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PLANT SPECIES MEDIATE CHANGES IN SOIL MICROBIAL N IN RESPONSE TO ELEVATED CO_2[1]

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Abstract. The effect of elevated CO_2 on plant–microbial interactions and nitrogen (N) cycling is critical to predicting plant growth responses to elevated CO_2, because plant growth is often N-limited. We investigated whether the effects of elevated CO_2 on plant–microbial N dynamics differed among six annual plant species: three European grasses that have invaded California grasslands, and one grass and two forbs native to California serpentine grassland. Elevated CO_2 altered plant N pools and $^{15}NH_4^+$ uptake, but the direction and magnitude of the changes were species dependent. The introduced grasses showed increased plant N pools and $^{15}NH_4^+$ uptake, whereas the native species showed smaller increases or even decreases in plant N pools and $^{15}NH_4^+$ uptake. Under nutrient enrichment, soil microbial N and $^{15}NH_4^+$ uptake differed among soils with different plant species, but they were not affected by elevated CO_2. At low nutrients, elevated CO_2 altered soil microbial N and $^{15}NH_4^+$ uptake, but the direction and magnitude of the changes were species dependent. The changes in soil microbial N were positively correlated with changes in the plant N pool, suggesting that there was no trade-off in N uptake between plants and microbes. These results also suggest that plant species composition will partly determine the direction of changes in soil N cycling in response to elevated CO_2.

Key words: annual grassland; elevated CO_2; functional groups; introduced vs. native species; Jasper Ridge, California; ^{15}N; nitrogen cycle; nitrogen immobilization, uptake, and partitioning; plant–microbe N interactions.

INTRODUCTION

Nitrogen (N) strongly limits plant growth in many terrestrial ecosystems (Vitousek and Howarth 1991), potentially constraining the response of terrestrial ecosystems to elevated CO_2 (Bazzaz and Fajer 1992, Field et al. 1992). Elevated CO_2 could modify N availability to plants, releasing or exacerbating this constraint on productivity. For example, increased C:N in litter produced under elevated CO_2 can slow nutrient release during decomposition (Bazzaz 1990, Coûteaux et al. 1991). Increased labile C input to soil resulting from higher root exudation or turnover under elevated CO_2 can stimulate microbial N immobilization and depress N availability to plants (Diaz et al. 1993). However, increased C input could also increase N availability to plants by stimulating protozoan predation and associated N mineralization, as protozoa respond to increased abundance of their microbial prey (Clarholm 1985, Zak et al. 1993). Also, elevated CO_2 can stimulate soil N cycling by increasing soil moisture, a result of decreased stomatal conductance and evapotranspiration under elevated CO_2 (Hungate et al., *in press*). We must investigate these effects of elevated CO_2 on N cycling and their feedbacks to plant growth in order to understand the potential for carbon sequestration by terrestrial vegetation in response to elevated CO_2.

Resource-balance models predict that plants respond to higher availability of aboveground resources by increasing relative allocation to nutrient acquisition (Chapin 1980, Garnier 1991). Thus, elevated atmospheric CO_2 is predicted (and sometimes observed) to increase carbon allocation to roots (Norby 1994, Rogers et al. 1994), especially when plant growth is nutrient limited (Stulen and den Hertog 1993). Increased labile soil C that results from increased root growth and rhizodeposition strongly influences the microbial processes that regulate nutrient cycling in soil and thus nutrient availability to plants. If elevated CO_2 increases rhizodeposition, changes in plant N availability will depend partly on how increased rhizodeposition alters these microbial processes.

Several studies have measured changes in soil N cycling under elevated CO_2 and have invoked increased rhizodeposition as the driving mechanism. Zak et al. (1993) found increased N mineralization in laboratory incubations of soil from poplar monocultures exposed to elevated CO_2. They suggested that increased soil carbon availability stimulated microbial activity and associated N mineralization, and that this stimulation would act as a positive feedback to increased plant growth under elevated CO_2. In contrast, Diaz et al. (1993) found increased microbial biomass N and symptoms of plant nutrient deficiency under elevated CO_2 in growth-chamber experiments with herbaceous grassland microcosms. They suggested that increased rhi-

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zodeposition stimulated microbial N immobilization, reducing N availability to nonmycorrhizal plants and limiting their growth response to elevated CO_2. Körner and Arnone (1993) found increased NO_3^- leaching loss under elevated CO_2, which could also limit plant response to elevated CO_2 by reducing N availability to plants.

Plant species differ in their effects on soil N cycling (Melillo et al. 1982, Pastor and Post 1986, Vitousek et al. 1987, Wedin and Tilman 1990, Hobbie 1992). However, less is known about whether plant species mediate differential changes in soil N cycling in response to environmental change. In this study, we investigated whether the effects of elevated CO_2 on rhizosphere N interactions differ among plant species, and whether these differences were predictable from a knowledge of species' biology.

We selected six annual species that exhibit a range of growth strategies: three slow-growing native species with the ability to thrive on extremely nutrient-limited serpentine soils, and three fast-growing introduced grasses with high resource requirements. *Plantago erecta, Lasthenia californica* (both forbs), and *Vulpia microstachys* (a grass) are native to serpentine grasslands in central coastal California. *Avena fatua, Bromus hordeaceus,* and *Lolium multiflorum* are introduced European grasses that dominate on nonserpentine soils. *Bromus hordeaceus* and *Lolium multiflorum* also occur on serpentine, but in low abundance (Huenneke et al. 1990). *Bromus hordeaceus* increases in abundance on serpentine soils during high rainfall years (Hobbs and Mooney 1991), and both species increase in abundance with experimental nutrient addition (Huenneke et al. 1990). We refer to these species by genus name only in the rest of this paper.

These plants grew in monocultures on serpentine soil at ambient and elevated atmospheric CO_2 for 5 mo. To determine how elevated CO_2 affects plant vs. microbial N acquisition, we measured N fluxes into plants and microorganisms over 24 h (using ^{15}N-labeled ammonium) and also measured total N pools in plants and microorganisms. Finally, we investigated whether the native and introduced plant species differed in their responses to elevated CO_2.

Methods

This research occurred at the Jasper Ridge Biological Preserve near Stanford, California (37°24′ N, 122°13′ W, 100 m elevation). The climate is mediterranean, with cool, wet winters and warm, dry summers. The work described here was conducted using the MicroEcosystems for Climate Change Analysis (MECCA), an outdoor facility consisting of 20 open-top chambers (1.3 × 1.3 × 3 m high), 10 with ambient CO_2 and 10 with elevated (710 μL/L) CO_2 (Field et al. 1996). Each MECCA chamber contains 27 or 30 polyvinyl chloride tubes (0.95 m tall × 0.2 m diameter) containing serpentine topsoil (0.15 m) overlying a rocky subsoil (0.8 m). All tubes in 10 chambers were amended (5 at ambient and 5 at elevated CO_2) with 20 g/m^2 each of N, P, and K (120-d Osmocote fertilizer). There were 7 replicate tubes for each treatment (species × CO_2 × nutrient combination). Tubes were seeded in October, when seed germination in the field begins, at densities approximating natural densities in the field: 3000 plants/m^2 for *Avena* and 9000 plants/m^2 for the other, smaller statured species.

We examined plant and microbial N pools and 24-h $^{15}NH_4^+$ partitioning in March 1993, when plants were in active vegetative growth, following the approach of Jackson et al. (1989). On 15 March for the high-nutrient treatment and 17 March for the low-nutrient treatment, we added 0.8 mg ^{15}N (99.9 atom % ^{15}N, i.e., 99.9% of the N is ^{15}N) to the top 9 cm of soil in each tube by injecting five 1-mL aliquots of 5.3 mmol/L aqueous $(^{15}NH_4)_2SO_4$ in an X pattern centered in a 5 cm diameter ring. For each injection, we inserted a 9 cm long side port spinal needle (Popper and Sons, Incorporated, New Hyde Park, New York) into the soil, and injected 1 mL of solution while raising and rotating the needle to distribute the $^{15}NH_4^+$ throughout the top 9 cm of soil.

After 24 h (16 March, high nutrients, and 18 March, low nutrients), we clipped plant shoots that fell within the ring, and sorted them into green leaves and other tissue (stems, reproductive tissues, and senesced leaves). From each tube, we took one soil core that was 1.9 cm diameter and nominally 15 cm deep, though total soil mass recovered was more consistent with a 10 cm deep core, reflecting the difficulty of sampling this very rocky soil. We removed roots by hand picking and washing. We dried all plant material at 60°C to constant mass, weighed it, then ground it to a fine powder and analyzed it for C, N, and ^{15}N content by combustion gas chromatography mass spectroscopy (Europa Scientific Limited, Crewe, UK). We mixed the green leaf and other shoot parts for *Lasthenia californica* before N analyses, so we only report shoot N and ^{15}N for this species. Root biomass values from our cores were small and highly variable compared to values from Jackson and Reynolds (1996) obtained from the same tubes a few days earlier but with a larger (3 cm diameter) core. Therefore, we used their root biomass and %N values, combined with our atom % ^{15}N values, to calculate root and total plant N and ^{15}N.

We measured microbial (chloroform-labile) N and ^{15}N using the chloroform-fumigation direct-extraction technique (Brookes et al. 1985). We extracted two 5–15 g soil subsamples with 50 mL of 0.5 moL/L K_2SO_4, one immediately, the other after exposure to $HCCl_3$ vapor for 24 h. We converted all N in the extracts to NH_4^+ by Kjeldahl digestion and determined total N by NH_4^+ analysis using a continuous flow autoanalyzer (Lachat Instruments, Incorporated, Milwaukee, Wisconsin). We collected NH_4^+ in the digests onto acidified filter disks using a diffusion procedure (Brooks et al.

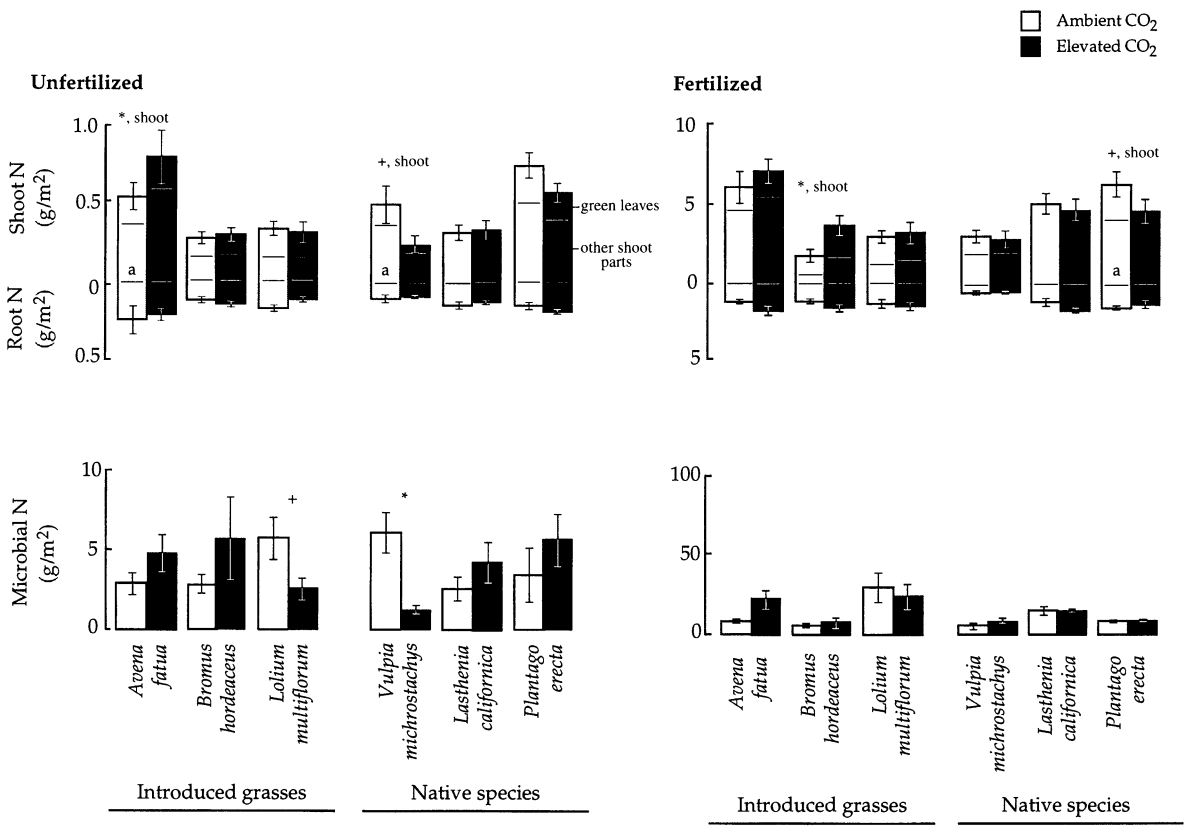

FIG. 1. Plant and microbial N pools (g N/m^2) for six monocultures grown at ambient (360 μL/L) and elevated (710 μL/L) CO_2 in unfertilized and fertilized soil (mean ± 1 SE, n = 5–7). For plants (top graph), bars show N pools in shoots (green leaf and nongreen leaf fractions, except for *Lasthenia californica* where only total shoot N was determined) and roots, determined 5 mo after seedling germination; error bars are ±1 SE for shoots and for roots. For microbes (bottom graph) bars show total microbial N pools ±1 SE. Significant differences for comparisons from Fisher's LSD post hoc tests are indicated by different letters for the green leaf and other shoot N pools, and by ** ($P < 0.01$), * ($P < 0.05$), and + ($P < 0.1$) for total shoot, total plant, and total microbial N. Note differences in scale between fertilized and unfertilized treatments.

1989) and determined ^{15}N content by combustion gas chromatography mass spectroscopy.

We calculated ^{15}N uptake in each ecosystem component by multiplying excess ^{15}N concentration (measured atom % ^{15}N − natural abundance [0.37%] ^{15}N) times N concentration times component mass. ^{15}N content was always >0.5 atom % ^{15}N, so variation in natural abundance of ^{15}N (0.36–0.37 atom % ^{15}N) should not affect the results. We calculated microbial (chloroform-labile) N and ^{15}N as the difference in total N and ^{15}N between chloroform-fumigated and nonfumigated subsamples, divided by 0.54 to correct for extraction efficiency (Brookes et al. 1985). We express microbial N and ^{15}N on a mass per soil area basis, using measured bulk density of 0.85 g/cm^2 for serpentine soil in these MECCA tubes (B. A. Hungate, *unpublished data*) and assuming a 10 cm deep soil core. We express ^{15}N recovered in the nonfumigated subsample as soil extractable ^{15}N. We calculated total ^{15}N recovery by summing total plant ^{15}N and total soil ^{15}N (includes microbial and soil solution ^{15}N) and dividing by the total ^{15}N added. We calculated soil-fixed ^{15}N as total soil ^{15}N minus microbial and soil solution ^{15}N.

We harvested all the high-nutrient tubes on 16 March, and all the low-nutrient tubes on 18 March. A rainstorm the night of 16 March increased soil moisture to 31.0 ± 0.6% of dry mass in the low-nutrient tubes on 18 March, compared to 21.8 ± 0.2% for the high-nutrient tubes. Because soil moisture strongly influences root and microbial activity and NH_4^+ diffusion, we were not confident that differences between high- and low-nutrient tubes in the short-term N assays reflect true nutrient effects. To avoid confounding soil moisture and the nutrient comparisons and interactions, we analyzed data at each nutrient level separately, using two-way ANOVAs for each response variable with species and CO_2 as main effects. We used a protected post hoc Fisher's Least Significant Difference test to determine where individual comparisons were statistically significant.

We calculated the relative response to elevated CO_2 for each species and nutrient combination by dividing

TABLE 1. Summary of ANOVA results for plant and microbial N and $^{15}NH_4^+$ uptake.

Response variable	Low nutrients						High nutrients					
	CO_2		Species		CO_2 × Species		CO_2		Species		CO_2 × Species	
	F_{df}	P	F_{df}	P	F_{df}	P	F_{df}	P	F_{df}	P	F_{df}	P
Plant N												
Total	$0.58_{1,53}$	0.45	$9.81_{5,53}$	<0.001	$0.99_{5,53}$	0.44	$0.75_{1,57}$	0.39	$10.28_{5,57}$	<0.001	$1.72_{5,57}$	0.15
Shoot	$0.24_{1,68}$	0.63	$10.38_{5,68}$	<0.001	$2.31_{5,68}$	0.05	$0.31_{1,70}$	0.58	$12.15_{5,70}$	<0.001	$2.09_{5,70}$	0.08
Green leaves	$0.63_{1,57}$	0.43	$3.10_{4,57}$	0.02	$0.89_{4,60}$	0.48	$1.15_{1,58}$	0.29	$3.80_{4,58}$	<0.01	$0.83_{4,58}$	0.51
Other shoot parts	$0.10_{1,60}$	0.76	$11.76_{4,60}$	<0.001	$2.63_{4,60}$	0.04	$<0.01_{1,59}$	0.99	$20.41_{4,59}$	<0.001	$2.83_{4,59}$	0.03
Root	$0.07_{1,54}$	0.79	$2.90_{5,54}$	0.02	$0.76_{5,54}$	0.76	$5.47_{5,57}$	0.06	$3.81_{1,57}$	<0.001	$1.18_{5,57}$	0.33
Plant ^{15}N												
Total	$0.03_{1,51}$	0.87	$6.16_{5,51}$	<0.001	$0.53_{5,51}$	0.75	$6.48_{1,55}$	0.01	$4.31_{5,55}$	<0.01	$1.59_{5,55}$	0.18
Shoot	$0.94_{1,67}$	0.94	$13.48_{5,67}$	<0.001	$0.99_{5,67}$	0.43	$7.58_{1,69}$	<0.01	$6.21_{5,69}$	<0.001	$0.63_{5,69}$	0.67
Green leaves	$0.04_{1,57}$	0.83	$13.84_{4,57}$	<0.001	$0.69_{4,57}$	0.60	$3.97_{1,58}$	0.05	$6.32_{4,58}$	<0.001	$1.42_{4,58}$	0.24
Other shoot parts	$0.37_{1,60}$	0.55	$16.31_{4,60}$	<0.001	$2.75_{4,60}$	0.04	$6.00_{1,59}$	0.02	$5.12_{4,59}$	<0.01	$0.54_{4,59}$	0.70
Root	$0.17_{1,53}$	0.69	$0.83_{5,53}$	0.53	$0.37_{4,53}$	0.87	$1.44_{1,55}$	0.24	$0.85_{5,55}$	0.52	$1.26_{5,55}$	0.30
Microbial N	$0.04_{1,69}$	0.85	$0.16_{5,69}$	0.98	$4.02_{5,69}$	<0.01	$2.64_{1,63}$	0.11	$7.34_{5,63}$	<0.001	$1.31_{5,63}$	0.27
Microbial ^{15}N	$0.10_{1,69}$	0.75	$0.88_{5,69}$	0.499	$2.99_{5,69}$	0.02	$3.24_{5,62}$	0.33	$0.95_{1,62}$	0.01	$0.59_{5,62}$	0.71
Soil extractable ^{15}N	$0.89_{1,68}$	0.35	$1.33_{5,68}$	0.26	$1.83_{5,68}$	0.12	$2.34_{1,61}$	0.12	$0.60_{5,61}$	0.70	$1.76_{5,61}$	0.13
Soil fixed ^{15}N	$0.25_{1,59}$	0.62	$0.93_{5,59}$	0.47	$1.19_{5,59}$	0.33	$0.61_{1,48}$	0.69	$0.05_{5,48}$	0.83	$0.88_{5,48}$	0.50
^{15}N recovery	$0.22_{1,51}$	0.64	$1.72_{5,51}$	0.14	$1.06_{5,51}$	0.39	$1.34_{1,53}$	0.25	$1.47_{5,53}$	0.21	$0.87_{5,53}$	0.51

Note: For the main effects of CO_2 and species and for their interaction, F ratios and degrees of freedom (subscript) and P values are noted. Green leaf and other aboveground N and $^{15}NH_4^+$ uptake have four degrees of freedom for the main effect of species and for the CO_2 × species interaction because *Lasthenia californica* was excluded from these tests (green leaf and other shoot fractions were combined before N and ^{15}N analyses for *Lasthenia*). See Figs. 1 and 4 and Table 5 for means and standard errors.

the difference between high and low CO_2 treatment means by the low CO_2 mean. We used this relative response to compare groups of species (introduced grasses vs. native species). To analyze for differences between introduced and native species, we analyzed low- and high-nutrient cases together in a two-way ANOVA with species group and fertilization as the main effects. This approach increased the statistical power to test for species group differences, and it did not interfere with the confounding fertilization effects because we found no significant interactions between the main factors. We also assessed the relationships between components of ecosystem responses to elevated CO_2 by calculating correlation coefficients (Pearson's) between the relative responses in plant and microbial N and ^{15}N to elevated CO_2.

RESULTS

Plant N pools

Elevated CO_2 did not significantly alter total plant N pools at either low or high nutrients, but caused

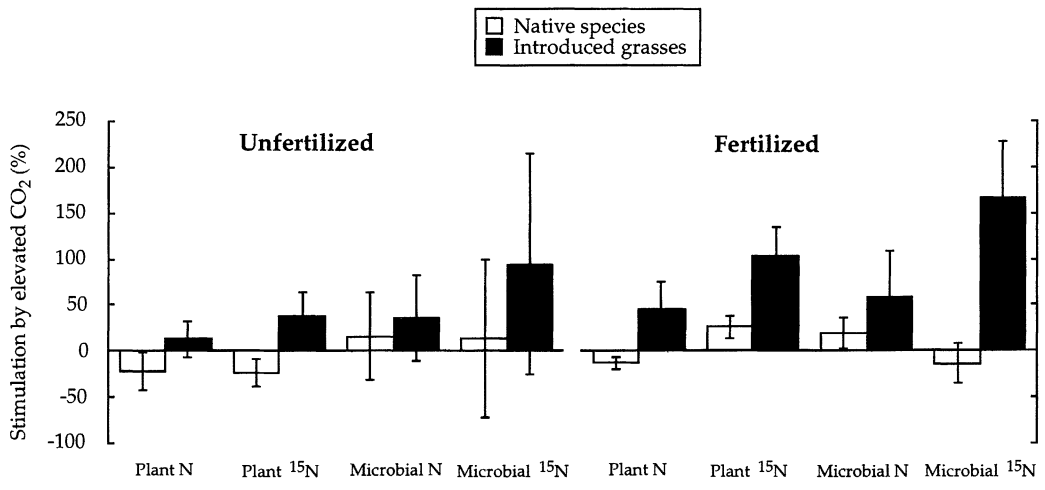

FIG. 2. Summary of relative CO_2 effects on plant and microbial N and $^{15}NH_4^+$ uptake for the three introduced grasses and the three serpentine natives, in unfertilized and fertilized soil. For each species and nutrient combination, we calculated the relative stimulation by elevated CO_2 as the difference between high and low CO_2 means divided by the mean value at low CO_2, expressed as a percentage. Values presented are means ± 1 SE (n = 3).

TABLE 2. Summary of two-way ANOVA results for introduced vs. native species' relative responses to elevated CO_2.

Response variable	Functional group		Nutrients		Functional group × nutrients	
	F	*P*	*F*	*P*	*F*	*P*
Plant N	5.01	0.06	1.04	0.34	0.32	0.59
Plant $^{15}NH_4^+$	9.35	0.02	6.25	0.04	0.10	0.77
Microbial N	0.47	0.52	0.09	0.78	0.04	0.84
Microbial $^{15}NH_4^+$	2.64	0.14	0.08	0.79	0.38	0.55

Note: F ratios and *P* values are shown. There is one degree of freedom for each main effect and the interaction, and eight residual degrees of freedom in all cases. See Fig. 2 for means and standard errors.

species-dependent changes in shoot N pools at both nutrient levels (Fig. 1, Table 1). In response to elevated CO_2, shoot N pools significantly increased in *Avena* at low nutrients and in *Bromus* at high nutrients, but decreased in *Vulpia* at low nutrients and in *Plantago* at high nutrients (Fig. 1). These changes in shoot N occurred primarily due to changes in N pools in stems, reproductive tissues, and senesced leaves (hereafter, "other" shoot parts); changes in green-leaf N were in the same direction, but were less pronounced (Fig. 1, Table 1).

Though the post hoc tests of CO_2 effects within each species were mostly not significant, the introduced grasses tended to increase N pools in response to elevated CO_2 (with the exception of *Lolium* at low nutrients), whereas the native species tended to decrease N pools (though only very slightly in *Lasthenia*). However, when expressed on a relative basis, the introduced grasses had significantly greater positive responses to elevated CO_2 than the native species for total plant N pools (Fig. 2, Table 2).

Plant C:N ratio

Overall, elevated CO_2 increased plant C:N ratios (grams of C per gram of N), though not significantly for all species (Tables 3 and 4). In all cases, increased C:N occurred because of decreased N concentrations rather than increased C concentrations (data not shown). Elevated CO_2 significantly increased whole-plant C:N in *Bromus, Plantago,* and *Vulpia* at low nutrients, and in *Lolium* and *Vulpia* at high nutrients. In *Lolium* (at high nutrients) and *Vulpia* (in both nutrient treatments), the largest increase in C:N ratios occurred in green leaves. At low nutrients, *Bromus* increased C:N primarily in other shoot parts, whereas *Plantago* increased C:N primarily in roots (Jackson and Reynolds 1996).

Where elevated CO_2 significantly increased plant C:N, shoot N pools either decreased (at low nutrients, significantly for *Vulpia* and nonsignificant trend for *Plantago*) or did not change significantly (*Bromus* at low nutrients, *Lolium* and *Vulpia* at high nutrients; Tables 3 and 4, Fig. 1). Elevated CO_2 did not significantly alter C:N where shoot N pools increased significantly (in *Avena* at low nutrients, and in *Bromus* at high nutrients; Tables 3 and 4, Fig. 1). Thus, elevated CO_2 altered C:N ratio more by reducing N uptake than by N dilution through growth.

Microbial N

At low nutrients, plant species determined the effects of elevated CO_2 on microbial N (species × CO_2 interaction, Table 1, Fig. 1); species and CO_2 alone were not significant main effects. Elevated CO_2 significantly decreased microbial N in *Lolium* and *Vulpia,* while increasing microbial N for the other species (nonsignificant trends). With the exception of *Plantago,* changes in microbial N pools paralleled changes in plant N pools (Fig. 1). Expressed on a relative basis, the effects of elevated CO_2 on plant and microbial N pools were positively correlated (Fig. 3). Thus, across a range of plant species that exhibit different growth strategies and under conditions where nutrients strongly limited plant growth, we observed a positive relationship between changes in plant and microbial N acquisition in response to perturbation by elevated CO_2. We found no evidence for a trade-off between plant and microbial N acquisition.

At high nutrients, plant species caused differences in microbial N, whereas CO_2 and the species × CO_2 interaction were not significant (Table 1, Fig. 1). *Lolium* and *Lasthenia* supported larger microbial N pools than *Plantago, Vulpia,* and *Bromus,* with *Avena* intermediate. Thus, species differences in microbial N pools did not correspond to ecological or taxonomic groups of plants. In contrast to low nutrients, the relative changes in plant and microbial N pools in response to elevated CO_2 at high nutrients were not correlated (Fig. 3), reflecting a decoupling of plant and microbial N acquisition when N was abundant.

Plant $^{15}NH_4^+$ uptake

At low nutrients, the effects of elevated CO_2 on short-term plant $^{15}NH_4^+$ uptake paralleled the effects of elevated CO_2 on plant N pools. Elevated CO_2 did not alter whole-plant $^{15}NH_4^+$ uptake (Fig. 4, Table 1), but caused species-dependent changes in ^{15}N allocation (Table 1). Elevated CO_2 tended to decrease ^{15}N allocation to other shoot parts in the native species, while increasing ^{15}N allocation to other shoot parts in the

TABLE 3. Plant carbon : nitrogen (C:N) ratios for six monocultures after 5 mo of growth at ambient (360 μL/L) and elevated (710 μL/L) CO_2 on serpentine soil under low- (unamended) and high-nutrient (20 g/m² NPK) conditions.

Species	Green leaves			Other shoot parts		
	Ambient (mean ± 1 SE)	Elevated (mean ± 1 SE)	Change (%)	Ambient (mean ± 1 SE)	Elevated (mean ± 1 SE)	Change (%)
Low nutrients						
Avena	26.4 ± 2.6	30.0 ± 2.4	14	50.1 ± 5.5	48.0 ± 6.5	−4
Bromus	19.2 ± 0.9	22.8 ± 1.0	19	37.3 ± 3.1	51.0 ± 4.3	**37**
Lolium	24.8 ± 0.8	25.1 ± 1.8	1	40.5 ± 2.7	43.8 ± 2.9	8
Lasthenia	...	...	...	...	...	...
Plantago	31.6 ± 2.0	35.0 ± 1.4	11	32.6 ± 1.4	37.8 ± 1.4	16
Vulpia	19.4 ± 1.2	32.4 ± 3.2	**67**	30.8 ± 3.1	38.4 ± 3.5	25
High nutrients						
Avena	19.0 ± 1.3	18.6 ± 1.0	−2	35.1 ± 2.4	39.8 ± 2.3	13
Bromus	19.0 ± 1.8	19.2 ± 0.8	1	34.7 ± 3.1	36.4 ± 1.2	5
Lolium	30.2 ± 1.4	38.5 ± 2.0	**28**	50.7 ± 1.9	57.5 ± 1.8	**13**
Lasthenia	...	...	...	...	...	...
Plantago	14.5 ± 2.2	16.5 ± 1.5	14	14.8 ± 1.6	15.4 ± 1.2	4
Vulpia	19.0 ± 1.1	26.7 ± 2.1	**40**	31.6 ± 3.7	41.2 ± 1.8	**31**

Note: Values are means ± 1 SE, n = 5–7, and the average percentage change in C:N ratio caused by elevated CO_2. Overall, elevated CO_2 significantly increased C:N ratios in all plant fractions, and species differed in response to CO_2 for changes in green-leaf C:N. Results from two-way ANOVAs for the main effects of elevated CO_2, species, and their interaction are presented in Table 4. Here, boldface indicates individual comparisons that were significantly different (at $P < 0.1$) according to the Fisher's LSD protected post hoc test.

introduced grasses (Fig. 4). At high nutrients, elevated CO_2 caused larger increases in plant $^{15}NH_4^+$ uptake (Fig. 4) than in plant N pools (Fig. 1). Elevated CO_2 stimulated whole-plant $^{15}NH_4^+$ uptake across all species by 64% (Fig. 4), though the individual comparisons were significant only for *Bromus* and *Lolium.* Newly absorbed $^{15}NH_4^+$ was allocated primarily to shoots; though ^{15}N in roots tended to increase in the introduced grasses, the changes were not significant.

The effects of elevated CO_2 on plant $^{15}NH_4^+$ uptake were predictable based on whether species were native or introduced. Across both nutrient levels, elevated CO_2 caused a larger stimulation of plant $^{15}NH_4^+$ uptake in the introduced grasses (70 ± 23%) than in the native species (1 ± 14%) (Fig. 2).

Microbial ^{15}N

As with microbial N, plant species determined the effects of elevated CO_2 on microbial $^{15}NH_4^+$ uptake at low nutrients (significant species by CO_2 interaction), whereas species and CO_2 alone were not significant main effects (Table 1, Fig. 4). Elevated CO_2 significantly increased microbial $^{15}NH_4^+$ uptake in *Avena* and *Lasthenia,* while decreasing microbial $^{15}NH_4^+$ uptake in *Vulpia* and *Plantago.*

At high nutrients, plant species caused differences in microbial $^{15}NH_4^+$ uptake, but CO_2 and the species × CO_2 interaction were not significant (Table 1, Fig. 4), just as observed with total microbial N pools (Table 1, Fig. 1). Microbial $^{15}NH_4^+$ uptake was highest in *Lasthenia,* then decreased in the order: *Lolium, Avena, Plantago, Bromus,* and *Vulpia* (Fig. 4). Species differences in microbial $^{15}NH_4^+$ uptake did not clearly correspond to whether species were native or introduced, nor to whether species were grasses or forbs.

N and ^{15}N distribution

Of the total amount of ^{15}N added, 102 ± 9% was recovered at low nutrients and 119 ± 13% at high nutrients (Table 5). Elevated CO_2 and plant species did not significantly affect total ^{15}N recovery, nor the amount of ^{15}N recovered in the soil solution and soil fixed pools (Table 1). Averaged across all treatments, the sum of ^{15}N recovered in the soil extractable and soil fixed pools was 30 times greater than ^{15}N recovered in plants (Table 5).

Similarly, on average, microbes took up 17 times more $^{15}NH_4^+$ than plants in the 24-h assay (Fig. 4, Table 5). Thus, small changes in microbial $^{15}NH_4^+$ uptake could substantially alter $^{15}NH_4^+$ availability to plants. However, there was little evidence for a trade-off between plant and microbial $^{15}NH_4^+$ uptake under low-nutrient conditions, with the exception of *Lasthenia,* where microbial $^{15}NH_4^+$ uptake increased with elevated CO_2, and plant $^{15}NH_4^+$ uptake tended to decrease with elevated CO_2. In all other cases at low nutrients, plant and microbial $^{15}NH_4^+$ uptake changed in the same direction. When expressed on a relative basis, changes in plant and microbial 24-h $^{15}NH_4^+$ uptake in response to elevated CO_2 were not significantly correlated (Fig. 3), though the trend was positive. As with microbial N and plant N, we found little evidence for a strong trade-off between plant and microbial N acquisition in our 24-h $^{15}NH_4^+$ assay.

Summary of results

Plant responses to elevated CO_2 were species dependent (Table 1, Figs. 1 and 4). However, responses within species groups were similar, with larger increases in plant N pools and plant $^{15}NH_4^+$ uptake in the

TABLE 3. Continued.

Total shoot			Total plant		
Ambient (mean ± 1 SE)	Elevated (mean ± 1 SE)	Change (%)	Ambient (mean ± 1 SE)	Elevated (mean ± 1 SE)	Change (%)
42.1 ± 4.2	42.2 ± 5.0	0	46.8 ± 4.9	46.7 ± 5.4	0
28.9 ± 1.6	37.4 ± 1.9	**29**	35.1 ± 1.3	43.7 ± 2.1	**25**
31.8 ± 1.8	34.5 ± 2.1	9	39.9 ± 2.5	43.6 ± 2.0	9
19.8 ± 0.9	19.2 ± 1.2	−3	30.8 ± 3.2	27.7 ± 2.7	−10
32.2 ± 1.2	36.7 ± 1.1	14	35.7 ± 0.8	44.4 ± 1.3	**24**
29.6 ± 3.0	35.4 ± 3.5	19	33.7 ± 2.8	44.1 ± 3.6	**31**
31.0 ± 2.2	34.9 ± 2.3	12	33.0 ± 2.2	34.4 ± 2.4	4
24.6 ± 2.5	27.0 ± 1.3	10	24.9 ± 1.9	27.2 ± 1.5	9
38.4 ± 1.6	46.6 ± 1.8	**21**	39.1 ± 1.4	43.8 ± 1.2	**12**
15.7 ± 0.7	19.0 ± 1.2	21	17.8 ± 1.1	20.2 ± 1.1	13
13.9 ± 1.8	15.9 ± 1.3	14	14.9 ± 1.9	16.5 ± 1.7	10
27.1 ± 2.8	37.0 ± 1.9	**36**	27.1 ± 3.6	34.3 ± 2.1	**26**

introduced grasses, and smaller increases or even decreases in plant N pools and plant $^{15}NH_4^+$ uptake in the native species (Fig. 2). Differences in microbial N and $^{15}NH_4^+$ uptake in association with different plant species were larger than between CO_2 levels at high nutrients (Table 1, Figs. 1 and 4). At low nutrients, the direction and magnitude of CO_2 effects on microbial N and $^{15}NH_4^+$ uptake depended on plant species (Table 1, Figs. 1 and 4). CO_2 effects on plant N were positively correlated with CO_2 effects on microbial N at low nutrients (Fig. 3). Similarly, CO_2 effects on plant $^{15}NH_4^+$ uptake were in the same direction as CO_2 effects on microbial $^{15}NH_4^+$ uptake (Fig. 3). Thus, we found no evidence for a trade-off between plant and microbial N acquisition over time scales of 24 h, or scales of several months.

DISCUSSION

These results support the hypothesis that changes in plant and microbial N pools and fluxes in response to elevated CO_2 depend on plant species. Changes in root biomass (Jackson and Reynolds 1996) and total plant biomass (C. B. Field et al., *unpublished manuscript*) in response to elevated CO_2 showed a similar species dependence in this experiment. The distinction between native and introduced species partly accounted for this species dependence. This distinction may reflect taxonomic differences, as the introduced group comprises solely grasses, whereas the native group comprises two forbs and one grass. However, the larger relative CO_2 stimulation of plant N pools and $^{15}NH_4^+$ uptake in the introduced grasses is probably not due to inherently greater CO_2 responsiveness in grasses. First, the largest negative responses to elevated CO_2 occurred in *Vulpia,* the one native grass included in our study. Second, in an extensive literature survey, Poorter (1993) found that C_3 grasses tend to have smaller responses to elevated CO_2 than C_3 forbs. Differences in seed size may provide a partial explanation for the different responses in native and introduced species in this study. The introduced grasses in this study have higher absolute growth rates due to their larger seeds. The greater ab-

TABLE 4. Summary of two-way ANOVA results for plant C:N ratios in ambient and elevated CO_2. Results are shown separately for fertilized and unfertilized soil.

	CO_2		Species		CO_2 × species	
Response variable	F_{df}	P	F_{df}	P	F_{df}	P
Low nutrients						
Total	$6.88_{1,53}$	0.01	$6.47_{5,53}$	<0.001	$1.46_{5,53}$	0.22
Shoot	$5.46_{1,68}$	0.02	$17.3_{5,68}$	<0.001	$0.93_{5,68}$	0.47
Green leaves	$17.7_{1,57}$	<0.001	$13.5_{4,57}$	<0.001	$2.92_{4,60}$	0.03
Other shoot parts	$5.54_{1,60}$	0.02	$5.37_{4,60}$	<0.001	$1.21_{4,60}$	0.32
High nutrients						
Total	$8.86_{1,57}$	0.004	$52.0_{5,57}$	<0.001	$0.67_{5,57}$	0.65
Shoot	$61.2_{1,70}$	<0.001	$20.6_{5,70}$	<0.001	$1.60_{5,70}$	0.18
Green leaves	$14.7_{1,58}$	<0.001	$45.3_{4,58}$	<0.001	$3.35_{4,58}$	0.02
Other shoot parts	$11.3_{1,59}$	0.001	$73.7_{4,59}$	<0.001	$1.21_{4,59}$	0.32

Note: Shown are F ratios, degrees of freedom, and P values for the main effects of CO_2, species, and their interaction. See Table 3 for mean C:N ratios ± standard errors for each treatment.

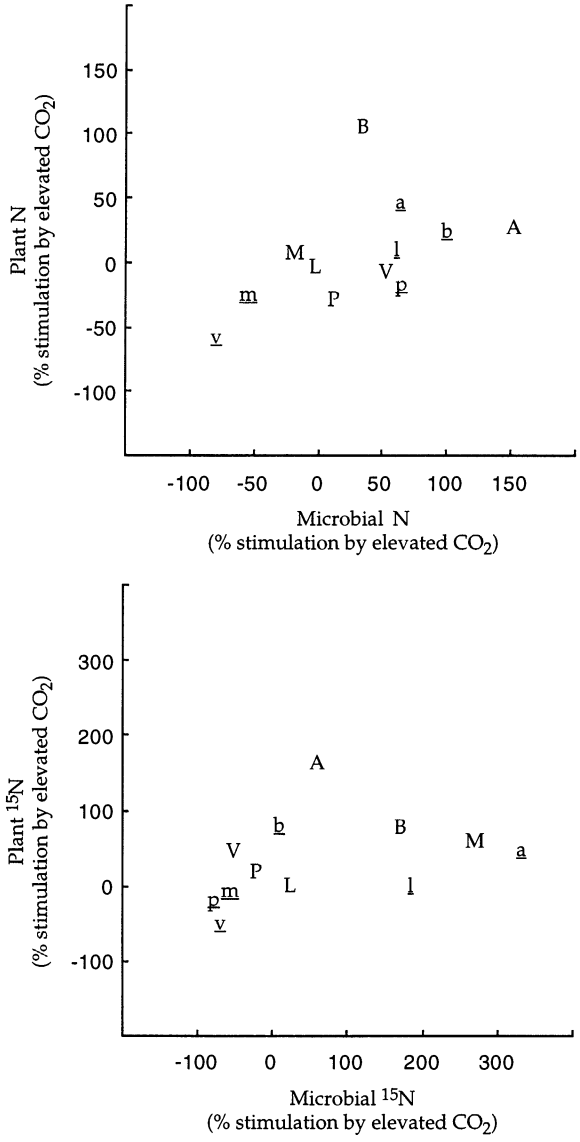

FIG. 3. Relationships between plant and microbial responses to elevated CO_2: the relative CO_2 stimulation of plant vs. microbial N (top) and of plant vs. microbial $^{15}NH_4^+$ uptake (bottom). We calculated relative responses as described in the legend to Fig. 2. Each letter indicates one of our six plant species: A (*Avena*), B (*Bromus*), M (*Lolium*), V (*Vulpia*), L (*Lasthenia*), and P (*Plantago*). Uppercase type indicates the high-nutrient treatment, and lowercase underlined type the low-nutrient treatment. The relationship between changes in plant and microbial N was significant at low nutrients (r = 0.81, P = 0.05) and for both high and low nutrients considered together (r = 0.49, P = 0.10), but not at high nutrients (r = 0.21, P = 0.69). The relationship between changes in plant and microbial $^{15}NH_4^+$ uptake were also positive, but were not significant (at low nutrients, r = 0.48, P = 0.42; at high nutrients, r = 0.23, P = 0.66; and for all points taken together, r = 0.36, P = 0.26).

solute growth rates early in the life of large-seeded plant species could explain their large CO_2 response, because this is the stage when CO_2 may exert its strongest effects (Bazzaz 1990).

Whatever the physiological basis, the difference in response to elevated CO_2 between the introduced grasses and the serpentine natives has implications for community composition on California serpentine soil in a high-CO_2 world. These grasses successfully invaded California's more productive grasslands during the last century (Baker 1989), and some of them, *Bromus* and *Lolium* in particular, can increase in abundance on serpentine soil when availability of belowground resources (N or water) increases (Huenneke et al. 1990, Hobbs and Mooney 1991). The results presented here suggest that availability of an aboveground resource, CO_2, may also favor the growth of these introduced grasses on serpentine soil, an important refuge for some of California's native plants.

In this study, we found that plant species determined the direction of CO_2-induced changes in microbial N pools and $^{15}NH_4^+$ uptake, indicating that species' differences are critical in determining changes in N cycling in response to elevated CO_2. The size of the microbial N pool reflects the balance of N mineralization and immobilization (Hart et al. 1994), in this experiment, integrated over 5 mo of growth. Microbial $^{15}NH_4^+$ uptake over 24 h reflects short-term N-immobilization (Jackson et al. 1989). Changes in these parameters will alter soil solution N concentrations, potentially affecting nitrification and denitrification, plant N availability, leaching, and trace gas N losses.

Previous studies have shown that elevated CO_2 can alter soil N cycling, but these studies contrast in the direction of the CO_2 effect. In a growth-chamber study with grassland microcosms, Diaz et al. (1993) found increased microbial N uptake under elevated CO_2, and a suggested decrease in N availability to plants. In poplar monocultures, Zak et al. (1993) found higher N-mineralization under elevated CO_2, suggesting increased N availability to plants. Our finding that species mediate changes in microbial N and $^{15}NH_4^+$ uptake in response to elevated CO_2 suggests a possible explanation for these contrasting results.

Although plant species treatments differed in microbial N and $^{15}NH_4^+$ uptake and in how these changed in response to CO_2, neither species' differences nor the interactive effects of species and elevated CO_2 were clearly (i.e., significantly) related to whether species were native or introduced. Thus, we cannot generalize how these plant species groups influence microbial N and $^{15}NH_4^+$ uptake, nor how they mediate changes in response to elevated CO_2. A potentially useful approach to further explore this question would be to quantify the plant traits that influence microbial N and $^{15}NH_4^+$ uptake (e.g., root exudation and turnover) across a range of species, and then to determine whether

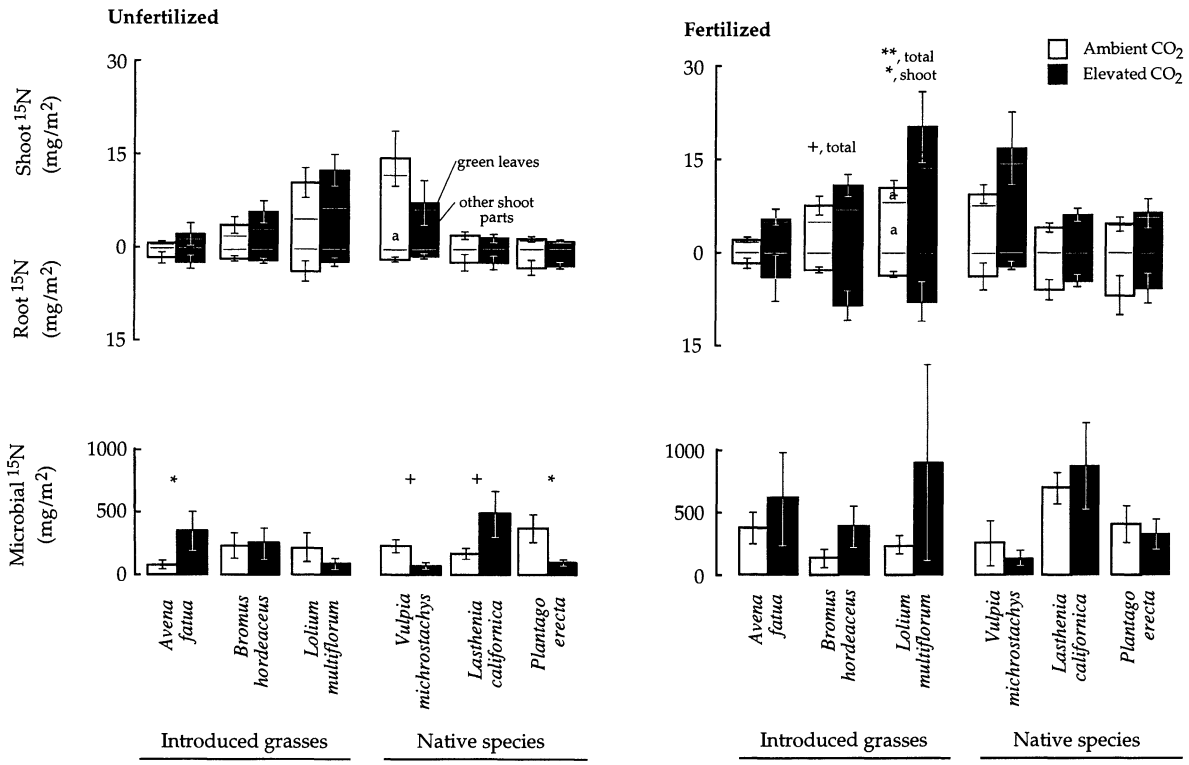

FIG. 4. Stand-level in situ plant and microbial $^{15}NH_4^+$ uptake (mg $N \cdot m^{-2} \cdot d^{-1}$) for six monocultures grown at ambient (360 μL/L) and elevated (710 μL/L) CO_2 in unfertilized and fertilized soil (mean ± 1 SE, n = 5–7). As in Fig. 1, bars for plants (top graph) show $^{15}NH_4^+$ uptake in shoots (green leaf and nongreen leaf fractions, except for *Lasthenia californica,* where only total shoot N was determined) and roots, determined 24 h after injecting $^{15}NH_4^+$ into the top 9 cm of soil. For microbes (bottom graph), bars show microbial $^{15}NH_4^+$ uptake ± 1 SE. Results from post hoc tests are summarized as in Fig. 1.

changes in these traits in response to elevated CO_2 vary among those species.

CO_2-induced changes in microbial N pools were positively related to CO_2-induced changes in plant N pools under strongly nutrient-limited conditions. The same pattern held for short-term plant and microbial $^{15}NH_4^+$ uptake, though the relationship was not significant. In this study, elevated CO_2 decreased plant and microbial N pools and $^{15}NH_4^+$ uptake just as often as it increased them. Thus, the positive relationship between plant and microbial responses to CO_2 reflects both parallel increases (e.g., *Avena*) and parallel decreases (e.g., *Vulpia*) in plant and microbial N pools. This relationship is consistent with the positive correlation between aboveground net primary productivity and soil microbial biomass across a broad array of terrestrial ecosystems (Myrold et al. 1989, Zak et al. 1994), and with the prediction that changes in plant production in response to climate change would cause changes of the same direction and magnitude in the microbial biomass (Zak et al. 1994).

TABLE 5. ^{15}N distribution and recovery. The percentage of added ^{15}N that was recovered in plants, soil microbes, soil extractable, soil fixed, and the sum of all plant and soil pools.

Percentage of added ^{15}N recovered in:	Low nutrients (%)	High nutrients (%)
Plants	1.9 ± 0.2	2.9 ± 0.3
Soil microbes	29.7 ± 4.0	50.8 ± 8.3
Soil extractable	14.0 ± 2.0	25.5 ± 4.3
Soil fixed	55.5 ± 6.5	40.3 ± 7.1
Total recovered	101 ± 9	119 ± 13

Note: Values are means ± 1 standard error for low- and high-nutrient treatments. Because neither species nor CO_2 treatments affected soil extractable, soil fixed, nor total ^{15}N recovered (Table 1), we present overall means for each nutrient treatment for simplicity (lumping CO_2 and species treatments at each nutrient level). See Figs. 1 and 4 and Table 1 for species and CO_2 effects on plant and soil microbial ^{15}N uptake.

Diaz et al. (1993) found that increased microbial N-immobilization limited plant N acquisition in elevated CO_2. In our study, however, the parallel responses of plant and microbial N pools argues against microbial sequestration of N as the mechanism causing decreased plant N acquisition. One possible explanation of decreased plant and microbial N and $^{15}NH_4^+$ uptake under elevated CO_2 in our experiment is that elevated CO_2 caused a feedback inhibition of photosynthesis due to starch accumulation in shoots (Poorter 1993). If such a feedback decreased C translocation to roots, both

plant N acquisition and root C input to soil (which partly drives microbial N uptake) would decline. Consistent with this explanation, decreased microbial $^{15}NH_4^+$ uptake (*Vulpia, Lolium,* and *Plantago* at low nutrients) and decreased microbial N (for *Vulpia* and *Lolium*) occurred when plant C:N ratios increased, but total shoot N pools decreased, suggesting that excess C may have limited plant N acquisition.

At low nutrients, parallel increases in plant and microbial N and $^{15}NH_4^+$ uptake occurred in *Bromus* (though the individual comparisons were not significant), and in *Avena,* where shoot N and microbial $^{15}NH_4^+$ increased significantly. These parallel increases in plant and microbial N acquisition show that elevated CO_2 can increase both plant and microbial N demand, but that there is not necessarily a trade-off between the two. In particular, increasing microbial N immobilization does not necessarily reduce plant N uptake. There are several mechanisms that could account for this relationship. First, plants are able to tap soil N resources over much larger spatial scales than microbes, so plants can capitalize on N-rich microsites. Thus, a more active root system could increase plant access to more N-rich microsites, as well as stimulate microbial N uptake by increasing C input to soil. Second, total microbial N includes N in extra-radicle mycorrhizal hyphae (Smith and Paul 1990), i.e., N that could be en route to plants (Read 1991). The six species in our study are vesicular–arbuscular mycorrhizal (VAM) plants. Although infection rates in other MEC-CA studies are lower than typical infection rates in the field (R. B. Jackson et al., *unpublished manuscript*), the mean ratio of fungal:bacterial biomass in the MEC-CA serpentine soils is 2 (B. A. Hungate, *unpublished data*), high compared to other grasslands (e.g., 0.8 in shortgrass prairie; Ingham et al. 1989), perhaps because extra-radicle VAM hyphae are a substantial part of the total microbial biomass. Parallel increases in plant and microbial N uptake in response to elevated CO_2 could reflect a role for VAM in providing N to these plants. Third, parallel increases in plant and microbial N uptake could be due to a "priming effect" (Clarholm 1985), where plant C input to soil increases microbial activity, causing a net transfer of N from recalcitrant soil organic matter to a more rapidly cycling pool in the microbial biomass, and, with subsequent microbial turnover, to plants. The CO_2 stimulation of root growth, microbial biomass C, and net N-mineralization that Zak et al. (1993) observed is consistent with a C priming of N-mineralization. These three mechanisms, alone or in combination, could explain the parallel increases in plant and microbial N acquisition that we observed. Each requires that elevated CO_2 increases rhizodeposition for these species. Elevated CO_2 did not significantly increase root biomass at low nutrients in our experiment (Jackson and Reynolds 1996). However, in a similar experiment, elevated CO_2 increased specific root mass in *Avena* (J. Canadell, *unpublished data*), suggesting that elevated CO_2 could increase root surface area (and thus perhaps root exudation) with no change in root biomass.

Under the low-nutrient conditions typical of this serpentine grassland, the effects of elevated CO_2 on N cycling are species dependent. Species mediation of CO_2 effects on microbial N, and associated changes in N availability to plants, may be critical in determining plant competitive interactions in nutrient-limited ecosystems in a high-CO_2 world. Under high-nutrient conditions, plant species differed in their effects on microbial N dynamics, but elevated CO_2 did not influence these effects. Species mediation of microbial N dynamics in response to elevated CO_2 may be less important in N-rich ecosystems, such as those affected by N-deposition. Consistent with numerous studies (Bazzaz 1990, Poorter 1993), plant responses to elevated CO_2 varied among plant species in this serpentine grassland. However, this variation was largely predictable according to whether species were native or introduced, suggesting that groups of plant species, e.g., "functional groups" (Chapin 1993), are useful in predicting responses to elevated CO_2. Such functional groups could simplify the daunting task of including characteristics of plant species in models of ecosystem responses to elevated CO_2. We examined species' responses to elevated CO_2 in monocultures: responses in mixtures may be quite different (Reynolds, *in press*), where positive effects on N availability by one species may favor the success of a competitor. However, if these results hold true for species mixtures and for other native vs. introduced species comparisons, elevated CO_2 may offer a competitive advantage to introduced species.

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