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Elevated CO_2 increases plant uptake of organic and inorganic N in the desert shrub *Larrea tridentata*

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Abstract Resource limitations, such as the availability of soil nitrogen (N), are expected to constrain continued increases in plant productivity under elevated atmospheric carbon dioxide (CO_2). One potential but under-studied N source for supporting increased plant growth under elevated CO_2 is soil organic N. In arid ecosystems, there have been no studies examining plant organic N uptake to date. To assess the potential effects of elevated atmospheric CO_2 on plant N uptake dynamics, we quantified plant uptake of organic and inorganic N forms in the dominant desert shrub *Larrea tridentata* under controlled environmental conditions. Seedlings of *L. tridentata* were grown in the Mojave Desert (NV, USA) soils that had been continuously exposed to ambient or elevated atmospheric CO_2 for 8 years at the Nevada Desert FACE Facility. After 6 months of growth in environmentally controlled chambers under ambient (380 μmol mol^{-1}) or elevated (600 μmol mol^{-1}) CO_2, pots were injected with stable isotopically labeled sole-N sources (^{13}C-[2]-^{15}N glycine, $^{15}NH_4^+$, or $^{15}NO_3^-$) and moved back to their respective chambers for the remainder of the study. Plants were destructively harvested at 0, 2, 10, 24, and 49 days. Plant uptake of soil N derived from glycine, NH_4^+, and NO_3^- increased under elevated CO_2 at days 2 and 10. Further, root uptake of organic N as glycine occurred as intact amino acid within the first hour after N treatment, indicated by ~1:1 M enrichment ratios of ^{13}C:^{15}N. Plant N uptake responses to elevated CO_2 are often species-specific and could potentially shift competitive interactions between co-occurring species. Thus, physiological changes in root N uptake dynamics coupled with previously observed changes in the availability of soil N resources could impact plant community structure as well as ecosystem nutrient cycling under increasing atmospheric CO_2 levels in the Mojave Desert.

Keywords Mojave Desert · ^{15}N · N uptake · Glycine · Growth chamber

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Introduction

Predicting plant and ecosystem responses to global changes requires a better understanding of the mechanisms controlling the acquisition of growth-limiting resources (Norby 1994; BassiriRad 2000). Enhanced growth due to increased photosynthetic rates under elevated atmospheric CO_2 is a common response in many woody plant species, but it is unclear how long this positive response can be sustained as resources become limiting. Thus, long-term positive growth responses can be expected only when the increased assimilation of carbon (C) is concomitant with that of soil nutrients, namely nitrogen (N) (BassiriRad et al. 1997; Stitt and Krapp 1999; Norby et al. 2001; Kimball et al. 2002).

One under-studied aspect of plant N nutrition in response to elevated atmospheric CO_2 is the uptake of soil organic N and its role in ecosystem N cycling. The uptake of soil organic N in the form of amino acids occurs in both mycorrhizal and non-mycorrhizal plants (Kielland 1994;

Raab et al. 1999; Lipson and Näsholm 2001). Organic N uptake is important in cold ecosystems with organic soils where inorganic N availability is highly constrained (Atkin 1996; Nordin et al. 2001; Persson et al. 2003; Schimel and Bennett 2004). Reports of significant plant organic N uptake in subtropical heaths (Schmidt and Stewart 1997), temperate grasslands (Bardgett et al. 2003), and temperate forests (Finzi and Berthrong 2005; Hofmockel et al. 2007), however, suggest that organic N uptake is also important in warmer ecosystems despite greater mineralization expected under higher temperatures (Jones et al. 2004; Schimel and Bennett 2004). In the Mojave Desert, total free amino-N concentrations under field conditions can be up to 20% of the total soil inorganic N during the growing season (Jin and Evans 2007). Further, more than 60% of total soil organic N in Mojave Desert soils is unbound, potentially hydrolysable proteins which could provide bioavailable amino compounds (Nadeau et al. 2007). No studies have considered the uptake of organic N by plants in hot arid ecosystems to date. Thus, the ubiquity of organic N uptake as a general mechanism of plant N acquisition is unknown. It is also unclear how plant N uptake generally will be affected by increasing atmospheric CO_2.

Woody shrubs are the dominant growth form in hot deserts of the southwestern USA and play a major role in potential responses of arid ecosystems to global change (Schlesinger et al. 1996; Reynolds et al. 1996). Aboveground productivity in the dominant desert shrub species creosotebush (*Larrea tridentata*) increases under elevated CO_2 (Smith et al. 2000), and is consistent with increases in N cycling rates and soil N availability in the Mojave Desert (Billings et al. 2002, 2004; Jin and Evans 2007; Schaeffer et al. 2007). The duration of enhanced plant productivity, however, is highly variable (Housman et al. 2006). This uncertainty in desert plant responses to elevated CO_2 limits our ability to predict the impacts of this global change driver on arid ecosystems, which cover >30% of land area worldwide (Noble et al. 1996).

In the present study, we examined N uptake responses of *Larrea tridentata* seedlings grown in ambient (380 μmol CO_2 mol^{-1}) or elevated CO_2 (600 μmol CO_2 mol^{-1}) under controlled environmental conditions. Our objective was to determine the effect of elevated CO_2 on the uptake of organic and inorganic N added as sole-N sources to plants grown in soils collected from the field. In addition to examining N uptake patterns, we also examined whether CO_2 and/or N treatments affected the plant growth and tissue N content over time. Plant communities in arid ecosystems are strongly controlled by interactions occurring belowground (Brisson and Reynolds 1997). Thus, understanding the effects of elevated CO_2 on root N uptake is critical for predicting both ecosystem- and community-level responses to this global change driver.

Materials and methods

Growth facility and conditions

Seeds of *Larrea tridentata* (Sessé and Moc. ex DC.) Coville (Comstock Seed, Gardnerville, NV, USA) were planted in plastic pots (5 cm diam. × 12 cm × 35 cm) filled with Mojave Desert soils that were collected from the Nevada Desert free-air carbon dioxide enrichment facility (NDFF), 15 km north of Mercury, NV, USA (36°49′N,115°55′W; elevation 965–970 m) (Jordan et al. 1999). Field soils (0–10 cm depth) were collected during dry conditions in October 2004 from plant interspaces in buffer areas immediately adjacent to experimental plots. Three replicate plots and their adjacent buffer areas have been continuously fumigated with either ambient atmospheric CO_2 (~380 μmol CO_2 mol^{-1}) or 1.5× ambient (~550 μmol CO_2 mol^{-1}) since 1997. Soils from each buffer area per treatment were sieved through a 1-cm mesh sieve and transported back to Washington State University. Soils were composited by CO_2 treatment and homogenized in a tumbler before filling pots. Pots were planted with *L. tridentata* seeds that had been leached under running water for 24 h to enhance germination.

Planted pots were placed in climate- and CO_2-controlled growth chambers (3 m × 2 m × 5 m; Enconair, Winnepeg, Canada). Pots with field soils that were exposed to ambient or elevated CO_2 in the field were assigned to chambers with ambient (380 μmol mol^{-1}) and elevated CO_2 (600 μmol mol^{-1}), respectively. Seeds were germinated under these conditions, and seedlings were grown for 6 months under a constant relative humidity of 70%. Ramping of temperature and light conditions simulated a 12-h photoperiod (daytime temperature = 35°C; nighttime temperature = 15°C; maximum photosynthetic photon flux density = 650 μmol s^{-1} m^{-2}). Pot locations within each chamber were shifted weekly, and CO_2 treatments alternated between the two chambers to minimize chamber effects. Plants were watered every other day, with a single deep watering each week to flush accumulated salts. Plants were fertilized every 2 months with 1:10 strength nutrient solution (15:30:15 N:P:K) to maintain seedlings until application of stable isotope labeling.

Isotopic labeling treatments and sample collection

The study was conducted as an incomplete factorial experiment with two CO_2 treatment levels (380 μmol mol^{-1}, 600 μmol mol^{-1}), four N treatments (no N, glycine, NH_4^+, NO_3^-), and two $^{13}CO_2$ labeling treatments (labeled, unlabeled). Plants were distributed in randomized complete blocks within each chamber. For each CO_2 treatment level, 25 replicate pots were randomly assigned

to each of the following treatments: (1) label control (no $^{13}CO_2$, no N); (2) N control ($^{13}CO_2$ added, no N); (3) unlabeled glycine ($^{13}CO_2$ added, N added); (4) ^{13}C-[2]-^{15}N glycine (no $^{13}CO_2$, N added); (5) $^{15}NH_4^+$ ($^{13}CO_2$ added, N added); or (6) $^{15}NO_3^-$ ($^{13}CO_2$ added, N added). Glycine was used as an analogue for soil organic N uptake because it has been used widely to examine organic N uptake in plant species from many ecosystems (Lipson and Näsholm 2001 and references therein) and because of the lack of amino acid-specific data about Mojave Desert soils. Glycine labeled at the 2-C position was selected over universally labeled glycine because the 1-C position is easily decarboxylated and lost via respiration (Näsholm and Persson 2001). Plants treated with isotopically labeled glycine to quantify intact amino acid uptake (treatment 4) were not labeled with $^{13}CO_2$ to avoid confounding glycine-^{13}C label with photosynthetically fixed ^{13}C. Experimental results from $^{13}CO_2$ labeling are reported elsewhere (Jin and Evans, in review).

Nitrogen treatments were injected into each pot with a 15-cm, 18-gauge side-port needle (Popper and Sons, Hyde Park, New York). Each pot received 12 mL of solution for each N treatment, adding 0.7 mg N per pot (99 atom percent ^{15}N). This amount corresponded to an expected increase of ~50% in soil total dissolved N (organic + inorganic) concentrations, based on measurements from field soils (Jin and Evans 2007). Label control and N control pots that received no N were injected with equivalent volumes of deionized water only. Pots were uniformly injected at four equidistant locations around the seedling (3 mL per injection), with syringes progressively emptied as needles were pulled from the soil. Aboveground tissues were kept covered during injections to prevent contact contamination of shoots. Plants in treatments 2, 3, 5, and 6 were then labeled with $^{13}CO_2$ for 4 h. Five replicate pots from each chamber per treatment were harvested over the course of the experiment (days 0, 2, 10, 24, 49). Day 0 plants for treatments 1, 3, 4, 5, and 6 were harvested within 1 h after injection and prior to $^{13}CO_2$ labeling. Day 0 plants for Treatment 2 were harvested within 1 h following $^{13}CO_2$ labeling.

Soil N pool size and estimating ^{15}N enrichment

Actual increases in soil N pools due to treatments were measured at each sampling time after plant harvest. All soil from each pot was transferred to a plastic bag and thoroughly homogenized. One 25-g subsample was extracted immediately with 100 mL 2 M KCl to determine soil N pool sizes of extractable NH_4^+ and NO_3^- using continuous flow colorimetry (Alpkem Autoanalyzer FS-3000; OI Analytical, College Station, TX) (Table 1). Glycine concentrations were not measured, so soil glycine pool concentrations in control soils were estimated conservatively as 1% of total inorganic N concentrations based on a review of available literature (Senwo and Tabatabai 1998; Jones et al. 2005b; Thompson et al. 2006; Amashukeli et al. 2007). Glycine concentrations in treated soils were estimated at ~1 μg N g^{-1} (i.e. 700 μg N added pot^{-1}/~700 g soil pot^{-1}), with negligible contribution from background soil glycine.

Pool ^{15}N enrichments for each N pool were calculated with a two end-member isotopic mixing model where (1) pool sizes for background N (i.e. control) and added N (i.e. treated) were measured from soil extracts, and (2) the ^{15}N composition of added N for all N forms was 99 atom percent. Background ^{15}N compositions for all N forms in control soils per CO_2 treatment were assumed to be equal to the average whole-plant δ^{15}N in control pots at day 0 ($\delta^{15}N_{ambient} = 5.9‰$; $\delta^{15}N_{elevated} = 5.6‰$). We made the assumption that all pools were equal to plant δ^{15}N because (1) whole-plant isotope composition integrates the δ^{15}N of all N sources when plant demand exceeds nitrogen supply, such as that expected under the control conditions (Evans 2001), and (2) potential differences in isotopic signatures between soil N pools at the natural abundance level were negligible relative to the ^{15}N label used. Variable enrichment in N pools was accounted for when calculating plant N uptake using an isotopic mixing model (Eq. 3, below).

Plant ^{15}N uptake calculations

Harvested plants were rinsed for 3 min with 0.5 mM $CaCl_2$ followed by a deionized water rinse to displace residual ^{15}N label on tissue surfaces. Plants were then separated into roots, stems, and leaves, flash-frozen in liquid N_2, lyophilized, and measured for dry weights. Dried plant tissues were ground into a fine powder to quantify C and N content and stable isotope composition (^{15}N, ^{13}C).

The fraction of plant tissue N derived from ^{15}N label at each harvest day was calculated with an isotopic mixing model (Robinson 2001) as:

$$x_{labeled} = \frac{\delta^{15}N_{sample} - \delta^{15}N_{control}}{\delta^{15}N_{label} - \delta^{15}N_{control}} \quad (1)$$

$$m_{labeled} = x_{labeled} m_{sample} \quad (2)$$

where $x_{labeled}$ is the fraction of label-derived ^{15}N in the tissue (unitless), and δ^{15}N is measured from the treated plant (i.e. sample), label, or control (i.e. background) plants at each harvest. The mass of label-derived ^{15}N in plant biomass at each harvest ($m_{labeled}$; μmol ^{15}N pot^{-1}) was calculated using Eq. 2, where m_{sample} is mass of total N in the plant (μmol $^{14+15}$N pot^{-1}). Molar concentrations of plant N were converted to mass (μg N pot^{-1}) for use in

Table 1 Soil N concentrations in day 0 control and treated soils, and estimated pool ^{15}N values in day 0 treated soils

CO_2 level	Control (mg N kg^{-1})	Treated (mg N kg^{-1})	N increase (%)	Est. pool ^{15}N (atom% ^{15}N)
Glycine–N				
Ambient	~0.03	~1.0	>1,000	96.2
Elevated	~0.04	~1.0	>1,000	95.1
NH_4^+–N				
Ambient	0.46 ± 0.04	2.23 ± 0.15	385	78.6
Elevated	0.36 ± 0.03	1.52 ± 0.09	322	75.8
NO_3^-–N				
Ambient	2.34 ± 0.59	4.28 ± 1.34	83	45.0
Elevated	2.79 ± 1.13	4.98 ± 0.96	78	43.9

Atom percent ^{15}N of added N was 99%, and initial glycine pool sizes were estimated as ~1% of total soil inorganic N concentrations

Eq. 3 by using atom% ^{15}N values corresponding with $\delta^{15}N$ values above.

A second isotopic mixing model was used to account for potential "fertilization effects" associated with adding the same amount of N to soil pools that differ in size (McKane et al. 2002). Plant uptake of background soil N that corresponded to treatment ^{15}N was calculated as:

$$m_{\text{background}} = m_{\text{labeled}} \frac{N_{\text{background}}}{N_{\text{labeled}}} \quad (3)$$

where N_{labeled} is the mass of ^{15}N-labeled nitrogen injected into the soil per treatment (g pot^{-1}), $N_{\text{background}}$ is the background mass of the target N pool measured in control soils (g pot^{-1}), $m_{\text{background}}$ is the total mass of background N taken up from the target N pool into plant biomass (mg pot^{-1}), and m_{labeled} is defined previously. We assumed that no fractionation occurred during N uptake. We also estimated background soil glycine pool concentrations as 1% of total inorganic N concentrations because glycine concentrations were not measured (Table 1).

To assess total N uptake over time, molar concentrations of the total N taken up were used to calculate specific absorption rates (SAR) of N in each N treatment (Eq. 4; Bailey 1999):

$$\text{SAR } (\mu\text{mol g dw}^{-1}\text{ day}^{-1}) = \frac{m_i - m_0}{D_i - D_0} \times \frac{\ln(R_0) - \ln(R_i)}{R_0 - R_i} \quad (4)$$

where m is plant N taken up from the target soil N pool ($m_{\text{background}} + m_{\text{labeled}}$; µmol pot^{-1}), D is day i, and R is root dry mass (g) at day i.

In addition to the SAR of glycine-N over time which reflected root uptake of both intact and mineralized glycine label, the fraction of glycine-N taken up that occurred as intact amino-acid N was calculated in plants treated with ^{13}C–[2]–^{15}N glycine by regressing excess molar concentrations of ^{13}C against ^{15}N in root tissue. The uptake of intact glycine molecules would be indicated by equivalent molar enrichments of ^{13}C: ^{15}N in root tissues (e.g. slope of 1) due to the 1:1 isotope label in the added glycine. Only day 0 plants were used to examine intact glycine uptake because day 0 plants were harvested within 1 h of labeling, minimizing the time for potential uptake of mineralized glycine. Day 0 plants were harvested before $^{13}CO_2$ labeling to ensure that all ^{13}C enrichment was derived from ^{13}C–[2]–^{15}N glycine only.

Statistical analysis

Two-way analyses of variance (ANOVA) were used to assess the fixed effects of CO_2 and N treatment on final plant biomass, root-to-shoot ratio (R:S), and tissue N contents. A three-way analysis of variance (ANOVA) was used to assess the fixed effects of CO_2, N treatment, and harvest day on SAR. Tissue nutrient concentrations and biomass were not different between label control and N control plants or between unlabeled glycine and ^{13}C–[2]–^{15}N glycine treatments. Plants were therefore pooled at each date ($n = 10$) for control and glycine treatments.

Data were tested for normality using the Shapiro–Wilk statistic and transformed when necessary. Post-hoc multiple comparisons between significant fixed treatment means were tested for differences using the Bonferroni-adjusted Fisher's least significant difference (LSD) procedure. Linear regressions were used to examine root uptake of intact glycine. All statistical tests were developed in consultation with the Statistics Department at Washington State University and performed using SAS 9.1 (SAS, Cary, NC, USA).

Results

Plant biomass and tissue N contents

Final plant biomass (g dw) was affected by both CO_2 and N treatments ($CO_2 \times N$ interaction; $P = 0.0044$).

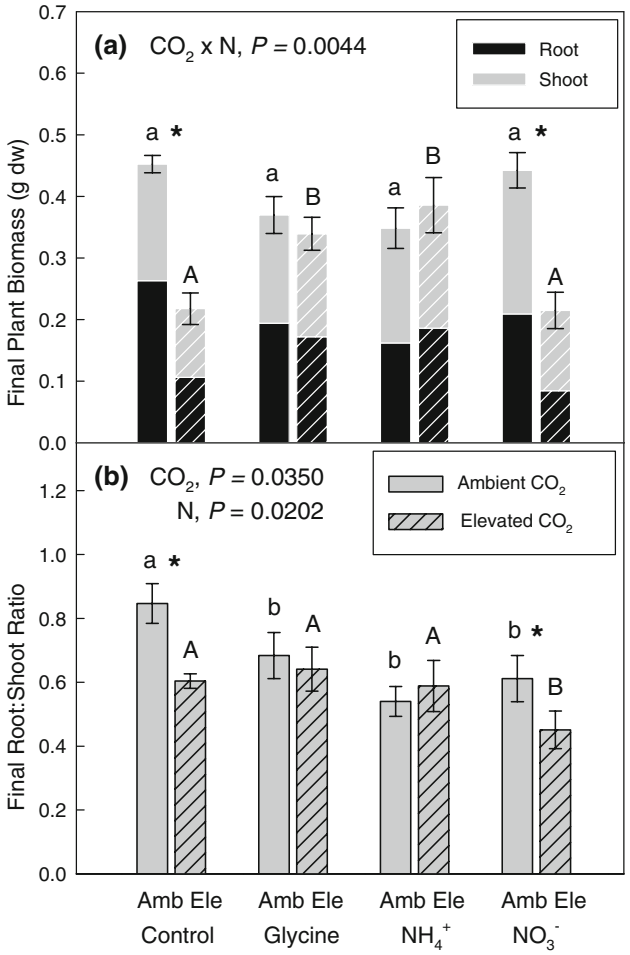

Fig. 1 Final values (day 49) for *Larrea tridentata* growing under ambient (*Amb*, *solid bars*) and elevated (*Ele*, *hatched bars*) CO_2 for **a** plant biomass (g dw), and **b** root:shoot ratio. Data are untransformed mean ± standard error. The standard error in panel **a** is shown for whole plant biomass. *Asterisks* indicate CO_2 treatment differences, and *different letters* indicate N-treatment differences ($P \leq 0.05$) for ambient (*lower case*) and elevated (*upper case*) CO_2 levels

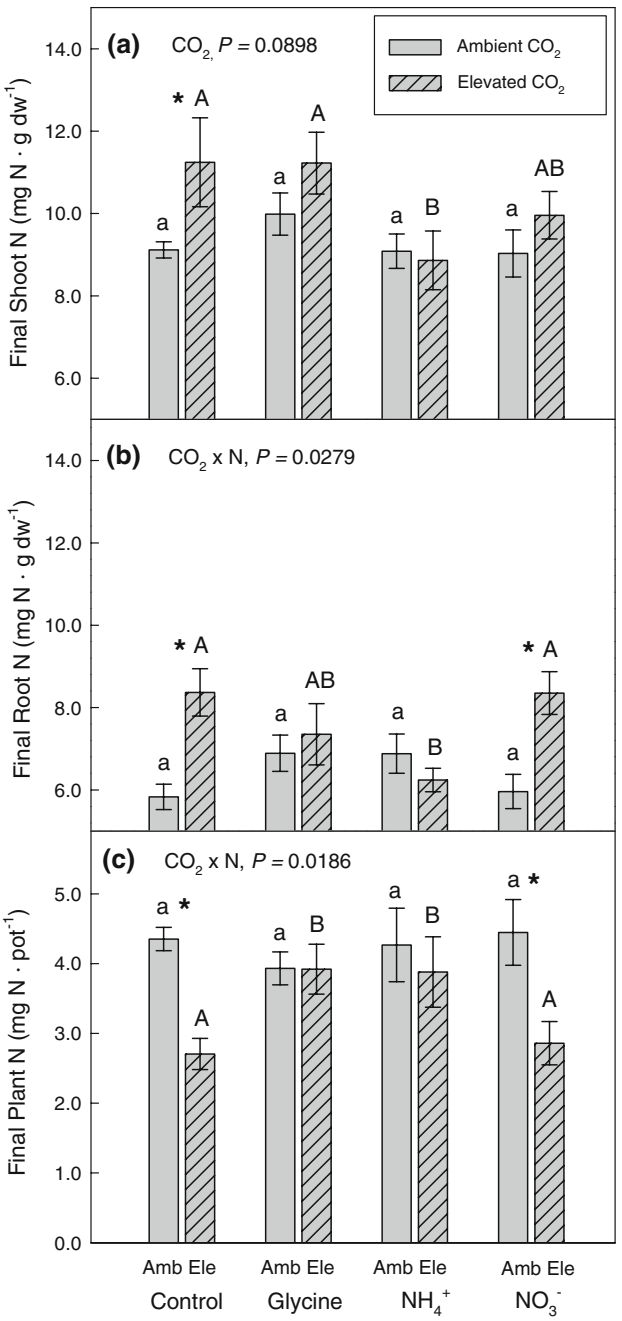

Fig. 2 Final values (day 49) for *Larrea tridentata* growing under ambient (*Amb, solid bars*) and elevated (*Ele, hatched bars*) CO_2 for **a** shoot N content (mg N g dw^{-1}), **b** root N content (mg N g dw^{-1}), and **c** total plant N (mg N pot^{-1}). Data are untransformed mean ± standard error. *Asterisks* indicate CO_2 treatment differences, and *different letters* indicate N-treatment differences ($P \leq 0.05$) for ambient (*lower case*) and elevated (*upper case*) CO_2 levels

Specifically, total plant biomass was not affected by N treatment in plants growing under ambient CO_2 (Fig. 1a), but elevated CO_2-grown plants had lower total biomass in control and NO_3^- treatments compared to glycine- and NH_4^+-treated plants. Final plant biomass in control and NO_3^--treated plants were lower under elevated CO_2 compared to ambient CO_2. Final R:S decreased under elevated CO_2 (CO_2 effect, $P = 0.0350$), and were also lower in NH_4^+ and NO_3^- treatments compared to control and glycine-treated plants (N effect, $P = 0.0202$) (Fig. 1b). A weak $CO_2 \times N$ treatment interaction ($P = 0.0931$) reflected lower R:S in all N-treated plants compared to controls under ambient CO_2 and in NO_3^--treated plants under elevated CO_2.

Shoot and root N content (mg N g dw^{-1}) and total plant N (mg N pot^{-1}) did not differ among N treatments in ambient-grown plants (Fig. 2a–c). Shoot N tended to increase under elevated CO_2 (CO_2 effect, $P = 0.0898$), and root N increased significantly in control and NO_3^--treated plants under elevated CO_2 compared to ambient CO_2 ($CO_2 \times N$ interaction; $P = 0.0279$). Shoot and root N

contents were lowest in NH_4^+-treated plants compared to other N treatments under elevated CO_2 only. Total plant N decreased in control and NO_3^--treated plants grown under elevated compared to ambient CO_2 ($CO_2 \times N$ interaction; $P = 0.0186$). Total plant N was also significantly higher in glycine- and NH_4^+-treated plants compared to the other N treatments under elevated CO_2.

Soil N pool size and ^{15}N enrichment

Initial background soil concentrations of NH_4^+–N and NO_3^-–N were not different between ambient and elevated CO_2 treatments for either N form. Relative increases in soil pool sizes differed between N forms and reflected initial differences in background soil N concentrations, even though the same amount of N was injected for each N treatment (Table 1). Measured increases in NH_4^+–N and NO_3^-–N pool sizes differed from expected increases ($\sim$1 μg N g^{-1}) which may have resulted from variation in total soil mass in pots and/or inconsistencies in N injection or soil homogenization. Relative increases in pool size between CO_2 treatments for each N form, however, were similar. Variable ^{15}N enrichments in N pools due to relative differences between background N and added N levels were accounted for when calculating plant N uptake.

Plant ^{15}N uptake of sole N sources

Specific absorption rates (SAR; μmol N g root dw^{-1} day^{-1}) of soil N varied by CO_2, N treatment, and day ($CO_2 \times N \times$ day interaction; $P = 0.0389$) (Fig. 3). SAR were highest for NO_3^- compared to glycine- and NH_4^+-treated plants under both CO_2 treatments on days 2, 10, and 24. SAR of glycine and NH_4^+ were not significantly different from each other within each CO_2 treatment level. SAR of all N forms was enhanced under elevated compared to ambient CO_2 at different times. Specifically, SAR was greater under elevated CO_2 compared to ambient CO_2 for N derived from glycine and NH_4^+ on day 2, and for glycine- and NO_3^--derived N on day 10. There were no differences in SAR of any N form under either CO_2 treatment by the end of the study period.

Regressions of glycine label-derived ^{13}C versus ^{15}N concentrations in *Larrea* roots within 1 h after N addition were not significant for either CO_2 treatment alone. For the two treatments combined, however, the regression was significant ($y = 1.033x + 125.7$, $R^2 = 0.4804$, $P = 0.0263$) (Fig. 4). The slope (1.033) approximated a $\sim$1:1 M enrichment ratio of ^{13}C:^{15}N, indicating that root uptake of glycine occurred as intact amino acid within the first hour after N treatment.

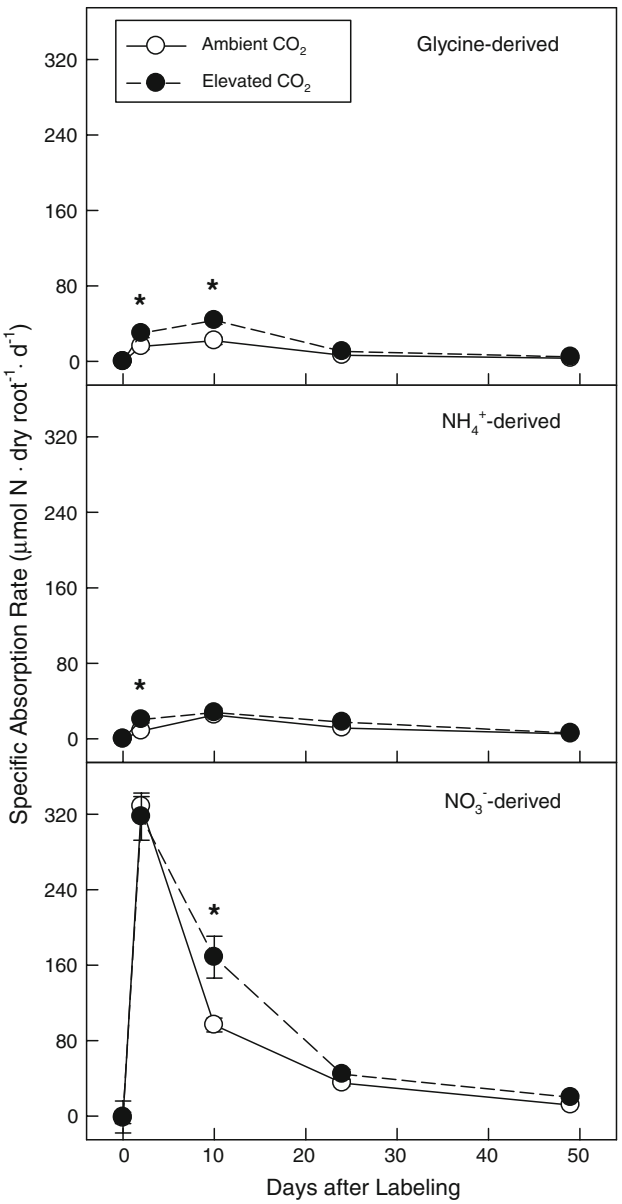

Fig. 3 *Larrea tridentata* specific absorption rates (SAR; μmol N g root dw^{-1} day^{-1}) of N derived from sole-N sources (background + label) over time. SAR of all N forms do not account for potential transformations of added N over time (i.e. glycine uptake likely reflects both uptake of glycine plus mineralized glycine). Data shown are untransformed mean ± standard error ($n = 5$). *Asterisks* indicate CO_2 treatment differences ($P \leq 0.05$)

Discussion

Short-term plant N uptake characteristics of *Larrea tridentata* seedlings were significantly altered by elevated CO_2. Elevated CO_2 positively affected root uptake N derived from all three N forms by day 10, with NO_3^--derived N taken up at the highest rates. In addition, added glycine was taken up as intact amino acid within 1 h of treatment application, indicating that *L. tridentata* can

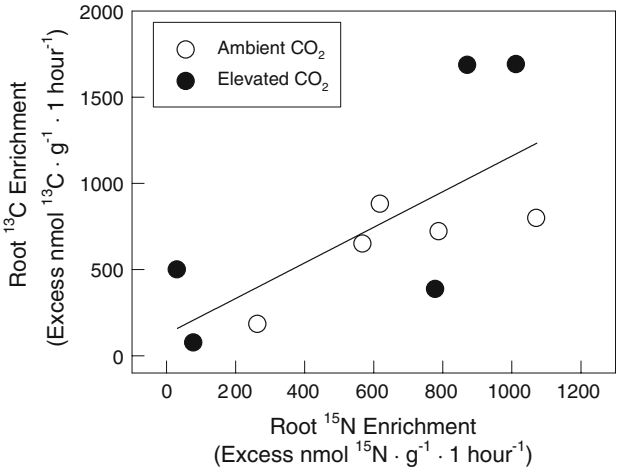

Fig. 4 Root concentrations of label-derived ^{13}C and ^{15}N (nmol g root dw^{-1}) by glycine-treated *L. tridentata* at day 0 under ambient and elevated CO_2 ($y = 1.033x + 125.7$, $R^2 = 0.4804$, $P = 0.0263$)

directly utilize soil organic N sources. To date, this study is the first to report organic N uptake by a plant species from a hot, arid ecosystem. While pool sizes of soil glycine were not measured directly here, assuming very low background concentrations of soil glycine coupled with the relatively large pool of labeled glycine injected provided the most conservative estimates plant glycine uptake to use in comparisons between N treatments and CO_2 levels. The level of added glycine used in this pot study likely increased available soil glycine concentrations many-fold, potentially saturating microbial uptake and decreasing microbial competition for this resource (Jones et al. 2005a). Plant uptake of intact amino acid, however, demonstrates the potential for increases in organic and inorganic N uptake to affect resource acquisition in *L. tridentata* in response to elevated atmospheric CO_2.

Organic N uptake has been studied extensively in arctic, boreal, alpine, and temperate ecosystems where plant uptake of amino acids from highly organic surface soils is important in influencing plant community structure and ecosystem N cycling (Schimel and Chapin 1996; McKane et al. 2002; Kielland et al. 2006; Finzi and Berthrong 2005). There is increasing consensus that organic N uptake could be a major plant N acquisition pathway (Lipson and Näsholm 2001; Schimel and Bennett 2004), with 10–90% of the total annual plant N requirement potentially met by the uptake of external soil organic N (Chapin et al. 1993; Kielland 1994; Jones and Darrah 1994). While organic N uptake likely includes some recapture of organic N lost through root exudation (Jones et al. 2005a, b), the proportion of amino acid-N resources taken up as intact organic molecules can range from 5–11% in arctic marsh species (Henry and Jeffries 2003), 12–52% in temperate grassland species (Näsholm et al. 2000; Bardgett et al. 2003), and 42–91% in various boreal forest species (Näsholm et al. 1998). In the present study, we found that ~100% of the glycine taken up by *L. tridentata* occurred as intact amino acid within the first hour after injection despite the absence of highly organic soils in this desert ecosystem.

Arid desert soils are generally depauperate in organic matter although soil microsites under shrubs can have greater organic matter content (i.e. "fertile island" effect) (Gallardo and Schlesinger 1992; Schlesinger et al. 1996; Titus et al. 2002). Total dissolved organic N or free amino-N, however, can make up 10–20% of total soil inorganic N concentration, respectively, in plant interspace as well as shrub microsites (Billings et al. 2004; Jin and Evans 2007). In addition, long-term exposure to elevated CO_2 has altered the quality and quantity of plant-derived C inputs into Mojave Desert soils, leading to higher extracellular enzyme activities indicative of a greater or more active soil fungal component (Jin and Evans 2007). Increased soil fungi may lead to the greater release of monomeric organic N under elevated CO_2, enhancing substrate availability for soil microbes as well as for plant uptake. In this study, we estimated that soil glycine concentrations were approximately 1% of total soil inorganic N (which did not differ between CO_2 treatments). The increase in plant glycine uptake observed under elevated CO_2 compared to the ambient treatment, therefore, may reflect greater soil glycine availability under elevated CO_2 as suggested above, but such conclusions cannot be supported unequivocally in the absence of soil glycine measurements. Regardless, *Larrea tridentata* covers ~20% of the land area in the Mojave Desert and has roots that extend beyond 5 m horizontally and vertically from the stem base (Jordan et al. 1999; Hartle et al. 2006). The direct uptake of monomeric organic N by *L. tridentata*, therefore, could play a significant role in plant nutrition and soil N cycling at the landscape scale. Further, our study suggests that organic N could become even more important in pulse-driven desert ecosystem N processes because the direct, short-term uptake of organic N or mineralized N derived from labile organic compounds by *L. tridentata* is enhanced under elevated atmospheric CO_2.

In the present study, *Larrea tridentata* grown in reconstituted field soils under elevated CO_2 had higher short-term SAR of both organic and inorganic N, but previous studies have reported decreased rates of NO_3^- uptake under elevated CO_2 (BassiriRad et al. 1997, 1999). The relative increase in N pool sizes due to N injections used here likely resulted in some fertilization effects, particularly for glycine and NH_4^+ uptake (Table 1). Potential fertilization effects may have contributed to greater whole plant biomass and whole plant N for these two N forms under elevated CO_2, as well as led to the expected general

decreases in root-to-shoot ratios in N-treated plants (BassiriRad et al. 2001). Potential fertilization effects, however, did not translate to overall increases in shoot or root N concentrations in N-treated plants relative to controls. Rather, tissue N concentrations increased under elevated CO_2 (control, NO_3^--treated plants), in contrast with other studies on *L. tridentata* (BassiriRad et al. 1997; Huxman et al. 1999) and other woody species (Constable et al. 2001). Tissue N concentrations are typically diluted by increased growth under elevated CO_2, so the increased tissue N concentrations in the present study were likely due to the decreased growth in control and NO_3^--treated plants.

Increased N uptake has been observed along with strong positive responses in the productivity of various temperate forest ecosystems to elevated CO_2, primarily due to increases in fine root production (Mikan et al. 2000; Finzi et al. 2007). Greater root growth can lead to a higher capacity for acquiring limiting soil resources, but increases in root biomass may not adequately reflect the actual root uptake capacity of nutrients (Chapin 1980; BassiriRad et al. 1997). In field-grown mature *L. tridentata*, increases in aboveground productivity under elevated CO_2 (Smith et al. 2000; Housman et al. 2003, 2006) were not accompanied by any changes in fine root biomass, resulting in decreased root-to-shoot ratios (Phillips et al. 2006). Root biomass of greenhouse-grown *L. tridentata* seedlings also show no response to elevated CO_2, with concomitant decreases in plant root-to-shoot ratios (BassiriRad et al. 1997). Similarly, we found decreases in root-to-shoot ratios in both control and NO_3^--treated plants under elevated CO_2. These reductions, however, resulted from significant decreases in root biomass rather than increases in shoot growth. It is unclear whether decreases in root biomass in control and NO_3^--treated plants under elevated CO_2 occurred due to decreased allocation to belowground growth or because of increases in other root C losses via increased root respiration or root exudation. While root respiration and exudation are beyond the scope of this study, Phillips et al. (2006) speculated that, in the absence of increased root growth or root respiration in field-grown *L. tridentata*, increased root exudation or allocation to mycorrhizal growth could contribute to the absence (or decrease, in this study) of root growth responses under elevated CO_2. Although root proliferation can be a major factor affecting resource acquisition in Larrea (BassiriRad et al. 1999), significant increases in root N concentrations in control and NO_3^--treated plants under elevated CO_2 observed in this study suggest that the up-regulation of nutrient uptake is as important for supporting enhanced aboveground productivity in arid ecosystems under elevated atmospheric CO_2.

Even very small changes in the spatial and temporal distribution and/or composition of N resources could impact plant growth responses to elevated CO_2 in resource-poor, arid ecosystems such as the Mojave Desert (Smith et al. 1997; Hamerlynck et al. 2004). Field constraints on seedling establishment in arid ecosystems can be relaxed under higher atmospheric CO_2 because of improved growth, gas exchange, and/or drought tolerance (Polley et al. 1996), but species-specific changes in the uptake of soil N resources may also affect seedling survival. The up-regulation of both organic and inorganic N uptake in seedlings of the dominant desert shrub, *L. tridentata*, suggests that root physiological responses to increasing atmospheric CO_2 could alter competitive belowground interactions, potentially shifting plant community compositions (BassiriRad et al. 1997; Berntson and Bazzaz 1998; BassiriRad 2000). Ultimately, the long-term effects of elevated atmospheric CO_2 in this Mojave Desert ecosystem will depend on how changes in productivity will interact with predicted shifts in precipitation to affect the feedbacks between soil nutrient availability and plant growth (BassiriRad et al. 1999; Barker et al. 2006; Housman et al. 2006).

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