 treatments were moisture driven, since no differences in soil moisture with elevated CO_2 have been observed (S. D. Smith, personal communication, 2000).

3.5. Soil Incubation Net N Mineralization and C Evolution

[22] Soils under shrubs experienced greater net N mineralization than soils under grasses and in plant interspaces, regardless of CO_2 treatment (Figure 5). Analyzing individual cover types revealed no CO_2 effects on cumulative net N mineralized on any date.

[23] There was no significant effect of CO_2 on soil $\delta^{15}N$ before or after the laboratory incubation. Mean values increased from 6.1 ± 0.4‰ to 7.3 ± 0.2‰ during the incubation. There was an effect of cover on soil $\delta^{15}N$ values, with soils under *Pleuraphis* having lower values than all other cover types except plant interspaces; there was no significant interaction effect. There was no cover type or CO_2 treatment effect on total soil N concentrations. Mean total N of all soils was higher before (0.55 ± 0.10 mg/g) than after the incubation (0.32 ± 0.03 mg/g).

[24] There was a significant interaction between cover type, date, and CO_2 on cumulative C evolution in soil incubations (Figure 6). *Lycium* soils produced 30% more CO_2 with elevated CO_2 from day 21 of the incubation through its end. Across the entire incubation, both elevated CO_2 and ambient *Larrea* soils produced more CO_2 than soils in interspaces or under *Pleuraphis*, and both elevated

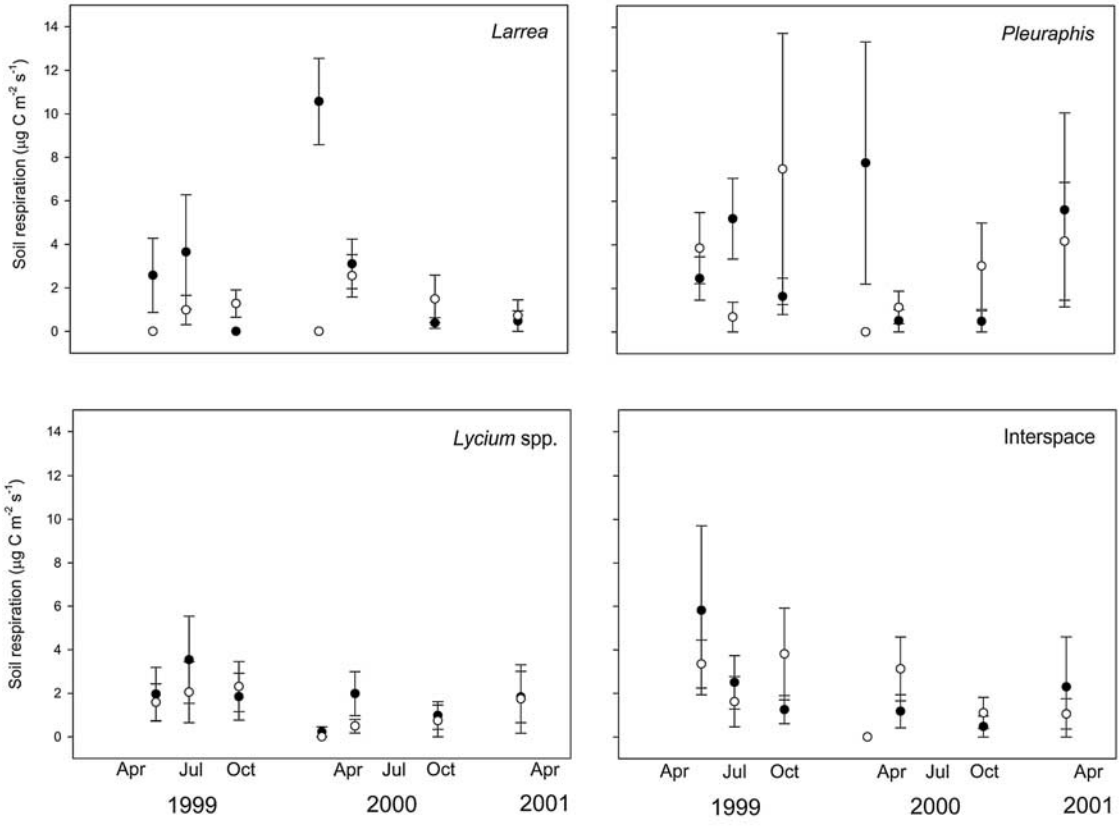

Figure 4. Field rates of soil respiration from May 1999 through March 2001 for *Larrea*, *Pleuraphis*, *Lycium* spp., and in plant interspaces under elevated (solid circles) and ambient (open circles) CO_2. Vertical lines are 1 standard error of the mean.

CO_2 and ambient *Lycium* soil produced more CO_2 than soils in interspaces.

[25] There was no CO_2 effect on soil $\delta^{13}C$ before or after the incubation. We did not expect a significant CO_2 effect on soil $\delta^{13}C$ prior to the incubation, since most soil organic matter was formed significantly before CO_2 treatment began. There was a significant interaction between soil cover type and time; soil $\delta^{13}C$ values under *Pleuraphis* and in interspaces were lighter following the incubation. *Pleuraphis* soils decreased from −21.6 to −22.9‰; interspace soils decreased from −21.4 to −24.0‰. There was an effect of cover type on soil organic C; soils from plant interspaces had lower C contents than soils from all other cover types (2.00 ± 0.30 versus 4.80 ± 0.50 mg/g before the incubation, respectively). Mean soil organic C content of all soils was higher before the incubation (4.08 ± 0.44 mg/g) than after the incubation (2.92 ± 0.24 mg/g).

3.6. Model Results

[26] Soils under *Larrea* had higher rate constants for the recalcitrant N pool (c_r, 0.5 ± 0.1 μg g^{-1} d^{-1}) than all other cover types (Table 1). Soils under *Lycium* had higher values (0.3 ± 0.1 μg g^{-1} d^{-1}) than those under *Pleuraphis* (0.1 ± 0.0 μg g^{-1} d^{-1}). No significant differences existed between model estimates of recalcitrant pool sizes or rate constants of the labile N pool.

4. Discussion

[27] Our results suggest three primary findings. First, plant $\delta^{15}N$ indicates significant perturbations in N dynamics with elevated CO_2. Second, variable increases in microbial biomass N, resin-available N, and soil respiration with CO_2 treatment are consistent with changes in microbial activity and/or population size with elevated CO_2 that may result in periodic increases in plant-available N. Third, model results suggest that understanding microbial utilization patterns of recalcitrant N pools are critical for assessing how N availability may respond to elevated CO_2. The data confirm others' conclusion that effects of elevated CO_2 on soil C and N dynamics are variable and complex, with many competing processes.

4.1. Foliar $\delta^{15}N$

[28] The increase in *Larrea* $\delta^{15}N$ suggests a perturbation in soil N cycling with elevated CO_2, enduring for 4 years after the treatment start date [*Billings et al.*, 2002]. Foliar $\delta^{15}N$ values significantly above soil $\delta^{15}N$ in treatment

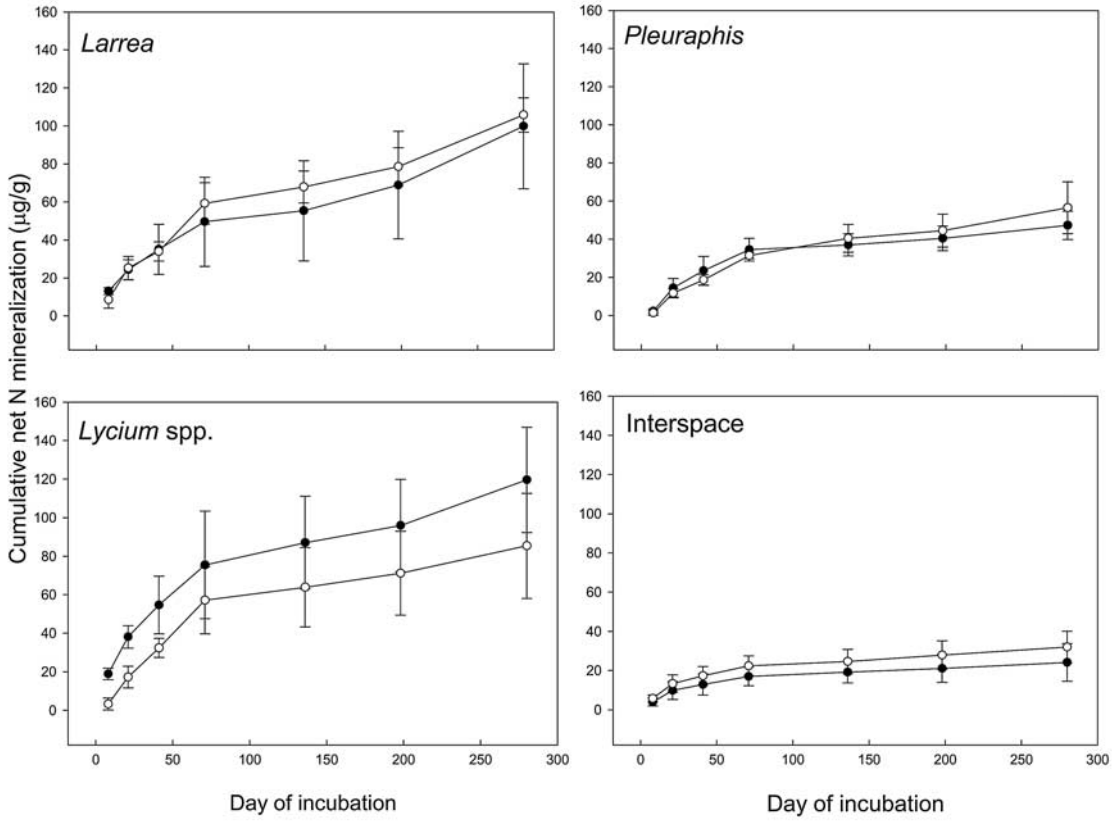

Figure 5. Mean net N mineralization from laboratory incubations by cover type, under elevated (solid circles) and ambient (open circles) CO_2 for *Larrea tridentata*, *Pleuraphis rigida*, *Lycium* spp., and interspace soils. Vertical lines are 1 standard error of the mean.

shrubs is consistent with *Johnson et al.* [2000], who found similar results in live and naturally senesced ponderosa pine needles with elevated CO_2. In conjunction with a seasonal fluctuation in both treatment and control shrubs, greater $\delta^{15}N$ values in foliage with elevated CO_2 can suggest that increased soil microbial activity may be responsible for the observed shift with elevated CO_2 [*Billings et al.*, 2002].

[29] Other possible mechanisms, such as changes in soil moisture, gaseous N loss, and plant use of stored N with elevated CO_2 likely would not induce this shift. Soil moisture data and plant pre-dawn water potentials do not suggest any differences in rooting depth with elevated CO_2 (S. D. Smith, personal communication, 2000), gaseous N loss is not altered with elevated CO_2 [*Billings et al.*, 2003b], and a shift in plant use of stored N with elevated CO_2 likely would result in more depleted foliar $\delta^{15}N$ values [*Billings et al.*, 2002]. A more complete discussion of these processes is detailed by *Billings et al.* [2002]. Because these mechanisms seem unlikely drivers of the observed increase in foliar $\delta^{15}N$ with elevated CO_2 in *Larrea*, we suggest that an increase in soil microbial activity may be increasing cycling rates of N compounds in the soil. Because enzymatic processes discriminate against ^{15}N, this can increase $\delta^{15}N$ of plant-available N [*Robinson*, 2001], and may be reflected as higher foliar $\delta^{15}N$. A shift in foliar $\delta^{15}N$ would be particularly prominent if the increase in microbial activity resulted in mineralization of older N sources, which tend to be enriched in ^{15}N [*Nadelhoffer and Fry*, 1988].

[30] Rooting patterns may explain the shift in vegetation $\delta^{15}N$ with elevated CO_2 observed in *Larrea*. *Larrea* has roots most concentrated in the top 0.5 m of the soil profile [*Yoder and Nowak*, 1999], and extending as far as 4.5 m from the plant base [*Gile et al.*, 1998]. This large, horizontal network of roots could provide *Larrea* with N from many different microbial communities, since soil microorganism community structure can vary significantly with cover type [*Belnap and Phillips*, 2001; *Kuske et al.*, 2002]. *Larrea* $\delta^{15}N$ thus reflects soil N processes from a large, microbially active area influenced by *Larrea* root activity. An increase in microbial activity across broad, heterogeneous patches of microbial communities with elevated CO_2 and the associated discrimination against ^{15}N could cause an increase in $\delta^{15}N$ of plant-available N [*Robinson*, 2001], resulting in a shift in plant $\delta^{15}N$. Because *Krameria* spp. are known to parasitize *Larrea* roots by tapping into root phloem [*Kearney and Peebles*, 1960; *Bowers and Wignall*, 1993], the significant shift in *Krameria* $\delta^{15}N$ values with elevated CO_2 is consistent with the *Larrea* data. The more negative values of *Krameria*'s $\delta^{15}N$ values likely represent fraction-

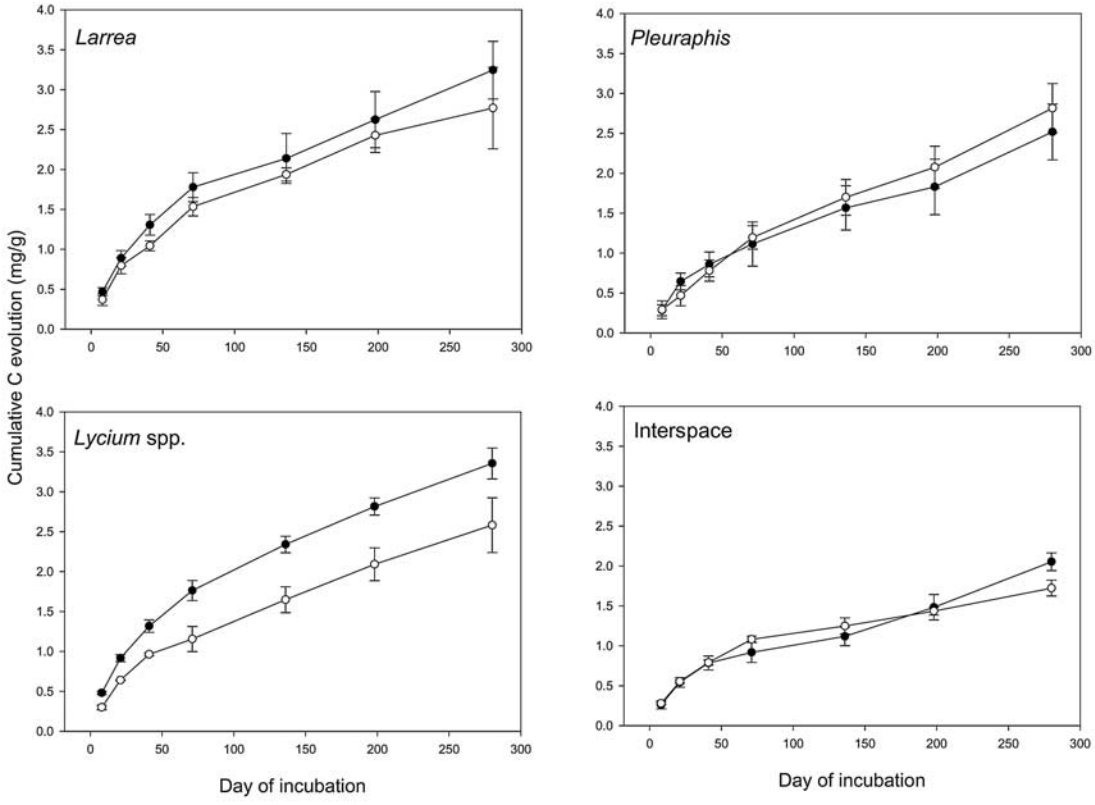

Figure 6. Mean C evolution from laboratory incubations by cover type, under elevated (solid circles) and ambient (open circles) CO_2 for *Larrea tridentata*, *Pleuraphis rigida*, *Lycium* spp., and interspace soils. Vertical lines are 1 standard error of the mean.

ation between the host and the parasite, also observed for C isotopes in facultative parasites of *Artemisia tridentata* [*Ducharme and Ehleringer*, 1996].

[31] Increases in root activity with elevated CO_2 in *Ambrosia* and *Lycium* perhaps are not sufficient to be reflected in their foliar $\delta^{15}N$ values because their rooting systems may not be as extensive as those of *Larrea*, with its lifespan of hundreds of years [*Vasek*, 1980]. In contrast, *Ambrosia* has a lifespan of 30–40 years [*Mahall and Callaway*, 1992]. Perennial grasses' $\delta^{15}N$ values also did not shift with elevated CO_2. Although *Pleuraphis* and *Achnatherum* roots function at shallow depths where soil microbial activity is high, their relatively low root biomass has less potential for influencing soil C inputs with elevated CO_2 than the extensive rooting systems of shrubs.

[32] It is somewhat surprising that a shrub species with an extensive lateral rooting system (*Larrea*) and its parasitic counterpart (*Krameria*) exhibit shifts in $\delta^{15}N$ compared to species with more restricted rooting zones, if the observed shifts are indicative of altered microbial activity in the soil. Intuitively, we might predict that shrubs with roots that take up N from a more homogeneous environment may more readily exhibit changes in integrative measures of N cycling like $\delta^{15}N$. That we did not observe this in species with more restricted rooting zones suggests that microbial activity affecting N cycling was altered in many microbial commu-

Table 1. Cumulative Net N Mineralized and Parameter Estimates From Exponential Model Applied to Laboratory Incubation Data[a]

	Larrea		Pleuraphis		Lycium		Interspace	
	550 μL/L	Ambient	550 μL/L	Ambient	550 μL/L	Ambient	550 μL/L	Ambient
Cumulative net N mineralized	99.84 (32.93)	105.76 (9.07)	47.24 (7.49)	56.52 (13.55)	119.61 (27.32)	85.36 (27.27)	24.12 (9.61)	31.95 (8.09)
N_l	48.76 (14.45)	64.63 (11.26)	75.54 (8.80)	72.89 (10.00)	93.86 (39.59)	132.26 (66.88)	82.48 (42.70)	61.59 (12.37)
h_l	0.09 (0.06)	0.04 (0.2)	0.02 (0.01)	0.02 (0.00)	0.07 (0.03)	0.02 (0.00)	0.03 (0.01)	0.04 (0.01)
c_r	0.42**a** (0.04)	0.49**a** (0.09)	0.10**c** (0.03)	0.14**c** (0.07)	0.37**b** (0.07)	0.21**b** (0.10)	0.07**bc** (0.16)	0.16**bc** (0.06)

[a]Means and standard errors of net N mineralization (μg/g) data over 280 days are presented. Parameter estimates with standard errors in parentheses are: N_l, the pool sizes for labile N (μg/g), h_l, the rate constant for the labile pool of N, and c_r, the rate of mineralization for recalcitrant pools of N (μg g^{-1} d^{-1}). Bold letters indicate significant differences between species ($P < 0.10$).

nities, in shallow soils where microbial activity tends to be highest. Such a response to elevated CO_2 may be related to increases in soil C availability [*Schaeffer et al.*, 2003; *Zak et al.*, 2000b].

4.2. Measures of Microbial Activity and N Availability

[33] Our measurements of potential C evolution during soil incubations likely reflect processes in addition to soil microbial activity. Incubation respiration data suggest a mean of 73% of organic C was respired, which is significantly higher than analogous numbers observed by *Frank and Groffman* [1998], and higher than the stability of most soil organic matter would predict [*Brady*, 1990]. Mean soil organic C contents before and after the incubation indicate that 1.16 ± 0.34 mg/g of C was lost via respiration; the mean total amount of CO_2-C evolved (2.63 ± 0.14 mg/g) suggests C evolution from a source other than organic matter during the incubations. Because soils from juxtaposed sites have carbonate concentrations ranging from 16 to 30% [*Romney et al.*, 1973], we suspect our C mineralization data may be confounded by high carbonate concentrations. Lighter soil $\delta^{13}C$ values after the incubation are consistent with carbonates precipitating early in the incubation and contributing to the CO_2 accumulation [*Mermut et al.*, 2000]; without the confounding effects of carbonates, we would expect soil $\delta^{13}C$ to increase with microbial reworking of soil organic matter [*Nadelhoffer and Fry*, 1988]. The C mineralization data serve as a reminder that incubations for assessing soil microbial respiration can be problematic with high pH soils.

[34] The sporadic increases in field rates of soil respiration with elevated CO_2 suggest that microbial activity and/or root respiration can increase with elevated CO_2. Elevated CO_2 can induce changes in soil microbial biomass when soil moisture is limiting, as demonstrated by the increase in microbial biomass in dry *Pleuraphis* soils, so increases in soil respiration under *Pleuraphis* may indicate increases in microbial activity. When soil moisture is more available, differences in microbial biomass induced by elevated CO_2 were eliminated, likely because the large response of soil microorganisms to moisture availability overwhelms any response to additional C availability. Increased field rates of soil respiration under *Larrea* with elevated CO_2 in March 2000 without a concomitant increase in microbial biomass could indicate an increase in root respiration, or increased activity levels of soil microorganisms with no augmentation of population size. If increases in soil respiration result from greater microbial activity in the soils surrounding *Larrea* roots, the rates are consistent with increased microbial activity in the rooting zone of *Larrea* driving foliar $\delta^{15}N$ data. This emphasizes how microorganisms' activity level is, in some cases, a more critical feature of soil metabolism than population size. These periodic increases in respiration with elevated CO_2 demonstrate the need for more studies with better resolution of root versus microbial respiration to resolve the effects of elevated CO_2 on soil biological activity.

[35] The periodic alterations in soil respiration and microbial biomass N are consistent with changes in soil microbial activity with elevated CO_2, which could affect both mineralizing and immobilizing microbial processes. Nitrogen availability during incubations and field data provide evidence consistent with these competing microbial processes. We observed no CO_2 effect on potential net N mineralization during the incubations, in spite of known alterations in some soils in microbial biomass N and an implied change in microbial population size. This likely reflects a positive effect of elevated CO_2 on both mineralization and immobilization. The lack of a CO_2 effect on field rates of resin-available N and the initial period of no CO_2 effect on cumulative N availability are consistent with this idea. *Arnone* [1997] and *Gloser et al.* [2000] report mineral N availability being unaffected by elevated CO_2 at a FACE site in a Swiss grassland, similar to the 1999 data presented here. These data, followed by the significant, positive effect of elevated CO_2 on cumulative N availability after January 2000, further suggest that CO_2 treatment can induce various, competing microbial responses.

[36] Soil moisture availability can result in increased rates of net N mineralization with elevated CO_2. When resin-available N increased with elevated CO_2 (January to March 2000), rainfall totaled 55 mm, the largest amount of rain during any resin bag installation period. In conjunction with the lack of response of microbial biomass N in wetted soils to elevated CO_2, this suggests that when soil moisture is available, net rates of N mineralization may increase with no changes in microorganisms' population size. This, like the field rates of respiration, implies that rates of microbial transformations are more critical determinants of N availability than population sizes of soil microbes.

4.3. Modeling Net N Mineralization

[37] *Wedin and Pastor* [1993] demonstrated that N turnover could be affected dramatically by changing the dynamics in a small fraction of soil N (2 to 3%) that was highly active in grassland soils. In contrast, model results in the current study do not suggest that changes in substrate availability affect turnover of the small fraction of soil N that is labile. Our model emphasizes that plant cover type and substrate availability drive the differences between mineralization rates of recalcitrant pools of N. If we assume that the recalcitrant fraction of soil N is the difference between total soil N and model estimates of labile N pool size, the mean estimate of the recalcitrant N pool across all soil types is 552 μg/g. This is more than 99% of total soil N. Changes in substrate availability with elevated CO_2 thus will have to affect a large fraction of soil organic matter to produce discernable differences in N mineralization in this ecosystem. Consistent with the hypothesis presented by *Zak et al.* [2000a], this is most likely to occur where root biomass can contribute a significant proportion of soil organic matter.

5. Conclusions

[38] This study presents a series of conclusions important for predicting the effects of elevated CO_2 on ecosystem and soil C and N processes, and for refining research needs. First, the study confirms that foliar $\delta^{15}N$ can be used as an integrator of changes in ecosystem N cycling processes. Values of foliar $\delta^{15}N$ may be most likely to reflect such

alterations in species with extensive rooting systems, distributed in soil volumes supporting the highest levels of microbial activity. Second, elevated CO_2 may increase root and/or soil microbial activity, which can result in periodic increases in resin-available N, particularly when soil moisture is available. This may translate into more plant available N at these times. The change in microbial biomass N with elevated CO_2 in dry but not in wetted soils indicates that shifts in microbial community structure or activity can occur that are masked when soil moisture is available. Though increased soil moisture availability may not foster a CO_2 effect on microbial biomass, it promoted increased net N mineralization in the field. If increases in plant-available N are maintained particularly when soil moisture is available, arid ecosystems may be able to sustain any increases in productivity induced by elevated CO_2. Third, an exponential model predicting labile pool sizes, labile pool rate constants, and rates of mineralization of recalcitrant pools of N predicts that substrate availability is critical in determining the turnover rates of recalcitrant N pools, which comprise more than 99% of soil N. To result in changes in N availability with elevated CO_2, any alterations in soil C must be sufficient to affect the dynamics of a large fraction of soil N; such effects are likely where rhizodeposition comprises a significant component of soil organic matter. Given these conclusions, we suggest that future studies exploring effects of elevated CO_2 on N availability examine N cycling in ecosystems with varied degrees of root colonization, shifts in microbial communities that may foster mineralization of older, recalcitrant organic matter, and the factors governing increases in soil respiration with elevated CO_2.

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