

# Carbon allocation in *Larrea tridentata* plant-soil systems as affected by elevated soil moisture and N availability

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## Abstract

**Background and Aims** Global change will likely express itself in southwestern United States arid lands through changes in amounts and timing of precipitation in response to elevated CO<sub>2</sub> concentrations. In addition, increased nitrogen (N) deposition may occur due to increased urban development. This study addressed the effects of water and N availability on C allocation in arid land soil-plant systems.

**Methods** Columns filled with Mojave Desert topsoil containing *Larrea tridentata* seedlings with two treatment levels each of N and soil moisture were labeled by exposure to <sup>13</sup>C-enriched CO<sub>2</sub>.

**Results** Increased soil moisture increased plant biomass, total <sup>13</sup>C uptake, <sup>13</sup>C levels in leaves, soil organic matter, and soil respiration, decreased relative C allocation to stems but increased allocation to soil organic matter. Increased soil N availability increased N uptake but decreased C allocation to soil respiration presumably due to decreased substrate supply for microbes. There was no detectable label in carbonate C, suggesting that this pool does not significantly contribute to ecosystem C fluxes.

**Conclusions** Our study indicates that increased water availability causes increased C uptake with increased C allocation to soil organic matter in *Larrea tridentata*-dominated communities while increased N deposition will have a minimal impact on C sequestration.

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## Introduction

Rising atmospheric CO<sub>2</sub> concentrations are predicted to cause an increase in atmospheric temperature by 4–5 °C in the next century in the arid lands of the southwestern U.S. while mean precipitation (PPT) is expected to decrease, with an increase in severity and frequency of droughts (Seager et al. 2007; Cayan et al. 2010; Fawcett et al. 2011; Notaro et al. 2012). In northern U.S. drylands, PPT is expected to change from a winter to a fall/spring-dominated regime (Solomon et al. 2007),

thereby shifting the timing of available moisture. In addition, high-intensity storms likely will become more common (Archer and Predick 2008). Climate change predictions are however highly uncertain for U.S. drylands both in terms of magnitude and direction of PPT as the processes responsible for climate forcing are poorly understood in this region (Friggens et al. 2012). Since biological processes in drylands typically are constrained by moisture availability, any changes in PPT amount and distribution likely will have important implications for C cycling in these ecosystems (e.g., Weltzin et al. 2003; Austin et al. 2004; Huxman et al. 2004). In addition to changes in PPT, ecosystem processes in the western U.S. may be impacted by rapid development of urban areas causing large increases in nitrogen (N) deposition in this region (e.g., Noy-Meir 1973; Smith et al. 1997), with deposition estimates ranging from 29 to 45 kg ha<sup>-1</sup> yr<sup>-1</sup> downwind from major cities (Fenn et al. 2003).

Despite arid regions covering a substantial portion of the earth's surface ( $\pm 40$  %; Reynolds 2001), relatively little is known about the role of these regions in the global C cycle and how their role might change in response to environmental change. Recently, several studies have suggested that arid ecosystems may represent an important sink for C (Jasoni et al. 2005; Wohlfahrt et al. 2008; Xie et al. 2009; Stone 2008). Potential C sinks that could contribute to C uptake include net primary productivity (e.g., Miller and Huenneke 1996; Rundel and Gibson 1996), biological crusts (Su et al. 2012; Li et al. 2012), and inorganic C (soil carbonates; Emmerich 2003; Xie et al. 2009); but none of these uptake mechanisms either by themselves or collectively appears to be large enough to explain uptake quantities obtained by ecosystem flux measurements (Schlesinger et al. 2009).

Generally, increases in resource availability appear to favor allocation of C from roots to leaves (e.g., Hunt et al. 1996; Poorter and Nagel 2000). Consequently, as resource limitations change, belowground C allocation may change as well which could affect soil C cycling through potential changes in root litter production and priming of soil organic matter decomposition (e.g., Dijkstra and Cheng 2007). Several studies show that plant photosynthesis as well as water and nutrient uptake in arid ecosystems increase in response to increased water and/or N availability (e.g., BassiriRad et al. 1999; Hamerlynck et al. 2000; Gebauer and Ehleringer 2002; Schwinning et al. 2002, 2005a, b; Snyder et al. 2004; Barker et al. 2006), but it is unclear how these changes affect total C uptake and/or C allocation.

Barker et al. (2006) showed increased photosynthetic activity following increases in summer rainfall and N deposition in a Mojave Desert ecosystem. This increased C uptake, however, was not accompanied by significant changes in aboveground biomass (Newingham et al. 2012) or fine root production (Verburg et al. 2013). Similarly, Clark et al. (2010) observed that fine root production and root respiration did not increase under elevated CO<sub>2</sub> concentrations despite measuring increased photosynthetic activity (Smith et al. 2000; Housman et al. 2006). They speculated that the extra C taken up may be released as root exudates since C-limited microbial processes were stimulated under elevated CO<sub>2</sub> (Billings et al. 2004; Jin and Evans 2007). These results suggest that the increased C uptake may not necessarily result in increased C sequestration in biomass as C may be respired at higher rates as well (e.g., Huxman et al. 2004; Cable et al. 2011). None of these studies assessed potential changes in C allocation to soil organic C pools in response to changes in resource availability.

Although responses of arid lands to changes in water and N availability appear modest, timing of resource availability can be critical. For instance, several studies show that plant production in the arid lands increased with increased spring moisture availability as most above- and belowground biomass production occurs during this period (e.g., Reynolds et al. 1999; Schwinning et al. 2005b; Phillips et al. 2006; Verburg et al. 2013). Consequently, for these ecosystems, changes in timing of resource availability in response to climate change may have a significant impact on C sequestration.

One mechanism that could potentially be important in arid lands that has received recent attention is sequestration of C in secondary carbonates (e.g., Stone 2008). In many arid soils, carbonate C is the largest C reservoir (Eswaran et al. 2000; Lal and Kimble 2000). Several field and laboratory studies have indicated a potential contribution of carbonate C to ecosystem C fluxes (e.g., Xie et al. 2009; Kuzyakov et al. 2006; Emmerich 2003; Stevenson and Verburg 2006). In contrast, Serna-Perez et al. (2006) found little evidence of C originating from carbonates in soil respiration measured in calcareous soils in New Mexico. Lapenis et al. (2008) proposed that carbonate dissolution and subsequent leaching could account for CO<sub>2</sub> uptake of 2.1–7.4 g C m<sup>-2</sup> yr<sup>-1</sup>, while Marion et al. (2008) suggested that sequestration rates of carbonate-C would be as low as 0.2 g m<sup>-2</sup> yr<sup>-1</sup>.

indicating a minor contribution to net ecosystem C exchange.

The main objective of our study was to quantify the effects of changes in water and N availability on C allocation in native desert shrubs under controlled chamber conditions using isotopic tracers. We exposed plants to  $^{13}\text{C}$ -enriched  $\text{CO}_2$  which allowed us to trace recently assimilated C through plant and soils and allowed us to detect relatively small changes in C flows in response to experimental treatments (e.g., Gorissen et al. 1995; Sangster et al. 2010). This approach is more sensitive than measuring total C in plants and soils since small changes in total C content are often difficult to detect against large background amounts of C in plants and soils especially when plants are slow-growing. We specifically hypothesized that (1) total net C uptake increases under increased soil moisture conditions; (2) relatively less C is being allocated belowground with increasing moisture levels, but absolute amounts may increase depending on the total increase in biomass; (3) increased N availability does not affect C allocation unless water availability increases as well; and (4) inorganic C can contribute to (belowground) C fluxes.

## Materials and methods

### Experimental setup

We used *Larrea tridentata* (DC.) Coville plants obtained from a nursery in the fall of 2006. The plants were approximately 20 cm tall. During the spring of 2007, 40 seedlings were transplanted into PVC columns (diameter 152 mm; height 250 mm) containing a mixture of 50 % Mojave Desert topsoil (0–20 cm) and 50 % Hydroton<sup>®</sup> extruded clay pebbles by volume. The soil was collected from the Mojave Global Change Facility (MGCF) located on the Nevada Test Site in the northern Mojave Desert, Nye County, Nevada (36°49'N, 115°55' W). Surface soils at MGCF are coarse-textured; gravel content is 40 % of the whole soil mass while the <2 mm fraction is dominated by sand (90 %). Soils are alkaline derived from calcareous alluvium (pH=8.2), and low in organic matter (organic C=1.0 %; inorganic C=2.5 %; Stevenson and Verburg 2006; Marion et al. 2008). The soils were sieved at 2 mm and homogenized. Given that size of the gravel varied widely we added Hydroton<sup>®</sup> to account for gravel content and to have a more even distribution of gravel material between the soil-plant

columns. Soil crust material was mixed within the soil and not left intact.

To assess the effect of moisture on C allocation, gravimetric moisture content in 20 columns was kept at 7 % (w/w), while moisture content in the remaining 20 columns was kept at 15 % (w/w). The amount of moisture was adjusted weekly by weighing the columns. We used de-ionized water for our watering treatments. While soil moisture content is highly variable in the field, we chose to keep moisture constant throughout the experiment but used levels similar to those observed in the field following average and wet winters (Holmes 2009). Consequently, these treatments would reflect conditions of both increased spring and summer moisture. Ten low and ten high moisture columns received 36.4 mg N, equivalent to 40 kg ha<sup>-1</sup> yr<sup>-1</sup> to simulate increased N deposition. This amount was chosen because it is similar to the amount of N added in a companion field study at the MGCF (Barker et al. 2006; Verburg et al. 2013) and reflects current N deposition rates in the vicinity of Los Angeles, California (Bytnerowicz and Fenn 1996). The N was added as 38-0-0 slow-release fertilizer (NITROFORM<sup>®</sup>). The remaining 20 columns acted as ambient N controls. The columns were randomly assigned to the water and N treatments. In May 2007, all columns were covered with a PVC lid. The lids were sealed with silicone rubber (Q3-3481; Dow Chemical) at the base of the plants to separate the shoot from the root and soil compartment. Each lid had a PVC tube (diameter 2 cm, length 10 cm) containing two layers of soda lime separated by a cotton plug allowing for free exchange of oxygen and trapping  $\text{CO}_2$  evolved from the soil surface (soil respiration). Approximately 8 g of soda lime were used in the lower layer to capture soil respiration. The upper layer, containing 6 g soda lime, prevented entrance of  $\text{CO}_2$  from the surrounding air (Verburg et al. 1998). A second tube (diameter and length 1 cm) acted as an access port to allow for watering the plants. This tube was sealed with a rubber stopper in between watering.

At the end of May 2007, the columns were divided over two growth chambers with each chamber containing columns subjected to all water and N treatment combinations. The  $\text{CO}_2$  concentrations inside the chambers were kept at 380  $\mu\text{l l}^{-1}$   $\text{CO}_2$  and temperatures were set at 25°C during the day and 15°C during the night. Temperature settings were triggered by ambient light levels. Maximum photon flux density was 1,866  $\mu\text{mol}$

photons  $\text{m}^{-2} \text{s}^{-1}$  during the middle of the day. To avoid chamber effects, plants were rotated between growth chambers on a weekly basis. Once per week, 20 plants from one chamber were put inside a UV-transparent acrylic chamber. To allow for tracking uptake and allocation of recently assimilated  $\text{CO}_2$ , we injected ten cc of pure  $^{13}\text{C}$ - $\text{CO}_2$  into the chamber using a syringe through a rubber septum, and the  $\text{CO}_2$  was allowed to assimilate for 2 h. The air inside the chamber was circulated using a small fan. The same plants were labeled each week. The 20 remaining plants were used as unlabeled controls.

To quantify the amount of C released from the autotrophic (root) and heterotrophic (microbial) respiration, we measured  $\text{CO}_2$  respiration bi-weekly throughout the study; and the amount of respired  $\text{CO}_2$  was calculated based on the weight increase in soda lime (e.g., Verburg et al. 1998; Serna-Perez et al. 2006). Samples were pooled to have three measurement periods during the experiment. Isotopic values of the respired  $\text{CO}_2$  were determined by dissolving the soda lime in concentrated phosphoric acid in an air-tight 250 ml glass jar and sampling the headspace of the jar after all soda lime was dissolved. We used fresh soda lime to account for any  $\text{CO}_2$  that may have been absorbed prior to adding the soda lime to the traps.

After being in the growth chambers for 6 months, plants were harvested and separated into leaves, shoots, and roots. All plant materials were weighed, and plant materials and soils were analyzed for C, N (plants only) and  $\delta^{13}\text{C}$  values to calculate C allocation in the different plant and soil compartments. Isotopic composition of soil organic C was measured after removal of carbonates using 1 M phosphoric acid. Total C, N (plants only) and isotopic values of vegetation material, respired  $\text{CO}_2$ , and organic C were measured at the Colorado Plateau Stable Isotope Facility at Northern Arizona University (Flagstaff, AZ). To estimate the amount of C incorporated in the inorganic C pool isotopic composition of inorganic C was measured at the University of Wyoming Stable Isotope Facility (Laramie, WY). Isotopic composition was reported as  $\delta^{13}\text{C}$ , and total  $^{13}\text{C}$  uptake of the label was calculated by using isotope ratio and total C data correcting for the amounts of  $^{13}\text{C}$  present in the unlabeled plant-soil systems (Verburg et al. 1998, 2004).

#### Data analysis

Our study included four experimental treatments: (1) low moisture and low N ( $-W-N$ ); (2) low moisture

and high N, ( $-W+N$ ); (3) high moisture and low N, ( $+W-N$ ); and (4) high moisture and high N ( $+W+N$ ) with each treatment initially having five replicates within each labeling treatment. During the experiment a five out of 40 plants died, but each treatment had a minimum of four replicates at the end of the study. We used analysis of variance (ANOVA) to assess effects of water and N treatments on measured parameters for the labeled plants. To test whether or not the isotope label had been incorporated in plants and soils, we used t-tests between control and labeled plant-soil columns. Patterns in respired  $\text{CO}_2$  as well as their isotopic composition were analyzed separately for control and labeled chambers using a repeated-measures ANOVA. Statistical analyses were carried out using DataDesk version 6.0.

## Results

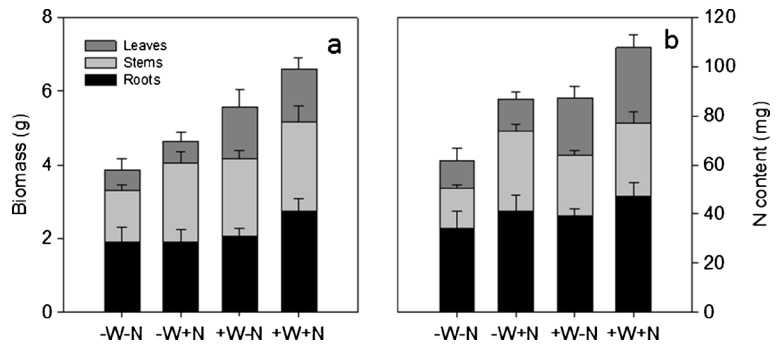
### Plant biomass and total soil respiration

Soil moisture did not affect stem or root biomass, but average leaf biomass increased by 140 % and total biomass increased by 36 % at higher soil moisture levels (Fig. 1). In both cases the increases were marginally significant ( $p=0.053$  for leaf biomass and  $p=0.073$  for total biomass; Table 1). Average stem biomass increased by 31 % while average total biomass increased by 21 % at higher levels of N availability (Fig. 1) but neither of the increases were significant (Table 1). Average shoot/root ratios increased by 47 % with higher N availability in the low soil moisture treatment ( $p=0.092$ ; Table 1).

Leaf N content significantly increased by 127 % while total plant N content tended to increase by 32 % ( $p=0.091$ ) at higher moisture availability (Fig. 1). Stem N content significantly increased by 52 % and total plant N content tended to increase by 31 % ( $p=0.099$ ) with increasing N availability.

Total soil respiration rates significantly decreased through time (Fig. 2). The N treatment did not significantly affect total soil respiration (Table 1), but the water treatments tended to affect respiration through time as indicated by a marginally significant interaction ( $p=0.062$ ) between “ $\text{H}_2\text{O}$ ” and “time” (not shown) with soil respiration being 60 % higher during the second and 78 % higher during the last time period in the high water treatments (Fig. 2).

**Fig. 1** Biomass (a) and N content (b) of *Larrea tridentata* in response to water and N addition treatments. ‘-W-N’=low water, low N, ‘-W+N’=low water, high N, ‘+W-N’=high water, low N, and ‘+W+N’=high water and high N. Error bars represent standard errors for roots, stems and leaves



Isotopic values of plants, soils and soil respiration

The labeling significantly increased  $\delta^{13}C$  values of leaves by 254‰ ( $p < 0.001$ ), stems by 92‰ ( $p < 0.001$ ), roots by 77‰ ( $p < 0.001$ ), and soil organic C by 2‰

( $p < 0.001$ ); but inorganic  $\delta^{13}C$  values were not significantly affected by the labeling ( $p = 0.701$ ; Figs. 3 and 4). None of the treatments affected isotopic values of the stems and leaves, but the  $\delta^{13}C$  values of the roots tended to be higher ( $p = 0.090$ )

**Table 1** Analysis of variance results for biomass and isotopic data using water (H<sub>2</sub>O) and N as main factors

	Leaf	Stem	Roots	Total	S/R	OC	IC	Resp
<b>Biomass<sup>a</sup></b>								
H <sub>2</sub> O	0.053	0.174	0.222	0.073	0.114	–	–	0.131
N	0.922	0.149	0.395	0.347	0.458	–	–	0.951
H <sub>2</sub> O*N	0.998	0.555	0.397	0.896	0.092	–	–	0.183
<b>N content<sup>b</sup></b>								
H <sub>2</sub> O	<b>0.013</b>	0.396	0.423	0.091	0.111	–	–	–
N	0.380	<b>0.007</b>	0.281	0.099	0.125	–	–	–
H <sub>2</sub> O*N	0.627	0.123	0.959	0.842	0.378	–	–	–
<b><math>\delta^{13}C^c</math></b>								
H <sub>2</sub> O	0.940	0.297	0.090	–	–	<b>0.005</b>	0.202	<b>0.013</b>
N	0.261	0.392	0.358	–	–	0.467	0.694	0.892
H <sub>2</sub> O*N	0.678	0.463	0.666	–	–	0.443	0.840	0.508
<b>Net <sup>13</sup>C uptake<sup>d</sup></b>								
H <sub>2</sub> O	<b>0.039</b>	0.139	0.101	<b>0.009</b>	0.935	<b>0.002</b>	–	<b>0.017</b>
N	0.415	0.128	0.190	0.309	0.617	0.332	–	0.839
H <sub>2</sub> O*N	0.518	0.781	0.462	0.531	0.354	0.762	–	0.462
<b><sup>13</sup>C allocation<sup>e</sup></b>								
H <sub>2</sub> O	0.346	<b>0.019</b>	0.318	–	–	<b>0.003</b>	–	0.172
N	0.491	0.413	0.523	–	–	0.180	–	<b>0.029</b>
H <sub>2</sub> O*N	0.678	0.518	0.605	–	–	0.258	–	0.165

Significant effects ( $p < 0.05$ ) are given in bold. Trends ( $0.1 > p > 0.05$ ) are shown in italics

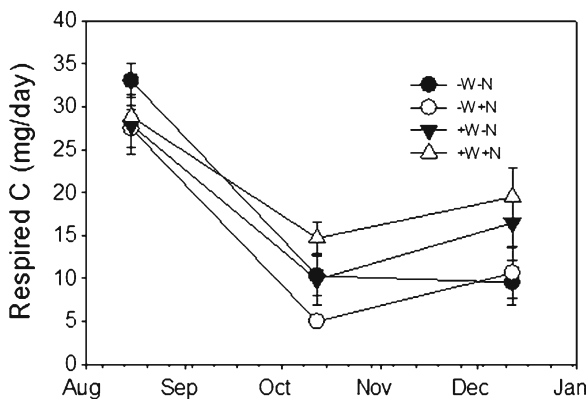
<sup>a</sup> Leaf, stem and root biomass in total grams, soil respiration in mgC/day

<sup>b</sup> Leaf, stem and root N content in mg/plant

<sup>c</sup>  $\delta^{13}C$  in ‰

<sup>d</sup> Net <sup>13</sup>C uptake in mg <sup>13</sup>C

<sup>e</sup> <sup>13</sup>C allocation in %



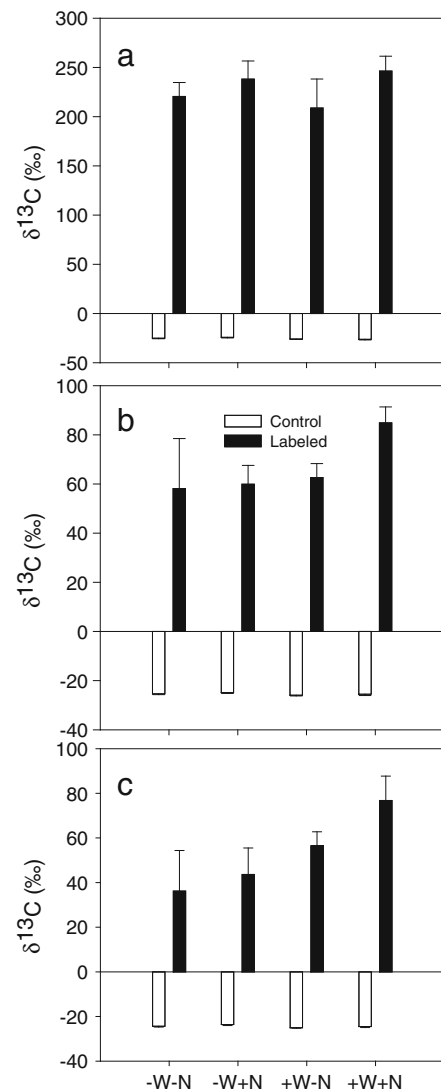
**Fig. 2** Soil-respired  $\text{CO}_2$  from soil-plant systems captured by soda lime in response to water and N addition treatments. ‘-W-N’=low water, low N, ‘-W+N’=low water, high N, ‘+W-N’=high water, low N, and ‘+W+N’=high water and high N

in high water treatments (66.6%) compared to the low water treatment (39.9%).

The  $\delta^{13}\text{C}$  values of soil organic C significantly increased with increasing soil moisture from  $-21.6$  to  $-19.8\text{‰}$  in the labeled plants, but the N treatments had no effect on the isotopic value of soil organic C. None of the treatments affected the  $\delta^{13}\text{C}$  of inorganic C. Isotopic values of the respired  $\text{CO}_2$  significantly increased from  $-35.4$  to  $-31.0\text{‰}$  through time in the unlabeled plants with respiration being  $0.4\text{‰}$  higher in the low moisture treatment at the end of the study. In the labeled treatments, isotopic values of respired  $\text{CO}_2$  significantly increased through time from  $-29.0$  to  $-10.2\text{‰}$ , with values being  $10\text{‰}$  higher in the high water treatments at the end of the study (Fig. 5).

#### Net $^{13}\text{C}$ uptake and $^{13}\text{C}$ allocation

Total net  $^{13}\text{C}$  uptake was about twice as high for the high moisture treatment with the increase mainly due to larger amounts being allocated to leaves, soil organic C, and soil respiration (Fig. 6). The  $^{13}\text{C}$  shoot/root ratio was not affected by any of the treatments, however. Relative C allocation to stems decreased from 27 to 17%, while allocation to soil organic C increased from 10 to 25% under higher soil moisture conditions (Fig. 6). In addition, relative C allocation to soil respiration decreased from 32 to 20% in the high N treatment. Specific uptake, defined as total  $^{13}\text{C}$  uptake per unit leaf biomass, increased from  $3.34$  to  $6.41 \mu\text{g g}^{-1}$  leaf biomass in the high moisture treatments but was not affected by the N treatment (Fig. 7). Since none of the  $^{13}\text{C}$  label was incorporated into the inorganic C pool, we



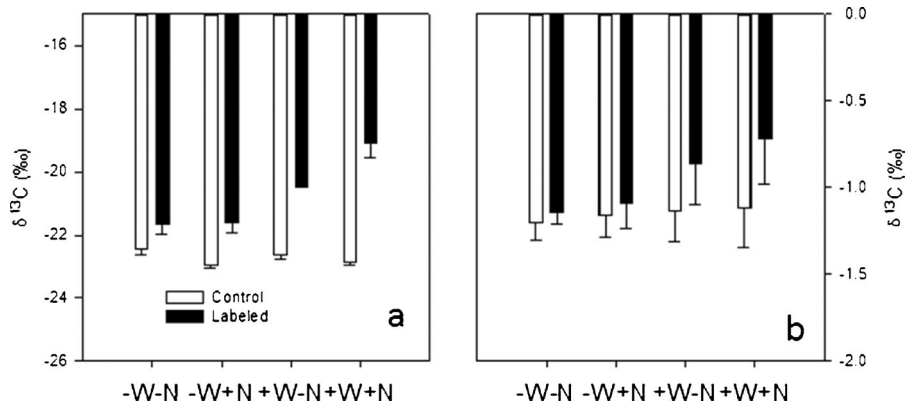
**Fig. 3** Isotopic composition of *larrea tridentata* leaves (a), stems (b) and roots (c) in unlabeled and  $^{13}\text{C}$ -labeled plant-soil systems in response to water and N additions. ‘-W-N’=low water, low N, ‘-W+N’=low water, high N, ‘+W-N’=high water, low N, and ‘+W+N’=high water and high N

did not include inorganic C in the  $^{13}\text{C}$  uptake and allocation calculations.

#### Discussion

Effects of water availability on biomass and C allocation

We initially hypothesized that (1) total net C uptake would increase under increased soil moisture



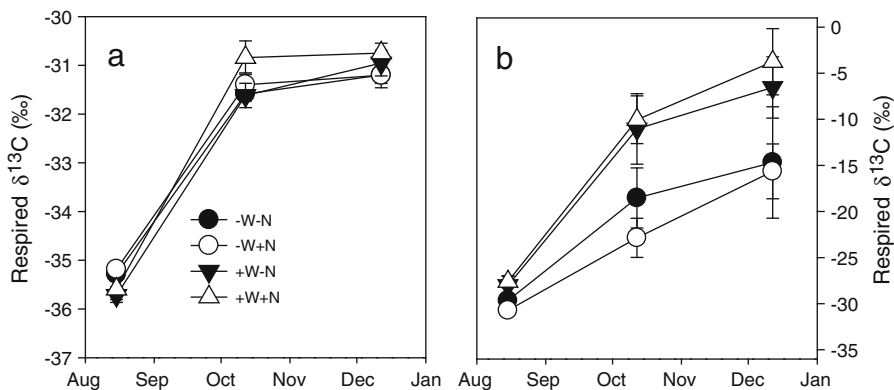
**Fig. 4** Isotopic composition of organic (a) and inorganic (b) soil C in unlabeled and  $^{13}\text{C}$ -labeled plant-soil systems in response to water and N additions. ‘-W-N’=low water, low N, ‘-W+N’=low water, high N, ‘+W-N’=high water, low N, and ‘+W+N’=high water and high N

conditions, and that (2) relatively less C would be allocated belowground with increasing moisture levels. The results from our study showed that leaf and total biomass, N content and  $^{13}\text{C}$  content as well as total  $^{13}\text{C}$  uptake either significantly increased or tended to increase with increasing soil moisture in *Larrea tridentata* (Figs. 1 and 6; Table 1) supporting our first hypothesis. In addition, soil respiration was higher in the high water treatment toward the end of the study (Fig. 2). Biomass and  $^{13}\text{C}$  shoot/root ratios were however not affected by the moisture treatments in contrast to our second hypothesis.

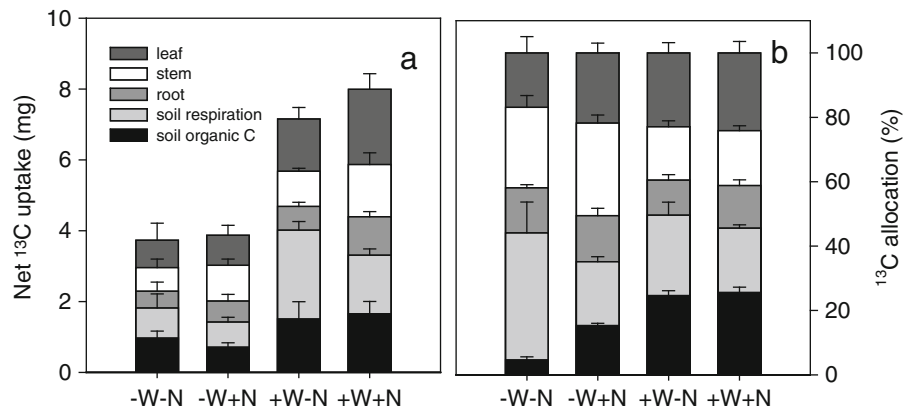
While the overall responses in biomass and soil respiration to changed water availability were not significant, the isotopic data showed clear effects of the water treatments. The absence of a significant effect on biomass may have been due to the relatively short growing period of about 5 months while seedlings were already 2 years old at the start of the study. In addition, the low

level of replication may have limited our ability to detect significant effects of moisture even though the absolute increases in leaf and total biomass were quite large (140 % for leaves and 36 % for total biomass). The increase in net C uptake was a direct function of total leaf mass but also leaf photosynthetic activity as indicated by the increased specific  $^{13}\text{C}$  uptake (Fig. 7), agreeing with Barker et al. (2006) who observed increased photosynthetic activity in response to summer irrigation in the Mojave Desert.

Increased moisture availability resulted in more  $^{13}\text{C}$  being allocated to soil organic matter and soil respiration (Fig. 6a). Relative C allocation (i.e., fraction of total  $^{13}\text{C}$  taken up allocated to specific plant-soil components) deviated from this pattern because a larger relative amount of C was allocated to soil organic matter at the expense of C allocated to stems (Fig. 6). Thus, increased moisture availability increased absolute belowground C allocation and proportionately more so to soil organic



**Fig. 5** Isotopic composition of soil-respired  $\text{CO}_2$  in unlabeled (a) and  $^{13}\text{C}$ -labeled (b) plant-soil systems in response to water and N additions. ‘-W-N’=low water, low N, ‘-W+N’=low water, high N, ‘+W-N’=high water, low N, and ‘+W+N’=high water and high N



**Fig. 6** Net  $^{13}\text{C}$  uptake (a) and  $^{13}\text{C}$  allocation (b) in plant-soil systems exposed to  $^{13}\text{C}$ -labeled  $\text{CO}_2$  in response to water and N additions. ‘-W-N’=low water, low N, ‘-W+N’=low water, high

N, ‘+W-N’=high water, low N, and ‘+W+N’=high water and high N. Error bars represent standard errors of each individual soil-plant compartment

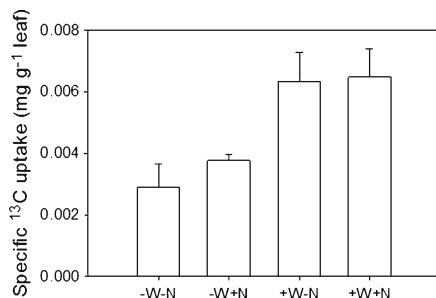
matter. When calculating the ratio of aboveground to belowground allocation as  $(\text{total } ^{13}\text{C}_{\text{shoots}} + \text{total } ^{13}\text{C}_{\text{stems}}) / (\text{total } ^{13}\text{C}_{\text{roots}} + \text{total } ^{13}\text{C}_{\text{soil organic matter}} + \text{total } ^{13}\text{C}_{\text{soil respiration}})$ , however, no effects of the treatments were found showing that—while total C uptake increased under higher moisture availability—the ratio of above-to-belowground allocation remained unchanged contrasting our second hypothesis.

Hunt et al. (1996) and Poorter and Nagel (2000) suggested that C allocation to roots decreases as resource availability increases. Several studies in arid environments have shown increased root proliferation in water and nutrient-rich patches, however (Eissenstat and Caldwell 1988; Jackson and Caldwell 1989; Caldwell et al. 1991). Reynolds et al. (1999) observed that root production in *Larrea tridentata* decreased with reduced summer moisture in a Chihuahuan Desert site in New Mexico. While no aboveground data were presented in these studies, they appear to contrast the idea that

resource limitations increase belowground C allocation. The data from our study suggest that, while increased moisture favors C uptake, no changes in C allocation occurred when considering biomass and  $^{13}\text{C}$  shoot/root ratios as well as total  $^{13}\text{C}$  allocation data in contrast to our second hypothesis. More C may, however, be allocated to soil organic matter under higher moisture availability suggesting that production rates of organic matter exceeded potential moisture-induced increases in decomposition rates.

#### Effects of N availability on biomass and C allocation

We hypothesized that increased N availability would only affect C allocation when moisture limitation were alleviated. Increased N availability did not impact biomass, net  $^{13}\text{C}$  uptake, specific  $^{13}\text{C}$  uptake, or C allocation even at higher soil moisture levels rejecting our initial hypothesis. Leaf and total biomass N content increased indicating that N availability affected N uptake but this did not result in a significant increase in biomass production even when moisture levels were higher. Increased N availability resulted in an increase biomass shoot/root ratios at low moisture levels but this pattern was not apparent in the  $^{13}\text{C}$  data. The lack of responses to increasing N availability was somewhat surprising but consistent with results from a meta-analysis conducted by LeBauer and Treseder (2008) who found no impact of increased N availability on net primary activity (NPP) in three desert ecosystems. In addition, previous studies found no effects of N additions on fine root dynamics (Verburg et al. 2013) or aboveground plant production (Newingham et al.



**Fig. 7** Specific  $^{13}\text{C}$  uptake per unit leaf biomass in plant-soil systems exposed to  $^{13}\text{C}$ -labeled  $\text{CO}_2$  in response to water and N additions. ‘-W-N’=low water, low N, ‘-W+N’=low water, high N, ‘+W-N’=high water, low N, and ‘+W+N’=high water and high N



2012). Barker et al. (2006) observed a significant increase in net photosynthesis in *Larrea tridentata* in response to N additions in combination with increased water availability during the summer at the site from which the soils were collected but this pattern was reversed during the spring. In our study, even at higher moisture availability we did not find an increase in net  $^{13}\text{C}$  uptake or specific  $^{13}\text{C}$  uptake suggesting that the patterns we found were more representative of increased spring moisture conditions.

The only effect of N availability observed in this study was a decrease in relative C allocation to soil respiration. Results from previous studies have shown reductions (e.g., Burton et al. 2004; Janssens et al. 2010) and increases (e.g., Deng et al. 2010) in soil respiration in response to N additions in forested environments. Burton et al. (2004) and Janssens et al. (2010) ascribed reductions in soil respiration to decreased belowground C allocation most likely resulting in reduced substrate supply to microbes, thereby reducing heterotrophic respiration. Deng et al. (2010) indicated that increased soil respiration occurring in the first year following N additions may have been caused by increased root biomass in response to N fertilization in a study using tree seedlings. This increased root biomass subsequently could result in increased belowground root litter production that could then stimulate heterotrophic respiration. Our study supports the suggestions from Burton et al. (2004) and Janssens et al. (2010) since previous studies in the Mojave Desert have shown no effects of water, N, and elevated  $\text{CO}_2$  concentrations on root biomass (Verburg et al. 2013; Ferguson and Nowak 2011; Newingham et al. 2012, 2013) or root respiration (Clark et al. 2010).

#### Contribution of inorganic C pools to C sequestration

Our last goal was to assess if carbonate C could play a role in ecosystem C sequestration in arid soils. The labeling did not result in a significant change in the  $\delta^{13}\text{C}$  value of the carbonate C suggesting that no new C was incorporated into the carbonate C pool. One potential reason for this lack of a change in the  $\delta^{13}\text{C}$  value may have been that the soils had a relatively large amount of inorganic C (2.5 %), so no clear change could be detected given the large background. Still, the amount of inorganic C was only 2.5 times higher than the amount of organic C; and the  $\delta^{13}\text{C}$  data showed that a significant amount of new C was incorporated into the

organic C pool especially under high soil moisture availability. In addition, the  $\delta^{13}\text{C}$  data of the respired  $\text{CO}_2$  in the labeled chamber suggested a significant amount of new C being present in soil  $\text{CO}_2$  which potentially could have been incorporated in the inorganic C pool. Our results agree with Marion et al. (2008) suggesting very little contribution of carbonate C to ecosystem C sequestration. Still, Xie et al. (2009) found large C uptake in carbonate C; but no clear explanation has been given in terms of the mechanisms that could explain this large uptake. In addition, Kuzyakov et al. (2006) observed increases in C sequestration in soil carbonate pools in a loess soil planted with maize. In that study, soil  $\text{CO}_2$  concentrations were much higher than those found in most arid soils potentially facilitating more rapid exchange of C between the atmosphere and carbonate pool.

#### Implications for C sequestration in arid lands

Our study showed that increased soil moisture had a larger impact on biomass production and C uptake and allocation than increased N availability with more C being taken up under wetter conditions supporting our initial hypotheses. The main pools that showed a net new C sequestration were the leaf and soil organic C pools, but the relative amounts of C allocated above- vs. belowground remained unchanged in contrast with our initial hypothesis. A substantial amount of the newly fixed C left the system as soil respiration but the amount of C sequestered in the soil-plant systems was still larger when moisture availability was high. While we observed active C uptake, particularly under wetter conditions, we cannot assess if the sink strength inferred from flux-based measurements (Jasoni et al. 2005; Wohlfahrt et al. 2008) can be explained by accumulations of C in biomass and/or organic matter as suggested by our study. First, we could not quantify the absolute amounts of new C that were incorporated into the various ecosystem pools because we conducted weekly labelings (i.e., a sequence of  $^{13}\text{C}$  pulses instead of exposure to a continuous  $^{13}\text{C}$  label; Sangster et al. 2010). Second, we did not measure C release through aboveground plant respiration which is included in flux-based net ecosystem measurements. Third, the plants used in our study were relatively young and most likely showed different C accumulation rates than mature shrubs. Finally, our study did not include intact biotic soil crusts which could potentially play an important role in C cycling in

arid ecosystems (e.g., Su et al. 2012; Li et al. 2012). Still, the isotopic data from our study show that these relatively slow-growing plants species actively accumulate C not only in biomass but also in soil organic C pools which could help explain net C uptake observed in previous studies.

The responses of soils and vegetation to moisture availability found in our study were larger than those observed in field studies carried out in the Mojave Desert ecosystems since Newingham et al. (2012) and Verburg et al. (2013) found little change in above- and belowground biomass production after 5–6 years of summer water additions in these systems. Above- and belowground biomass also did not respond to 10 years of exposure to elevated CO<sub>2</sub> concentrations at a site in close proximity to the previously mentioned studies (Newingham et al. 2013; Ferguson and Nowak 2011). The lack of responses to N additions was however similar in our study and related field studies. One potential explanation for the discrepancy between results from our study and previous field studies may be that in our study moisture content was held constant throughout our experiment. As a result, the effects of increased moisture availability are likely to be magnified in our study since under field conditions changes in soil moisture may be more short-lived (episodic). Also, our study was conducted with 2 year old seedlings rather than mature shrubs. Still, Verburg et al. (2013) showed significant effects of winter precipitation on fine root dynamics while summer precipitation did not impact fine roots showing that timing of moisture availability is critical for these ecosystems. Given that we started our study in early spring, our water treatments were more representative of changes in spring water availability when most above- and belowground biomass production occurs. Consequently, if climate change results in differences in winter moisture, C sequestration may be impacted. Comparing results from our study with those from field studies suggest that our N treatments are likely to be more representative of field conditions where N deposition in wet and/or dry forms occurs throughout the year.

Overall, the results from our study suggest that changes in soil moisture may have an impact on C uptake by plants that could result in changing biomass and soil organic C levels in arid systems. These changes could contribute to increased C sequestration under wetter conditions. In contrast, increased N availability in response to atmospheric N deposition is not likely to

have a major impact on C uptake in these ecosystems. The data from our study further suggested that inorganic C may not contribute significantly to C cycling in these systems compared to plants and soil organic C.

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