Soil microbial activity and N availability with elevated CO₂ in Mojave Desert soils

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We examined the effects of elevated CO₂ on soil nitrogen (N) dynamics in the Mojave Desert by measuring plant N isotope composition (δ¹⁵N), soil microbial biomass N, soil respiration, resin-available N, and C and N dynamics during soil incubations. With elevated CO₂, foliage of Larrea tridentata and Krameria erecta had mean δ¹⁵N 2.1 and 1.1% higher with elevated CO₂, respectively, and elevated CO₂ increased microbial biomass N in dry soils under a perennial grass (6.8 ± 1.4 versus 3.7 ± 0.3 μg/g). Elevated CO₂ significantly increased cumulative resin-available N in the field by 12%, driven by available soil moisture. Rates of soil respiration with elevated CO₂ 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 CO₂ and may result in increases in plant-available N when soil moisture is available.

INDEX TERMS: 1615 Global Change: Biogeochemical processes (4805); KEYWORDS: elevated CO₂, soil microorganisms, soil carbon, nitrogen cycling, Mojave Desert, nutrient availability


1. Introduction

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 CO₂ [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 CO₂ [Zak et al., 1993, 2000a].

Numerous studies show increases in plant C allocation to roots, rhizosphere respiration, and rhizodeposition with elevated CO₂ [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 CO₂.

Zak et al. [2000b] recently concluded that few studies have found significant increases in microbial respiration with elevated CO₂, and that there is high variation in microbial response to elevated CO₂. This implies that the microbes responsible for soil N transformations have varied responses to elevated CO₂.

The response of N cycling to rising atmospheric CO₂ concentrations in arid ecosystems is equally unclear, even though arid ecosystems are expected to experience large increases in productivity with elevated CO₂ [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 CO₂ 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 CO₂ 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 CO₂ may be limited by N availability. Alternatively, these effects could be mitigated by increases in net N availability.
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 CO2 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 CO2 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 CO2 soils would be relatively less C limited than their ambient counterparts; this would be reflected in higher vegetation δ15N 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. Hitech.), 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 N2, 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 [CO2] 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 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 H3PO4 to remove carbonates. To ensure that organic matter was not lost during acid washing, we compared total organic N and soil δ15N in acid washed and non-acid washed soils. No differences were found. Both vegetation and soil samples were analyzed for δ15N 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.

2.2. Carbon and Nitrogen Content and Isotope Composition

[8] We sampled foliage for δ15N 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 H3PO4 to remove carbonates. To ensure that organic matter was not lost during acid washing, we compared total organic N and soil δ15N in acid washed and non-acid washed soils. No differences were found. Both vegetation and soil samples were analyzed for δ15N 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 K2SO4 after the incubation period. A second replicate set was fumigated with CIC4 for 5 days in a dessicator before extraction. Extracts were subjected to a Kjeldahl digest and analyzed for total ammonium-N (NH4-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 CIC4 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 (NO3-N) on an Alpkem autoanalyzer. Microbial biomass N was calculated as the difference in NH4-N or NO3-N between fumigated and unfumigated samples, divided by the constant 0.69 [Brookes et al., 1985; Gallardo
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 × 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, 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

$$ N_t = N_i (1 - e^{-kt}) + c_t, $$

where $N_i$ is the cumulative amount of N mineralized at time $t$, $N_i$ 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’ $^{15}$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 $^{15}$N before and after the incubation. An
analysis of variance was also used to determine the effect of CO₂ 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

Foliation of Larrea shrubs with elevated CO₂ 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 CO₂ and ambient shrub \( \delta^{15}N \) values was \( 2.1 \pm 0.1\% \). Foliation \( \delta^{15}N \) did not change with elevated CO₂ for other species.

3.2. Microbial Biomass N

Soils under Pleuraphis had higher microbial biomass N with elevated CO₂ 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 CO₂ treatment and no interaction between cover type and CO₂ 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

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 CO₂ treatment.
and date. From January 2000 through May 2001, elevated CO2 had a positive effect on cumulative resin-available N. At the end of the final collection period, soils with elevated CO2 had experienced a 12% increase in resin-available N, largely driven by a shift with elevated CO2 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 CO2 treatment, soil cover type, and measurement date on field rates of soil respiration (Figure 4). Soil respiration was significantly higher with elevated CO2 on three occasions of 28 sampling units (four cover types x 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 CO2 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 CO2 treatments were moisture driven, since no differences in soil moisture with elevated CO2 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 CO2 treatment (Figure 5). Analyzing individual cover types revealed no CO2 effects on cumulative net N mineralized on any date.

[23] There was no significant effect of CO2 on soil δ15N 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 δ15N 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 CO2 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 CO2 on cumulative C evolution in soil incubations (Figure 6). Lycium soils produced 30% more CO2 with elevated CO2 from day 21 of the incubation through its end. Across the entire incubation, both elevated CO2 and ambient Larrea soils produced more CO2 than soils in interspaces or under Pleuraphis, and both elevated
CO₂ and ambient Lycium soil produced more CO₂ than soils in interspaces. There was no CO₂ effect on soil δ¹³C before or after the incubation. We did not expect a significant CO₂ effect on soil δ¹³C prior to the incubation, since most soil organic matter was formed significantly before CO₂ treatment began. There was a significant interaction between soil cover type and time; soil δ¹³C 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 (r_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 δ¹⁵N indicates significant perturbations in N dynamics with elevated CO₂. Second, variable increases in microbial biomass N, resin-available N, and soil respiration with CO₂ treatment are consistent with changes in microbial activity and/or population size with elevated CO₂ 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 CO₂. The data confirm others’ conclusion that effects of elevated CO₂ on soil C and N dynamics are variable and complex, with many competing processes.

4.1. Foliar δ¹⁵N

[28] The increase in Larrea δ¹⁵N suggests a perturbation in soil N cycling with elevated CO₂, enduring for 4 years after the treatment start date [Billings et al., 2002]. Foliar δ¹⁵N values significantly above soil δ¹⁵N in treatment
shrubs is consistent with Johnson et al. [2000], who found similar results in live and naturally senesced ponderosa pine needles with elevated CO2. In conjunction with a seasonal fluctuation in both treatment and control shrubs, greater δ15N values in foliage with elevated CO2 can suggest that increased soil microbial activity may be responsible for the observed shift with elevated CO2 [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 CO2 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 CO2 (S. D. Smith, personal communication, 2000), gaseous N loss is not altered with elevated CO2 [Billings et al., 2003b], and a shift in plant use of stored N with elevated CO2 likely would result in more depleted foliar δ15N 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 δ15N with elevated CO2 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 15N, this can increase δ15N of plant-available N [Robinson, 2001], and may be reflected as higher foliar δ15N. A shift in foliar δ15N would be particularly prominent if the increase in microbial activity resulted in mineralization of older N sources, which tend to be enriched in 15N [Nadelhoffer and Fry, 1988].

[30] Rooting patterns may explain the shift in vegetation δ15N with elevated CO2 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 δ15N thus reflects soil N processes from a large, microbiologically active area influenced by Larrea root activity. An increase in microbial activity across broad, heterogeneous patches of microbial communities with elevated CO2 and the associated discrimination against 15N could cause an increase in δ15N of plant-available N [Robinson, 2001], resulting in a shift in plant δ15N. 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 δ15N values with elevated CO2 is consistent with the Larrea data. The more negative values of Krameria’s δ15N values likely represent fraction-
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 CO₂ in *Ambrosia* and *Lycium* perhaps are not sufficient to be reflected in their foliar δ15N 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’ δ15N values also did not shift with elevated CO₂. 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 CO₂ 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 δ15N 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 δ15N. 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-

**Figure 6.** Mean C evolution from laboratory incubations by cover type, under elevated (solid circles) and ambient (open circles) CO₂ for *Larrea tridentata*, *Pleuraphis rigida*, *Lycium* spp., and interspace soils. Vertical lines are 1 standard error of the mean.

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

<table>
<thead>
<tr>
<th>Species</th>
<th>550 µL/L Ambient</th>
<th>550 µL/L Ambient</th>
<th>550 µL/L Ambient</th>
<th>550 µL/L Ambient</th>
<th>550 µL/L Ambient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative net N mineralized</td>
<td>99.84 (32.93)</td>
<td>105.76 (9.07)</td>
<td>47.24 (7.49)</td>
<td>56.52 (13.55)</td>
<td>119.61 (27.32)</td>
</tr>
<tr>
<td>( N_0 )</td>
<td>48.76 (14.45)</td>
<td>64.63 (11.26)</td>
<td>75.54 (8.80)</td>
<td>72.89 (10.00)</td>
<td>93.86 (39.59)</td>
</tr>
<tr>
<td>( h_i )</td>
<td>0.09 (0.06)</td>
<td>0.04 (0.2)</td>
<td>0.02 (0.01)</td>
<td>0.02 (0.00)</td>
<td>0.07 (0.03)</td>
</tr>
<tr>
<td>( c_r )</td>
<td>0.04 (0.04)</td>
<td>0.49a (0.09)</td>
<td>0.10c (0.03)</td>
<td>0.14c (0.07)</td>
<td>0.37b (0.07)</td>
</tr>
</tbody>
</table>

*Means and standard errors of net N mineralization (µg/g) data over 280 days are presented. Parameter estimates with standard errors in parentheses are: \( N_0 \), the pool sizes for labile N (µg/g); \( h_i \), 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 carbones 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 resins-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 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂. 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 CO₂, 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 CO₂ 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 CO₂.

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