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Nitrate and ammonium uptake for single- and mixed-species communities grown at elevated CO_2

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Abstract Sustained increases in plant production in elevated CO_2 depend on adequate belowground resources. Mechanisms for acquiring additional soil resources include increased root allocation and changes in root morphology or physiology. CO_2 research to date has focused almost exclusively on changes in biomass and allocation. We examined physiological changes in nitrate and ammonium uptake in elevated CO_2, hypothesizing that uptake rates would increase with the amount of available CO_2. We combined our physiological estimates of nitrogen uptake with measurements of root biomass to assess whole root-system rates of nitrogen uptake. Surprisingly, physiological rates of ammonium uptake were unchanged with CO_2, and rates of nitrate uptake actually decreased significantly ($P<0.005$). Root biomass increased 23% in elevated CO_2 ($P<0.005$), but almost all of this increase came in fertilized replicates. Rates of root-system nitrogen uptake in elevated CO_2 increased for ammonium in nutrient-rich soil ($P<0.05$) and were unchanged for nitrate ($P>0.80$). Root-system rates of nitrogen uptake were more strongly correlated with physiological uptake rates than with root biomass in unamended soil, but the reverse was true in fertilized replicates. We discuss nitrogen uptake and changes in root biomass in the context of root nutrient concentrations (which were generally unchanged with CO_2) and standing pools of belowground plant nitrogen. In research to date, there appears to be a fairly general increase in root biomass with elevated CO_2, and little evidence of up-regulation in root physiology.

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Introduction

For ecosystems that respond positively to increased levels of CO_2, sustained increases in plant production depend on the capture of adequate belowground resources (Chapin et al. 1987; Bazzaz 1990; Field et al. 1992; Körner and Arnone 1992; Polley et al. 1995). Numerous studies have shown an interaction between resource availability and elevated CO_2, with growth responses to CO_2 sometimes mitigated or eliminated under low-nutrient conditions (e.g. Zangerl and Bazzaz 1984; Larigauderie et al. 1988). Possibly in consequence, plant responses to CO_2 in the field have been inconsistent (e.g. Tissue and Oechel 1987; Curtis et al. 1989; Idso et al. 1991; Mooney et al. 1991; Norby et al. 1992; Owensby et al. 1993; Jackson et al. 1994).

The ability of plants from low-resource systems to acquire additional soil resources in the presence of elevated CO_2 may depend upon the exchange of carbon for a more limiting currency, particularly water or nutrients. Mechanisms for converting carbon into belowground resources include increased root allocation and changes in root morphology and physiology (e.g. Fitter 1987; Chapin et al. 1988; Jackson et al. 1990; Berntson and Woodward 1992). Research to date has focused almost exclusively on changes in root biomass, generally showing an increase with elevated CO_2 (Rogers et al. 1994). Potentially just as important for nutrient uptake is the physiological activity of the roots, and whether plants of resource-limited environments can increase this activity in elevated CO_2. To our knowledge, there have been no published studies of root uptake kinetics and increased nutrient capture in elevated CO_2.

In this study, we examined physiological rates of ammonium and nitrate uptake (nitrogen uptake per gram root) for one mixed-species and six single-species com-

munities grown at ambient and elevated CO_2. Nitrogen is the nutrient that most limits growth in our system in the field (Huenneke et al. 1990). Because of the potential interaction between CO_2 and nutrient availability, we included both unamended and fertilized soil. We hypothesized that plants in elevated CO_2 would increase their physiological rates of nutrient uptake, particularly at low nutrient availability. Plant community uptake for a nutrient is a function of not just the physiological rates of uptake, but also of the quantity of roots in the system. We combined our physiological flux rates with estimates of root biomass to compare rates of nitrogen uptake at the stand level (termed "whole root-system flux rates") for each single- and mixed-species community. We discuss these parameters in the context of root nutrient concentrations and standing pools of belowground plant nitrogen.

Materials and methods

This research was part of the Jasper Ridge CO_2 project, located at the Jasper Ridge Biological Preserve near Stanford, Calif., USA (37°24'N, 122°13'W, 100 m elevation). The site has cool, wet winters and warm, dry summers, with a 15-year average precipitation of 579 mm. The experiments were carried out using 20 open-top chambers (1.3-m square) that received either ambient or ambient plus 350 ppm CO_2. Each chamber contained an array of approximately 30 1-m deep tubes. The tubes were 0.2 m wide and 0.95 m deep, with an upper 0.15 m of shredded serpentine topsoil and a lower 0.80 m of crushed serpentine rock. Half of the tubes in each CO_2 treatment received additional nutrients (20 $g \cdot m^{-2}$ nitrogen, phosphorus and potassium as 120-day time-release Osmocote fertilizer, with nitrogen supplied equally as ammonium and nitrate). Extractable macronutrient concentrations per kilogram of unfertilized topsoil were 4.6 mg phosphorus and 97.8 mg potassium and the percentage total nitrogen and organic carbon in the soil were 0.11% and 1.15%, respectively (Table 1). Extractable nitrate and ammonium concentrations were approximately 1.5 and 3.0 mg nitrogen per kilogram of soil in unfertilized replicates and 10.0 and 11.5 mg nitrogen per kilogram of soil in fertilized replicates (B. Hungate, unpublished 2 M KCl extractions). Values of extractable nitrogen for unfertilized replicates were quite similar to those reported by Koide and Mooney (1987) for undisturbed serpentine topsoil in the field. Except for one 38-mm watering to initiate germination, no supplementary water was added during our experiment. The tubes were prepared and the plants germinated in November 1992, corresponding approximately with germination in the field.

Table 1 Serpentine soil properties from the upper 15 cm of each tube (*TN* Total Kjeldahl nitrogen, *OC* soil organic carbon, *CEC* cation exchange capacity, *EC* electrical conductivity, *SP* saturation percentage). Also given are quantities of extractable phosphorus and potassium; pH; the % CEC occupied by CA^{2+}, Mg^{2+} and Na^{+}; and soil texture (% sand, silt and clay). P and K were extracted with 0.5 M sodium bicarbonate while CEC measurements were based on 1.0 M ammonium acetate extractions. Where appropriate, all determinations are expressed on a soil dry-mass basis

Soil properties	Value
% TN	0.110±0.002
P ($mg \cdot kg^{-1}$)	4.6±0.2
K ($mg \cdot kg^{-1}$)	97.8±3.9
% OC	1.15±0.02
pH	6.7±0.10
CEC ($mEq \cdot 100\ g^{-1}$)	36.3±1.5
Ca (% CEC)	14.5±1.0
Mg (% CEC)	64.6±3.7
Na (% CEC)	0.15±0.02
EC ($mS \cdot cm^{-1}$)	0.63±0.05
% Sand	38.0±0.4
% Silt	29.0±0.4
% Clay	33.0±0.0
SP ($g\ H_2O \cdot g^{-1}$)	0.945±0.031

We grew monocultures of six annual species common to Jasper Ridge grasslands. The six species included four C3 grasses, *Avena fatua, Bromus hordeaceus, Lolium multiflorum,* and *Vulpia microstachys*, and two C3 forbs, *Lasthenia californica* and *Plantago erecta* (Hickman 1993). At Jasper Ridge, *Lasthenia, Plantago*, and *Vulpia* grow only on serpentine soil and *Avena, Lolium,* and *Bromus* occur in non-serpentine grasslands. *Bromus* is also present at a low density on the serpentine, and can greatly increase in abundance during wet years (Hobbs and Mooney 1991) and with nutrient addition (Huenneke et al. 1990). In addition to these six species, we also grew a mixed community of *Bromus, Lasthenia*, and *Plantago* (equal proportions of each by number). The density for all treatments was 300 plants per tube (roughly 9000 $plants \cdot m^{-2}$), except for the large-statured *Avena*, which had 95 plants per tube. These densities were designed to approximate the density and total production seen in the field. Further description of the experimental system can be found in Field et al. (1995).

We conducted our nitrogen uptake experiment in March 1993. One 3-cm diameter, 15-cm deep soil core was taken from each replicate tube, and the roots from each core were washed from the soil. We placed random subsamples of the roots in small cheesecloth bags, equilibrated them for 20 min in 0.5 mM $CaCl_2$ at the assay temperature of 20° C, then placed the subsamples in solutions containing either 100 μM $^{15}NH_4Cl$ or 100 μM $K^{15}NO_3$ (99 atom% ^{15}N) for 30 min. Although we did not have measurements of actual soil-solution concentrations, we estimate soil-solution nitrate in unfertilized tubes to be between 100 μM and 450 μM (based on the extractable nitrate concentrations, gravimetric water, and the assumption that all nitrate in the extracts came from the soil solution). For most low-nutrient and all *Vulpia* replicates, there were sufficient roots to quantify only ammonium uptake. All solutions were well mixed and aerated, adjusted to pH 6.0, and contained both 0.01 M sucrose as an energy source and 0.5 mM $CaCl_2$ for membrane integrity (Jackson et al. 1990). After incubation, each subsample was rinsed in several solutions of 1 mM KCl to remove any ^{15}N adsorbed to the root surfaces. Roots were blotted dry, oven-dried at 75° C, weighed, ground, and analyzed for ^{15}N content, %N, and %C by mass spectrometry. The nutrient uptake assay was completed less than 2 h after soil coring to minimize the effect of root excision on nitrate and ammonium uptake (Bloom and Caldwell 1988).

Physiological rates of nitrogen uptake are expressed per unit dry mass of root ($\mu mol \cdot g^{-1} \cdot h^{-1}$). Whole root-system flux rates were obtained by multiplying physiological fluxes by root biomass; they are expressed on a soil surface area basis ($mg \cdot m^{-2} \cdot h^{-1}$) to facilitate comparison between uptake measurements and biomass/production estimates ($g \cdot m^{-2}$). (Each 3-cm diameter core had a soil surface area of 7.1 cm^2). Total belowground pools of plant nitrogen were obtained by multiplying root biomass and respective nitrogen concentrations. Each variable was analyzed statistically with a three-factor analysis of variance using the SAS general linear models procedure (SAS 1985). Where appropriate, protected post hoc comparisons were made with Tukey's multiple comparison test.

Results

Elevated CO_2 affected physiological rates of ammonium and nitrate uptake quite differently (Figs. 1, 2). Increased CO_2 had no apparent effect on ammonium uptake for any

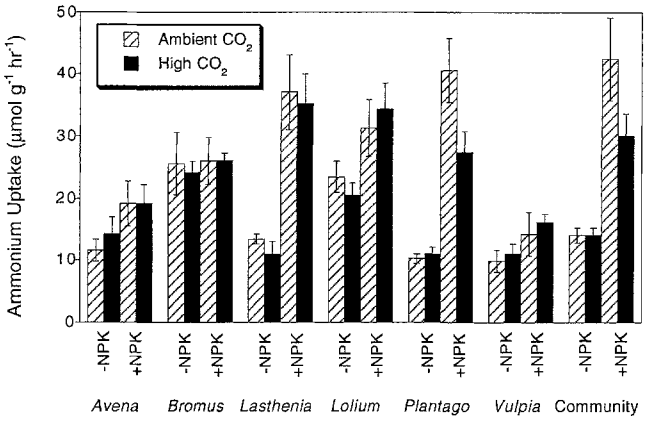

Fig. 1 Physiological rates of ammonium uptake (μmol N g^{-1}·h^{-1}, root dry-mass basis) for six single- and one mixed-species communities grown in unamended and fertilized soil and high and low concentrations of CO_2 (mean±SEM, n=4–6). The two CO_2 concentrations were ambient and ambient +350 ppm CO_2 (approximately 360 ppm and 710 ppm for ambient and high CO_2, respectively). Roots were placed in solutions containing 100 μM $^{15}NH_4Cl$ for 30 min and then analyzed for their ^{15}N content. CO_2 had no apparent effect on physiological rates of ammonium uptake for any combination of nutrients or species (P>0.30 for the main effect of CO_2 and its interactions)

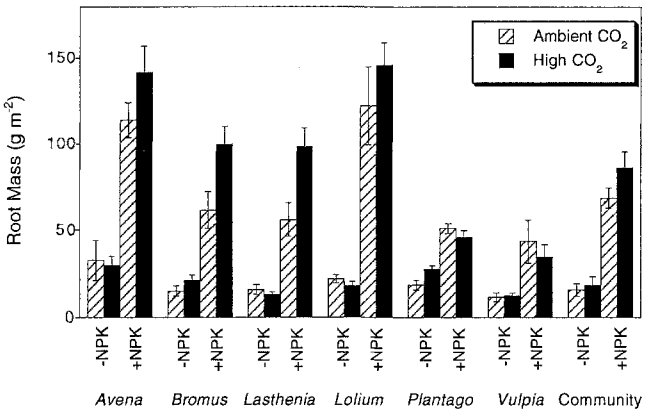

Fig. 3 Root dry mass (g·m^{-2}, soil surface area basis) for six single- and one mixed-species communities grown in unamended and fertilized soil and high and low concentrations of CO_2 (mean±SEM, n=4–6). The roots were washed from 3-cm diameter, 15-cm deep soil cores harvested in March 1993. Elevated CO_2 increased root biomass 23% overall (P<0.005), but most of the increase came in fertilized replicates (28% and 7% increases for fertilized and unfertilized treatments, respectively, in elevated CO_2). Fertilization had a much stronger effect than did CO_2 on root biomass

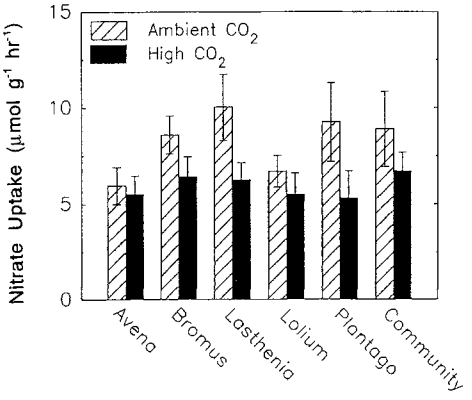

Fig. 2 Physiological rates of nitrate uptake (μmol N g^{-1}·h^{-1}, root dry-mass basis) for six single- and one mixed-species communities grown in fertilized soil and high and low concentrations of CO_2 (mean±SEM, n=4–6, ambient and ambient +350 ppm CO_2). Roots were placed in solutions containing 100 μM $K^{15}NO_3$ for 30 min and then analysed for their ^{15}N content. There was not enough roots in the soil cores from unamended soil to measure nitrate uptake, so the data presented are only for fertilized replicates. CO_2 significantly decreased nitrate uptake (P<0.005), a 28% reduction overall

combination of nutrients or species (Fig. 1, P>0.30 for the main effect of CO_2 and its interactions). In contrast and contrary to expectation, nitrate uptake decreased significantly in elevated CO_2 (Fig. 2, P<0.005), a 28% reduction overall and a decrease on average for every species examined.

There were strong fertilization and species effects on physiological rates of ammonium uptake. Ammonium uptake rates tripled with fertilization for the two forb species, *Plantago* and *Lasthenia*, (Fig. 1, P<0.0001), but increased only 37% on average for the four grasses (5%, 46%, 49%, and 49% increases for *Bromus, Vulpia, Lolium*, and *Avena*, respectively). *Bromus* and *Lolium* had significantly higher rates of ammonium uptake in unamended soil than did the other species (Fig. 1, P<0.05); in fertilized soil, rates were significantly lower for *Avena* and *Vulpia* (Fig. 1, P<0.05). Despite strong species effects for ammonium uptake (P<0.0001), there were no species differences for nitrate uptake (Fig. 2, P>0.35). Rates of ammonium and nitrate uptake for the mixed-species community tended to be similar to or intermediate between uptake for the component species (*Plantago, Lasthenia,* and *Bromus*), and this holds for all variables discussed below.

Elevated CO_2 increased root biomass 23% overall (Fig. 3, P<0.005), but most of the increase came in fertilized replicates (28% and 7% increases for fertilized and unfertilized treatments, respectively, in elevated CO_2). Fertilization had a much stronger effect than did CO_2 on root biomass; leading to a fourfold increase in fertilized tubes (Fig. 3, P<0.0001). Species differed both in total root biomass and in root responses to nutrients and CO_2. In fertilized soil, *Lolium* and *Avena* had three times the root biomass of *Vulpia* and over twice the biomass of *Plantago*. *Plantago* showed the smallest species response to nutrients, only doubling its root biomass with fertilization, and it showed no response to CO_2 in nutrient-rich soil. Root biomass of the mixed-species community was intermediate between values of the component species.

Whole root-system ammonium uptake increased 22.3% overall in elevated CO_2 (Fig. 4, P<0.05), though as with root mass, most of this increase came with fertilization (25.9% and 5.3% increases for fertilized and unfertilized replicates, respectively, in elevated CO_2).

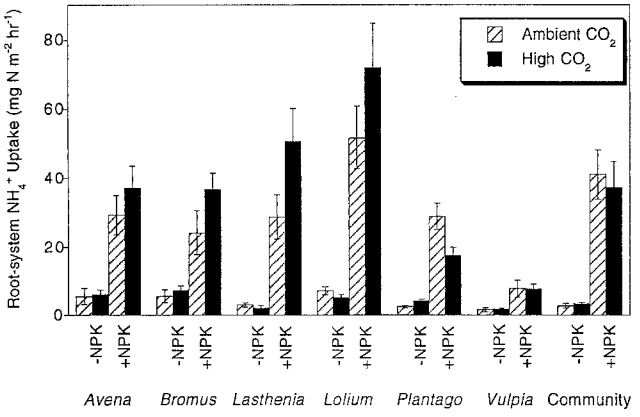

Fig. 4 Whole root-system rates of ammonium uptake (mg N m^{-2}·h^{-1}, soil surface area basis) for six single- and one mixed-species communities grown in unamended and fertilized soil and high and low concentrations of CO_2 (mean±SEM, n=4–6). Whole root-system rates of ammonium uptake are obtained by multiplying respective physiological fluxes by root biomass and are based on 3-cm diameter, 15-cm deep soil cores. Root-system ammonium uptake increased 22.3% overall with elevated CO_2 (P<0.05), though, as with root mass, most of this increase came with fertilization (25.9% and 5.3% increases for fertilized and unfertilized replicates, respectively, in elevated CO_2)

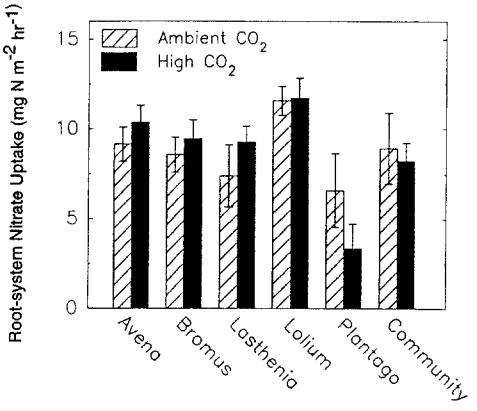

Fig. 5 Whole root-system rates of nitrate uptake (mg N m^{-2}·h^{-1}, soil surface area basis) for six single- and one mixed-species communities grown in fertilized soil and high and low concentrations of CO_2 (mean±SEM, n=4–6). There were not enough roots in the soil cores from unamended soil to measure nitrate uptake, so the data presented are only for fertilized replicates. There were no differences in rates of root-system nitrate uptake in elevated CO_2 (P>0.80)

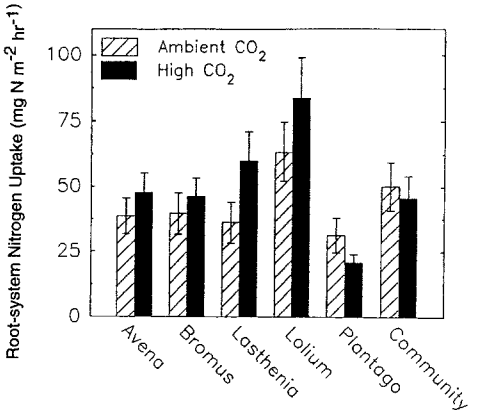

Fig. 6 Whole root-system rates of nitrogen uptake (mg N m^{-2}·h^{-1}, soil surface area basis) for six single- and one mixed-species communities grown in fertilized soil and high and low concentrations of CO_2 (mean±SEM, n=4–6). Root-system rates of nitrogen uptake are obtained by multiplying respective physiological fluxes for ammonium and nitrate by root biomass and summing them together; the calculations are based on 3-cm diameter, 15-cm deep soil cores. There were not enough roots in the soil cores from unamended soil to measure nitrate uptake, so the data presented are only for fertilized replicates

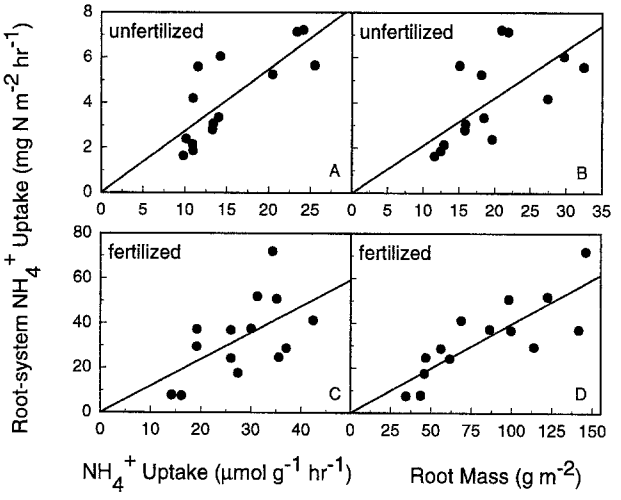

Fig. 7 Whole root-system rates of ammonium uptake (mg N m^{-2} h^{-1}, soil surface area basis) in fertilized and unfertilized replicates as a function of physiological rates of ammonium uptake (left two panels) and root mass (right two panels). The 14 points comprising each panel are the mean values for each of the six single-species and one mixed-species communities grown at high and low CO_2.

Again, the plants reponded much more strongly to nutrients than to CO_2. The combination of greater physiological uptake and increased root mass led to an eightfold increase in ammonium uptake in nutrient-rich soil (Fig. 4, P<0.0001). The extremely low rate of root-system ammonium uptake by *Vulpia* reflected both a low physiological uptake rate and low root biomass. Despite the significant decrease in physiological nitrate uptake with CO_2 (Fig. 2), the increase in root mass in the presence of CO_2 and a nutrient-rich soil exactly compensated for this decrease, and there were no differences in root-system nitrate uptake in elevated CO_2 (Fig. 5, P>0.80, average nitrate uptake only 0.49% higher in the presence of CO_2). The only significant species difference in terms of nitrate uptake was between *Lolium* and *Plantago* (over twice as high for *Lolium*), but this was due exclusively to differences in root biomass. Root-system rates of nitrogen uptake (ammonium plus nitrate) were significantly higher overall in elevated CO_2 (Fig. 6, P<0.05), but not for *Plantago* individually. Because the physiological capacities for nitrate uptake were only about 30% of those for ammonium uptake, ammonium probably accounted for most of the nitrogen uptake by the roots in our experiment.

Table 2 The percentage of total nitrogen (N) and carbon (C) in roots (dry-mass basis), C:N ratios, and root N pools in the soil (grams of N per m^2, soil surface area basis) for one mixed-species and six single-species communities grown in unamended and fertilized soils and in the presence of high and low concentrations of CO_2 (mean±SEM, n=5–8)

Conditions			Parameter			
Species	Nutrients	CO_2	%N	%C	C:N Ratios	Root N pools ($g \cdot m^{-2}$)
Avena fatua	Low	Low	0.78±0.11	39.7±1.32	55.7±7.88	0.237±0.087
	Low	High	0.67±0.08	39.2±0.68	63.1±6.95	0.201±0.038
	High	Low	1.00±0.05	38.4±0.44	39.1±2.24	1.140±0.122
	High	High	1.22±0.15	39.2±0.43	34.5±4.38	1.720±0.288
Bromus hordeaceus	Low	Low	0.86±0.08	41.4±0.44	49.7±4.67	0.175±0.028
	Low	High	0.77±0.06	41.4±0.60	55.0±3.87	0.157±0.019
	High	Low	1.89±0.19	42.3±0.42	23.5±2.19	1.100±0.160
	High	High	1.51±0.12	42.4±0.24	29.2±3.12	1.520±0.216
Lasthenia californica	Low	Low	0.90±0.08	43.6±1.15	49.9±5.22	0.138±0.021
	Low	High	1.03±0.12	42.5±1.20	43.0±3.95	0.129±0.012
	High	Low	2.16±0.43	41.4±0.84	23.8±4.94	1.140±0.238
	High	High	1.77±0.19	40.2±0.53	24.2±2.80	1.658±0.158
Lolium multiflorum	Low	Low	0.80±0.08	43.3±0.92	56.3±4.92	0.174±0.021
	Low	High	0.70±0.04	44.9±1.17	65.3±4.15	0.126±0.017
	High	Low	1.02±0.04	41.5±0.46	41.0±1.59	1.280±0.280
	High	High	0.97±0.09	41.0±0.58	44.5±5.59	1.450±0.228
Plantago erecta	Low	Low	0.76±0.03	42.9±0.88	56.9±2.64	0.133±0.022
	Low	High	0.66±0.03	44.9±0.43	68.5±2.95	0.181±0.014
	High	Low	2.59±0.41	42.7±0.80	20.9±6.04	1.279±0.238
	High	High	2.83±0.64	42.8±0.68	19.0±3.54	1.298±0.259
Vulpia microstachys	Low	Low	0.74±0.10	40.7±1.61	59.3±8.22	0.095±0.027
	Low	High	0.70±0.03	44.0±0.66	63.7±2.78	0.086±0.010
	High	Low	1.18±0.17	41.3±0.95	38.8±6.50	0.497±0.126
	High	High	1.48±0.17	41.6±0.82	29.6±3.31	0.473±0.081
Community	Low	Low	0.97±0.06	43.8±0.81	45.8±2.91	0.149±0.027
	Low	High	0.86±0.10	43.8±0.88	53.1±4.99	0.176±0.069
	High	Low	1.80±0.20	41.2±0.50	23.9±2.69	1.242±0.163
	High	High	1.55±0.35	41.2±0.57	31.9±6.40	1.325±0.314

Elevated CO_2 had no apparent effect on root nitrogen concentrations (Table 2, P>0.45 for all CO_2 and CO_2 interaction terms), nor on root carbon concentrations (Table 2, P>0.20). There was some indication of an increase in root carbon:nitrogen ratios (Table 2, P=0.10, 7% overall), with this increase associated exclusively with low concentrations of nutrients (an 11% and 1% increase for unfertilized and fertilized replicates, respectively, in elevated CO_2). Not surprisingly, there were a number of strong species and fertilization effects on root tissue concentrations. Fertilization doubled root nitrogen concentrations (Table 2, P<0.0001), but the relative increase with fertilization was eight times as great for *Plantago* as for *Avena* (P<0.0001 for the interaction between species and nitrogen). *Plantago* and *Lasthenia* had significantly higher nitrogen concentrations with fertilization than did *Vulpia, Avena*, or *Lolium* (P<0.05).

Standing pools of belowground plant nitrogen increased 19% in elevated CO_2 (Table 2, P<0.05), with the increase occurring entirely in fertilized replicates. Since the standing pools of belowground nitrogen are a function of root biomass and nitrogen concentrations, our increases are due primarily to the increase in root biomass, rather than to changes in nitrogen concentrations (Fig. 3, Table 2).

Discussion

Our results do not support our initial hypothesis that physiological rates of nitrogen uptake would increase with elevated CO_2. We found that ammonium uptake was unaffected by CO_2, and that nitrate uptake was down-regulated. We cannot account for this down-regulation of nitrate uptake based on nutritional changes in the plants, since the nitrogen concentrations of roots and shoots were relatively unaffected by CO_2 (Table 2; and Field et al. 1995), as were root:shoot ratios. Some studies have shown a change in the architecture or fineness of roots with CO_2 (e.g. Berntson and Woodward 1992), which can alter nutrient uptake expressed on a root dry-mass basis. We also consider this explanation unlikely, since ammonium uptake rates in subsamples were unchanged (Fig. 1), and no changes were observed for our species in root length or surface area for a given root mass (Z.G. Cardon and R.B. Jackson, unpublished data).

One intriguing possibility for the down-regulation of nitrate uptake is a change in soil-solution nitrogen availability, possibly mediated by altered microbial activity (see Diaz et al. 1993, Zak et al. 1993). Possible evidence for this change can be found in resin bag data from fertilized replicates (J. des Rosiers and S. Thayer, unpublished data). The amount of ammonium captured on resin bags was twice as high in ambient as in elevated CO_2, potentially reflecting greater plant uptake of ammonium in the

presence of CO_2. In contrast, the amount of nitrate on resin bags was 50% greater in the presence of elevated CO_2. Ammonium is the dominant form of available nitrogen in California grassland soils (Jackson et al. 1988), and gross ammonium mineralization and microbial numbers increased in the field at Jasper Ridge with CO_2 (B. Hungate in preparation). Fertilized plants grown in the presence of a high level of CO_2 may have met their increased nitrogen demand more readily with ammonium uptake, and down-regulated nitrate uptake.

Although we found no effects of CO_2 on ammonium uptake, we did find strong, species-specific fertilization effects (e.g. Fig. 1). The striking up-regulation of ammonium uptake observed for *Plantago* and *Lasthenia* in fertilized soil is surprising for two reasons. First, other studies (e.g. Lee 1982) have shown down-regulation of nutrient absorption with increasing fertility of the growth medium. Secondly, species typical of low-fertility habitats (such as *Plantago* and *Lasthenia*) have sometimes been characterized as being less responsive to changes in external nutrient concentration than species of higher fertility habitats (Chapin 1988). That we saw much less up-regulation in the grass species when grown in fertilized soil, even for those grasses typical of higher fertility soil, may suggest a taxonomic constraint on the plasticity of ammonium uptake.

Because whole root-system ammonium and nitrate uptake are the product of physiological uptake rates and the root mass of the system, root-system uptake should be positively correlated with physiological rates and root biomass. Interestingly, the relative strength of the correlations depended on soil fertility (Fig. 7). In the presence of a low concentration of nutrients, root-system ammonium uptake for each species at low and high concentrations of CO_2 was much more strongly correlated with physiological ammonium uptake (r^2=0.61, P=0.003) than with root biomass (r^2=0.38, P=0.03). In nutrient-rich soil, root mass became much more dominant in the root-system calculation (r^2=0.70, P=0.0006 for root mass; r^2=0.35, P=0.04 for physiological ammonium uptake). There was no evidence for any correlation between root mass and physiological ammonium uptake (r^2=0.0008 and 0.005 for unfertilized and fertilized treatments, respectively).

Plantago was the only species that did not increase its rate of root-system nitrogen uptake with CO_2 when fertilized (Fig. 6), and it showed one of the smallest root mass responses to fertilization (Fig. 3). Of the species examined here, *Plantago*, also showed the smallest total biomass response to CO_2 in nutrient-rich soil (Field et al., in preparation). It is not clear why the species was unable to respond to elevated CO_2 when nutrients were abundant, but these results suggest that *Plantago's* relative importance in this community could decline with CO_2 if nutrient availability increased. For the community treatment (*Plantago, Bromus*, and *Lasthenia*), uptake for the community was generally similar to or intermediate between the rates of the three component species. This suggests that, for this system at least, community uptake rates can be estimated from the knowledge of the physiology of individual species, without assuming strong interactions within species in the mixtures.

Although our study focused primarily on belowground phenomena, the strong interaction between CO_2 and nutrients we observed for root biomass is mirrored in the pattern observed for shoot biomass and nutrient concentrations (Field et al. 1995; C.B. Field, in preparation). Elevated CO_2 increased shoot biomass 15% an average in nutrient-rich soil, but only 5% at low nutrient concentrations, and there were no consistent changes in root:shoot ratios in elevated CO_2 at the end of the growing season. These results imply that the species were unlikely to respond very much to CO_2 unless additional nutrients were added. The lack of change at low nutrient concentrations of total root nitrogen (Table 2) or aboveground nitrogen pools implies the plants were unsuccessful in converting additional carbon into increased nitrogen uptake. Since nitrogen and other soil resources limit productivity in many terrestrial ecosystems, responses in natural systems may be smaller than predicted from studies of well fertilized plants (Williams et al. 1988; Idso et al. 1991; Norby et al. 1992).

Our study is, to our knowledge, the first to examine nutrient uptake kinetics in elevated CO_2, so we cannot address whether plants in general fail to up-regulate nutrient uptake with CO_2. Israel et al. (1990) showed that total nitrogen uptake during 27 days of soybean growth increased with CO_2, but nitrogen uptake efficiency (nitrogen uptake per unit root mass) was unchanged for the same time period. Rogers et al. (1992) showed similar results for an 18-day soybean experiment. Plants possess other physiological mechanisms for regulating nutrient uptake, including changes in mycorrhizal infection (Harley and Smith 1983; St. John et al. 1983) and increased exudation of nutrient-mobilizing compounds such as phosphatases and siderophores (Tarafdar and Jungk 1987; Treeby et al. 1989). Z.G. Cardon and R.B. Jackson (in preparation) examined root phosphatase activity for four of the species studied here, and found strong nutrient effects, but no CO_2 effect on phosphatase activity. Mycorrhizal responses to CO_2 have been examined more extensively. While ectomycorrhizal symbioses have frequently increased in elevated CO_2 (e.g. O'Neill et al. 1987), vesicular-arbuscular mycorrhizae have usually shown very limited or negligible responses to CO_2 (e.g. O'Neill et al. 1989; Whitbeck 1994; though see one species in Monz et al. 1994). To date, despite fairly consistent increases in belowground biomass and allocation with increased CO_2 (e.g. Rogers et al. 1994), there is little evidence for up-regulation in root physiology.

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