

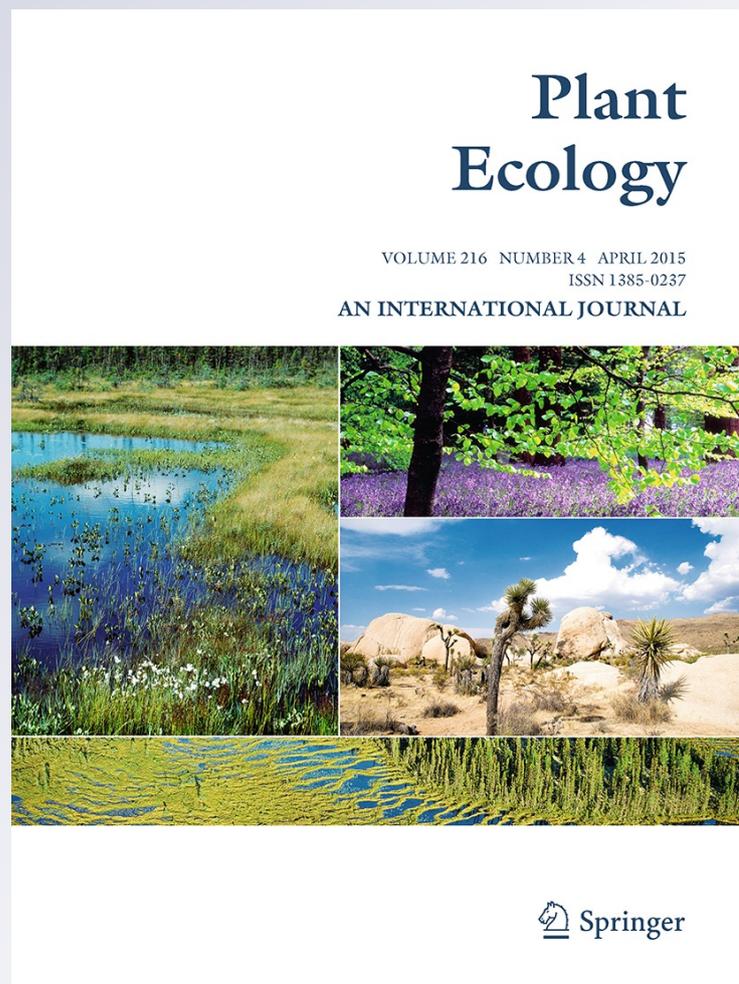
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# Root biomass and soil $\delta^{13}\text{C}$ in $\text{C}_3$ and $\text{C}_4$ grasslands along a precipitation gradient

Courtney L. Angelo · Stephanie Pau

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**Abstract** Many studies have investigated the above-ground distributions of  $\text{C}_3$  and  $\text{C}_4$  grasses along climatic gradients because they illustrate complex interactions between abiotic and biotic controls on ecosystem functions. Yet few studies have examined belowground components of these distributions, which may present very different patterns compared with aboveground measures. In this study, we surveyed grass species cover and collected soil and root samples from field plots at 100–150-m elevation intervals along a climatic gradient in Hawai'i. We examined how the relationship between soil carbon isotopic composition ( $\delta^{13}\text{C}$ ), a proxy for  $\text{C}_4$  dominance, and %  $\text{C}_4$  cover changed along a climatic gradient. We also evaluated root biomass to determine if belowground dominance reflects aboveground patterns under climate variation. Results showed that soil  $\delta^{13}\text{C}$  under predicted  $\text{C}_4$  dominance in wetter sites. The relationship between %  $\text{C}_4$  cover and soil  $\delta^{13}\text{C}$  became more negative with increasing mean annual

precipitation (MAP) based on a linear mixed-effects model ( $F_{1,34} = 12.25$ ,  $P < 0.01$ ). Soil  $\delta^{13}\text{C}$  in wetter sites indicated a larger  $\text{C}_3$  contribution than estimated by aboveground cover, which was in part due to  $\text{C}_3$  root biomass increasing ( $P < 0.05$ ), whereas  $\text{C}_4$  root biomass did not change along the precipitation gradient.  $\text{C}_3$  and  $\text{C}_4$  grasses appear to allocate disproportionately belowground; thus, a different understanding of  $\text{C}_4$  ecological dominance (biomass or productivity) may emerge when considering both the above and belowground components. Our results show that belowground allocation and interpretation of soil  $\delta^{13}\text{C}$  need to be more carefully considered in global vegetation and carbon models and paleoecological reconstructions of  $\text{C}_4$  dominance.

**Keywords** Belowground allocation · Climate variation · Dominance patterns · Ecosystem function · Grasses · Hawaiian Islands

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C. L. Angelo (✉) · S. Pau  
Department of Geography, Florida State University,  
113 Collegiate Loop, Bellamy Building, Room 323,  
Tallahassee, FL 32306-2190, USA  
e-mail: courtneyangelo@gmail.com

## Introduction

$\text{C}_3$  and  $\text{C}_4$  plants are two plant functional types (PFTs) with distinct biochemistry and physiology, which scale up to important differences in ecosystem functioning (Sage and Monson 1999; Still et al. 2003). The  $\text{C}_4$  photosynthetic pathway is most abundant in the grass family (Poaceae); with almost half the species having the pathway, while the remainder utilize the  $\text{C}_3$

photosynthetic pathway (Sage and Monson 1999). The global expansion of  $C_4$  grasslands occurred 3–8 million years ago (Cerling et al. 1997; Edwards et al. 2010) and has been predominately attributed to a decline in atmospheric  $CO_2$  concentrations (Cerling et al. 1997; Ehleringer et al. 1997).  $C_4$  plants are estimated to account for roughly 20–25 % of the global terrestrial primary production (Still et al. 2003), although comprising only 3 % of vascular plant species (Sage et al. 1999a). The most extensive natural  $C_4$  biomes are the various grasslands of the tropics, subtropics, warm temperate zones, while  $C_3$  grasses dominate cooler temperate regions (Sage et al. 1999b). The global distributions of  $C_3$  and  $C_4$  grasses influence global water, carbon, and nitrogen cycles due to different biochemical and physiological processes associated with the  $C_3$  and  $C_4$  photosynthetic pathways (Sage and Monson 1999; Still et al. 2003). For example,  $C_4$  plants have higher water-use efficiency than  $C_3$  plants (Sage and Kubien 2003); thus, regions dominated by  $C_4$  plants typically have lower canopy conductance and latent heat fluxes than  $C_3$ -dominated regions influencing land surface temperatures and humidity (Sellers et al. 1992; Still et al. 2003). At the site level, the composition of  $C_3$  and  $C_4$  grasses control various ecosystem functions such as primary productivity, decomposition rates, fire regimes as well as nutrient and water cycling (D'Antonio and Vitousek 1992; Díaz and Cabido 2001).

The environmental sorting of  $C_3$  and  $C_4$  grasses has been primarily explained based on a physiological model called *quantum yield* (Ehleringer et al. 1997; Collatz et al. 1998), which simply stated, reasons that the efficiency of  $C_4$  photosynthesis should be greater than  $C_3$  photosynthesis under conditions of high temperatures, high light, and greater aridity, and this advantage translates into the competitive success of  $C_4$  plants under these conditions.  $C_4$  plants gain this advantage by internally concentrating higher levels of  $CO_2$ , generating photosynthetic efficiency under low  $CO_2$  concentrations and eliminating photorespiration compared with  $C_3$  plants. Mechanistic models have predicted that  $C_4$  plants will have a competitive advantage resulting in greater abundance in warm climates where the mean monthly air temperature exceeds 22 °C and precipitation is at least 25 mm (Collatz et al. 1998; Still et al. 2003). In addition to temperature, differences in precipitation may modify where  $C_3$  plants can grow, allowing them to coexist

with  $C_4$  plants in warmer regions likely due to reduced water loss and photorespiration under moist conditions. Field studies have shown the transition of  $C_3$ – $C_4$  plants to be in the temperature range of 21–31 °C for the mean warmest month of the year maximum (Rundel 1980; Hattersley 1983; Wentworth 1985; Cavagnaro 1988).

Numerous studies have since examined how temperature and precipitation determine the  $C_3$ – $C_4$  composition of grasslands in both temperate and tropical settings (e.g., Teeri and Stowe 1976; Paruelo and Lauenroth 1996; Murphy and Bowman 2007; Bremond et al. 2012). In tropical regions, classic work on  $C_3$ – $C_4$  turnover (i.e., the replacement of  $C_3$  species by  $C_4$  species) along an elevational gradient has been conducted on the Hawaiian Islands (Rundel 1980), as well as other tropical regions (Chazdon 1978; Tieszen et al. 1979). Rundel (1980) showed in Hawai'i that  $C_4$  grasses were dominant in warm low-mid elevation sites, whereas,  $C_3$  grasses dominated cool mid-high elevation sites. Both PFTs were found to span a precipitation gradient with  $C_3$  and  $C_4$  grasses being found in both dry and moist regions (Rundel 1980). Distributional patterns of  $C_3$  and  $C_4$  grasses are predicted to shift in the future in response to increasing atmospheric  $CO_2$  concentrations (Ainsworth and Long 2005; Morgan et al. 2011) and changing climate (Epstein et al. 2002); thus understanding aboveground and belowground dominance patterns will help better predict how ecosystem functions may shift in the future.

A considerable gap in our knowledge of  $C_3$  and  $C_4$  grass biogeography and associated ecosystem functions is our understanding of belowground dominance (i.e., root biomass and soil carbon contribution), even though in some regions, grasses allocate up to 87 % of their net primary production belowground (Jackson et al. 1996; Gill and Jackson 2000; Mokany et al. 2006). Root allocation reflects the balance between acquisition of belowground (water and nutrients) and aboveground (light and carbon dioxide) resources; thus, plants in water-limited systems may allocate more biomass to roots (Canadell et al. 1996; Shipley and Meziane 2002; Mokany et al. 2006). In the tallgrass prairies of North America, Nippert and Knapp (2007a, b) showed greater root differentiation between  $C_3$  species and  $C_4$  grasses in regions of limited water availability and this belowground allocation was not proportional to aboveground

productivity (Nippert et al. 2011). Similarly, seasonal dynamics of root growth vary, and root biomass has been shown to be greatest in the dry season (McNaughton et al. 1998).

In contrast to  $C_3$  or  $C_4$  turnover reflecting differences in physiology and abiotic tolerances, coexisting  $C_3$  and  $C_4$  plants may instead be more strongly affected by competition for limited resources. Consequently, differences in root allocation of coexisting  $C_3$  and  $C_4$  species may indicate resource partitioning (Fargione and Tilman 2005; Nippert and Knapp 2007a, b). Previous studies have shown that  $C_4$  root characteristics and water use do not vary with differences in water availability (Nippert and Knapp 2007a, b; Nippert et al. 2012).  $C_4$  species appeared to rely on surface soil water regardless of water availability (Nippert et al. 2012), whereas  $C_3$  species were more plastic in their response to water availability (Nippert and Knapp 2007a, b). Furthermore, greater competition for water resources between  $C_3$  and  $C_4$  plants has also been shown in areas where water is plentiful (Saunders et al. 2006; Nippert and Knapp 2007b).

Carbon isotopic values ( $\delta^{13}\text{C}$ ) in soil organic matter (SOM) can provide time-integrated information on both the composition of  $C_3$  and  $C_4$  plants, as well as the environmental conditions in which the plants grow (Sage et al. 1999b). These unique values are the result of differences in discrimination ( $\Delta$ ) of the heavier  $^{13}\text{C}$  isotope by their respective photosynthetic carboxylation enzymes and diffusional fractionations. Due to differences in carboxylation enzymes in  $C_3$  and  $C_4$  photosynthesis [i.e., ribulose biphosphate carboxylase–oxygenase (Rubisco) and phosphoenolpyruvate carboxylase (PEP)],  $C_4$  plants discriminate less against  $^{13}\text{C}$  than  $C_3$  plants resulting in more enriched  $\delta^{13}\text{C}$  values (Farquhar et al. 1982; Farquhar 1983; Farquhar and Cernusak 2012). The carbon isotopic values ( $\delta^{13}\text{C}$ ) of  $C_3$  plants can vary widely in different environmental conditions. In water-stressed environments,  $\delta^{13}\text{C}$  values of  $C_3$  plants can range from as high as  $-21$  to  $-22$  ‰ and be as low as  $-35$  ‰ in closed canopies, relative to the average value of  $-27$  ‰ (Cerling et al. 1997). In comparison,  $C_4$  plants have a smaller range of  $\delta^{13}\text{C}$  values, from  $-10$  to  $-14$  ‰, with the most enriched values found in the NADP-ME, one of the three biochemical subtypes found in  $C_4$  plants, and which typically grows in locations with greater precipitation (Ghannoum et al. 2002).  $C_4$

plants retain a narrow range of  $\delta^{13}\text{C}$  values under varying environments, with  $\delta^{13}\text{C}$  deviating less than 4 ‰ (Buchmann et al. 1996; Ghannoum et al. 2002). Therefore, carbon isotope values in soils can be used as an indication of  $C_3$  and  $C_4$  vegetation patterns.

The decomposition of plant tissues (leaves, stems, and roots), root exudation (Balesdent et al. 1993; Ehleringer et al. 2000; Chapin et al. 2011), along with soil processes such as microbial activity and respiration (Tieszen and Archer 1990; Townsend et al. 1997) determine  $\delta^{13}\text{C}$  values in SOM. Thus, although  $\delta^{13}\text{C}$  values of SOM should accurately reflect  $C_4$  dominance (reviewed in Sage et al. 1999b), several processes could bias this relationship: (1) an enrichment of  $\delta^{13}\text{C}$  may occur at greater soil depths due to a change in the  $^{13}\text{C}/^{12}\text{C}$  ratio in atmospheric  $\text{CO}_2$  after increased fossil fuel burning during the industrial period compared with pre-industrial period, called the “Suess effect” (Suess 1955; Ehleringer et al. 2000); (2)  $C_3$  and  $C_4$  plants may differ in their allocation to belowground production due to abiotic (Nippert and Knapp 2007a, b; Luo et al. 2013) or biotic factors (Fargione and Tilman 2005; Nippert and Knapp 2007a, b); (3) an increase in total soil carbon can be associated with a decrease in  $\delta^{13}\text{C}$  values of SOM (Balesdent et al. 1993; Bird and Pousai 1997); (4) decomposition rates of  $C_3$ - and  $C_4$ -derived carbon may vary (Wynn and Bird 2007); (5)  $C_3$  species may be depleted in  $\delta^{13}\text{C}$  across substrate age or precipitation gradients due to changes in intrinsic water-use efficiencies (iWUE) (Cordell et al. 1998; Wynn and Bird 2008); and (6) recent shifts in  $C_3$ – $C_4$  composition.

Limited research has evaluated belowground patterns of  $C_3$  and  $C_4$  grasses in tropical systems (Jackson et al. 1996; Hui and Jackson 2006; Mokany et al. 2006); thus, this has been identified as an area of opportunity for ecological research (Wilson 2014). The interpretation of  $\delta^{13}\text{C}$  values for understanding modern spatial distributions and paleoecological reconstructions of  $C_4$  dominance can be improved with careful consideration of differences in root allocation along environmental gradients. Here we examine the relationships between  $\delta^{13}\text{C}$  values of SOM, aboveground %  $C_3$ – $C_4$  cover, and root biomasses along a climatic gradient in Hawai‘i. We aimed to address two questions: (1) Are soil  $\delta^{13}\text{C}$  values proportional to the percent cover of  $C_4$  grasses (i.e., the relative abundance of  $C_4/C_3$  plants)? and (2) Do  $C_3$  and  $C_4$  root allocation patterns change along climatic

gradients? To address these questions we collected soil, root, and foliar samples to extend our current understanding of  $C_3$ – $C_4$  turnover from sites that have been repeatedly surveyed for aboveground %  $C_3$ – $C_4$  cover, first examined by Rundel (1980) and more recently by Angelo and Daehler (2013, 2015). The Hawaiian Islands are an ideal location for investigating  $C_3$  and  $C_4$  patterns because they vary along climatic gradients over relatively short distances.

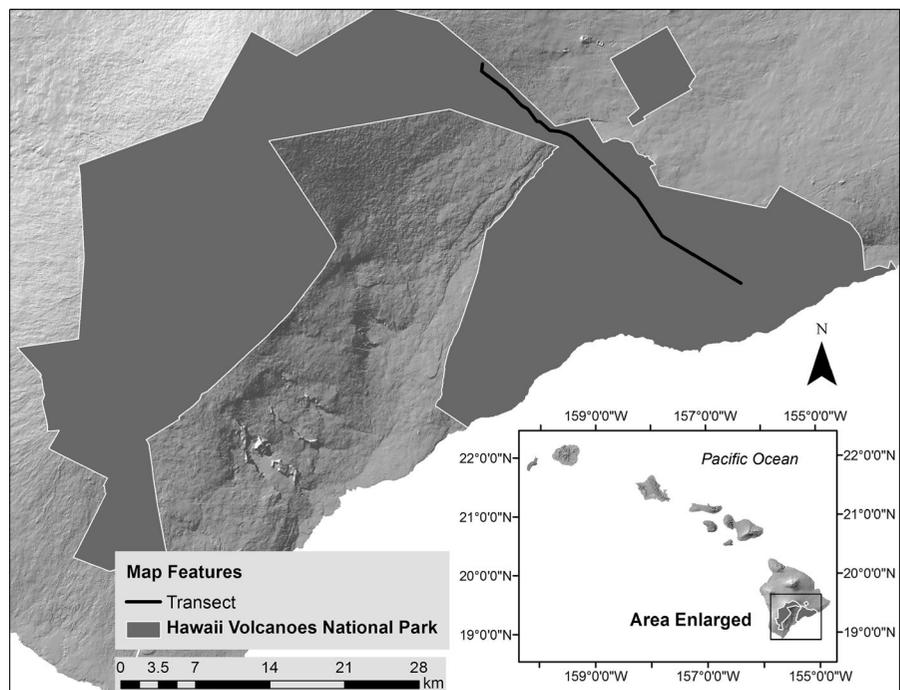
## Materials and methods

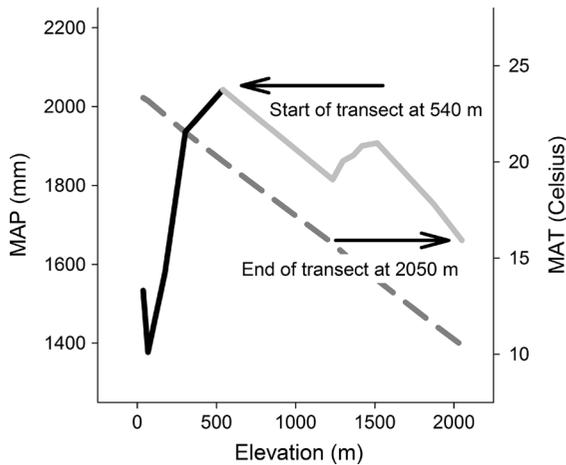
### Study site and field methods

During late July to early August 2013 (Hawaiian dry season), field plots were surveyed along a climatic gradient on Mauna Loa, Hawai'i (Fig. 1). Field surveys were done within Hawai'i Volcanoes National Park (HAVO) along the Chain of Craters and Mauna Loa Roads following Angelo and Daehler (2013, 2015). Along the Mauna Loa gradient, precipitation peaks at mid-elevations (Giambelluca et al. 2013) because the trade wind inversion layer (TWI) found in Hawai'i limits cloud formation above it, thus creating a moist layer below it and dry air above (Giambelluca

and Nullet 1991; Cao et al. 2007) decoupling precipitation and temperature patterns. Due to the climate patterns along the Mauna Loa gradient,  $C_3$  and  $C_4$  grasses in our transect are found coexisting under moist conditions at mid-elevations, while  $C_3$  dominance is found at cooler, drier conditions at the highest elevations (1600 mm MAP, 2050 m) and  $C_4$  dominance is found at warmer, wetter areas at the lowest elevations (2043 mm MAP, 540 m) (Fig. 2).  $C_4$  grasses are abundant at elevations below 540 m where conditions are drier (Fig. 2); however, extensive lava cover at these sites made soil extractions unfeasible. The substrate age (age of soil parent material) of our sites were of intermediate to old age (Vitousek et al. 1992) and ranged from 500 to 8000 years old (years) (Wolfe and Morris 1996). Plot selection was based on minimizing differences in soil carbon and nitrogen, and substrate age across the Mauna Loa gradient. Plots  $50 \times 2$  m in size (nine plots total) were established in natural habitats ( $\sim 30$  m away from the road) every 100–150-m change in elevation along the Mauna Loa gradient. Plots were divided into five  $10 \times 2$ -m subplots at each site. Percent cover estimates are a standard measure in previous works examining  $C_3$ – $C_4$  grass distributional patterns (e.g., Rundel 1980; von Fischer et al. 2008),

**Fig. 1** Transect line (black line) on the island of Hawai'i, all within Hawai'i Volcanoes National Park





**Fig. 2** The distribution of mean annual precipitation (MAP) (solid lines) (Giambelluca et al. 2013) and mean annual temperature (MAT) (dark gray dashed line) (Giambelluca et al. 2014) along the Mauna Loa elevational gradient within Hawai'i Volcanoes National Park (HAVO), Hawai'i. The solid gray line denotes MAP for our transect spanning from 540 m (%  $C_4$  cover is high) to 2050 m (%  $C_3$  cover is high). Along the Mauna Loa gradient, precipitation peaks at mid-elevations (Giambelluca et al. 2013) because the TWI found in Hawai'i limits cloud formation above it, thus creating a moist layer below it and dry air above (Giambelluca and Nullet 1991; Cao et al. 2007), decoupling precipitation and temperature patterns. Based on surveys by Angelo and Daehler (2013, 2015), %  $C_4$  cover remains high below 540 m, but due to extensive lava cover these sites were not available for soil  $\delta^{13}C$  or root analysis in this study

and provide ecological information that is not available in floristic analyses (Sage et al. 1999b). All plant species (including grass, woody, and forb  $C_3$  plants;  $C_4$  plants are all grasses) found in the subplots (every 10 m) were recorded, and visual cover estimates for each species were made in subplots based on one of six percent cover categories: 0–1, 2–5, 6–25, 26–50, 51–75, and 76–100 (Mueller-Dombois and Ellenberg 1974). The photosynthetic pathway of all grasses was determined using the  $C_4$  genera list in Sage et al. (1999a). The relative aboveground cover of  $C_4$  grasses to  $C_3$  vegetation (grass, woody, and forb species) was determined (hereafter, %  $C_4$  cover), along with the percent cover of  $C_3$  woody species. A proxy for  $C_3$  and  $C_4$  leaf litter contributions at each site was determined by estimating species' plant heights and leaf areas based on field observations and species' descriptions in Wagner et al. (1999). For SOM  $\delta^{13}C$  samples (hereafter, soil  $\delta^{13}C$ ), soil cores were taken from 0 to 15-cm depths with a 5-cm diameter using an AMS

sliding hammer corer (American Falls, ID, USA), and bulked for soil  $\delta^{13}C$  analysis. Fifty-four total soil cores were extracted as point measurements every 10 m for a total of six cores at each site (at the beginning and end of each subplot: 0, 10, 20, 30, 40, and 50 m). The soil  $\delta^{13}C$  values at the beginning and end of each subplot were averaged to get a single value for each subplot ( $n = 45$ ). Our soils were of volcanic nature and very shallow with volcanic rock being found at depths circa 15 cm at most sites; thus, the majority of roots in our study were captured at this depth, and visual inspection did not reveal inadequate root sampling. Furthermore, it has been shown that 60–70 % of root biomass is within the top 15 cm of the soil profile (Weaver et al. 1935; Gill et al. 1999).

For root samples, soil cores (5-cm diameter and 15-cm length) were taken as point measurements directly under the plant for all dominant grass species (at least 25 % cover) at each site. The number of dominant species varied by site, with a maximum of four possible grass species per site. Species richness was low; thus, at almost all sites, all grasses were sampled in a plot. Grass species that were not sampled for root biomass had less than 5 % cover. Root and foliar samples (three replicates per species each) were taken from the same mature individual plant species. Mature individual plants were chosen based on size and the presence of flowering stalks. All soil and root cores were stored in a cooler on ice, and foliar samples were stored in paper bags during transport to the Natural Resource and Environmental Management Department and the Stable Isotope Biogeochemistry Lab at the University of Hawai'i at Manoa (UHM).

#### Lab analysis

Soil and foliar samples were taken to the Stable Isotope Biogeochemistry Lab at UHM for  $\delta^{13}C$  analysis. Soils were sieved, and the <2 mm fraction was dried, ground, acidified via fumigation using 12 N HCl to remove inorganic carbon, and weighed. During lab analysis, replicate foliar samples were combined to get an average  $\delta^{13}C$  value per species per site. Foliar samples were dried, ground, and weighed. Both soil and foliar data were then analyzed on a Costech ECS 4010 Elemental Combustion System (Costech Analytical Technologies Inc., Valencia, CA) coupled with a ThermoFinnigan DeltaXP isotope ratio mass spectrometer (Thermo Electron Corporation, Bremen

Germany) to determine the isotopic ratio of  $^{13}\text{C}:^{12}\text{C}$ . Quantification and isotopic corrections were prepared by the Stable Isotope Biogeochemistry Lab. Isotopic ratios are expressed as a  $\delta^{13}\text{C}$  value with respect to a standard (VPDB) where

$$\delta^{13}\text{C}_{\text{sample}} = \left( \frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}} - (^{13}\text{C}/^{12}\text{C})_{\text{std}}}{(^{13}\text{C}/^{12}\text{C})_{\text{std}}} \right) \quad (1)$$

In Eq. 1,  $\delta^{13}\text{C}_{\text{sample}}$  is the sample of interest,  $^{13}\text{C}/^{12}\text{C}_{\text{sample}}$  is its  $^{13}\text{C}/^{12}\text{C}$  ratio, and  $^{13}\text{C}/^{12}\text{C}_{\text{std}}$  is the  $^{13}\text{C}/^{12}\text{C}$  ratio of the standard (Farquhar et al. 1989). The standard for stable carbon isotope ratios is Vienna Pee Dee Belemnite (VPDB) (Brand et al. 2014). All stable isotope ratios are reported as per mil (‰). Soil carbon content was also subsequently analyzed. For root cores, soil was removed from live root tissue through a series of sieves (0.5-mm mesh and smaller for fine roots). Live root samples were oven dried (60 °C) for 1 week and weighed for root biomass ( $\text{g}/\text{m}^2$ ).

### Statistical analysis

A Pearson's correlation was used to evaluate the overall relationship between soil  $\delta^{13}\text{C}$  and %  $\text{C}_4$  cover. We next examined the relationship between soil  $\delta^{13}\text{C}$  and %  $\text{C}_4$  cover using linear mixed-effects models with "site" as a random effect because data for each subplot at each site were not independent. We also examined whether the relationship between soil  $\delta^{13}\text{C}$  and %  $\text{C}_4$  cover changed with mean annual precipitation and/or temperature (MAP and MAT) (Giambelluca et al. 2013, 2014) by including an interaction term for the climatic variables (Table 1). Akaike's information criterion (AIC) was used to compare models with the interaction term for MAP and MAT to more parsimonious models. Models are typically considered equivalent if the difference in their respective AIC values is 2 or less, and in this case the most parsimonious model using the fewest parameters is selected as the best-fit (Burnham and Anderson 2002).

The relationships between total  $\text{C}_3$  and  $\text{C}_4$  root biomass ( $\text{g}/\text{m}^2$ ) and climate (MAP and MAT) were examined using Pearson's correlations. Relationships with root biomass excluded sites where there were no  $\text{C}_3$  or  $\text{C}_4$  species with cover  $\geq 25\%$  (i.e., we excluded

**Table 1** Linear mixed-effects models of soil  $\delta^{13}\text{C}$  along a climatic gradient on Mauna Loa with each site as a random effect (see "Methods" section)

Models	Fixed parameters	AIC	df
a	% $\text{C}_4 \times \text{MAP} + \% \text{C}_4 \times \text{MAT}$	132.54	8
<b>b</b>	<b>% <math>\text{C}_4 \times \text{MAP}</math></b>	<b>132.06</b>	<b>6</b>
c	% $\text{C}_4 \times \text{MAT}$	136.65	6
d	% $\text{C}_4$	138.18	4

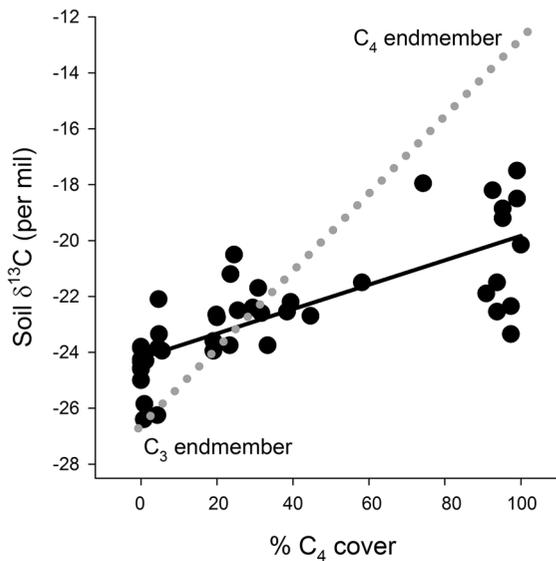
Models a and b are considered equivalent, but the interaction with temperature was not significant, and Model b (bold) is more parsimonious ( $F_{1,34} = 12.25$ ,  $P < 0.01$ ). Although the main effect of %  $\text{C}_4$  cover on soil  $\delta^{13}\text{C}$  is positive (see Fig. 3), the interaction term with MAP in the best-fit model was negative, indicating that the slope between soil  $\delta^{13}\text{C}$  and %  $\text{C}_4$  cover became more negative with increasing MAP ( $\beta = -0.02$ )

zeros), which resulted in six sites with  $\text{C}_3$  root samples and eight sites with  $\text{C}_4$  root samples. Three replicate root samples per species per site were used to determine total  $\text{C}_3$  and  $\text{C}_4$  root biomass ( $\text{g}/\text{m}^2$ ).

Additional Pearson's correlations with soil carbon content (%), substrate age, soil and foliar  $\delta^{13}\text{C}$ , and MAP were performed to demonstrate that these correlations were not confounding factors with other relationships that we show between %  $\text{C}_4$  cover, root biomass, soil  $\delta^{13}\text{C}$ , and MAP. Pearson's correlations were also used to examine aboveground biomass of  $\text{C}_3$  and  $\text{C}_4$  species based on plant height (cm) and leaf area ( $\text{cm}^2$ ) values with MAP. Soil data were originally 45 samples, but averaged for each site to avoid pseudo-replication. All statistical analyses were performed in R (R-version 3.0.2, Welthandelsplatz, Austria).

### Results

Soil  $\delta^{13}\text{C}$  became enriched with increasing %  $\text{C}_4$  cover ( $P < 0.001$ ,  $r = 0.77$ ,  $n = 45$ ; Fig. 3) as expected based on  $^{13}\text{C}$  discrimination of  $\text{C}_4$  plants and common use of  $\delta^{13}\text{C}$  to infer %  $\text{C}_4$  productivity (Sage et al. 1999b). However, there was large variability in this relationship, particularly in plots with coexisting  $\text{C}_3$  and  $\text{C}_4$  species, as well as in plots receiving higher MAP (Fig. 4). In  $\text{C}_3$ -dominated sites, the average within-site soil  $\delta^{13}\text{C}$  variability ( $\pm 1$  SD) from the mean was  $\pm 0.4\%$ , whereas in wetter  $\text{C}_4$ -dominated sites, within-site variability was higher, averaging

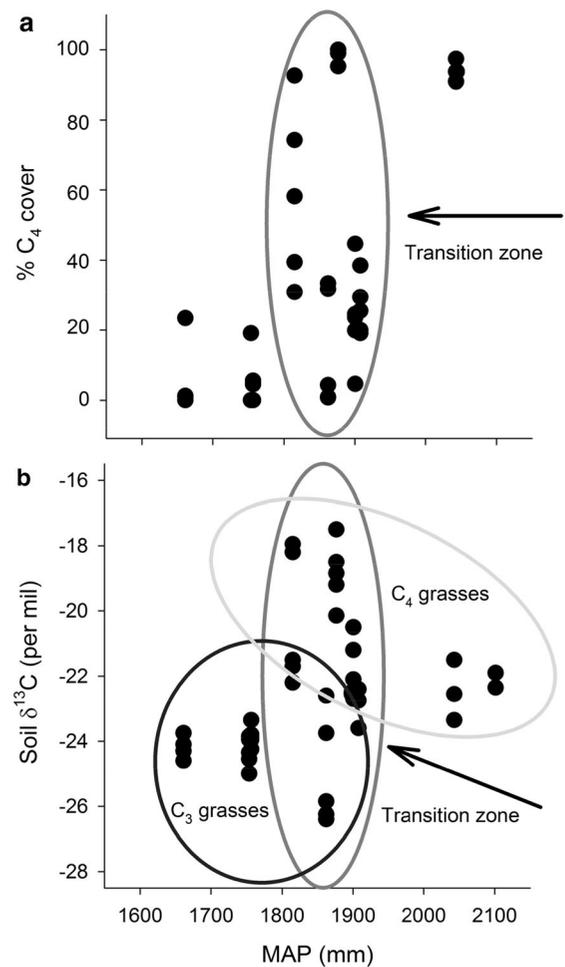


**Fig. 3** Relationship between % C<sub>4</sub> cover and soil δ<sup>13</sup>C (per mil) (solid line) and the expected relationship (gray dotted line) based on widely used C<sub>3</sub> (−26.7 ‰ ± 2.3) and C<sub>4</sub> (12.5 ‰ ± 1.1) endmembers (Cerling et al. 1997). See Table 1

±0.72 ‰, and the mixed C<sub>3</sub>–C<sub>4</sub> sites had within-site variability averaging ±1.6 ‰.

Indeed the best-fit model based on AIC and parsimony included the interaction with precipitation and showed that as precipitation increased, % C<sub>4</sub> cover contributed less to belowground soil δ<sup>13</sup>C values (Table 1;  $F_{1,34} = 12.25$ ,  $P < 0.01$ ). Sites with MAP between 1800 and 1900 mm were in the C<sub>3</sub>–C<sub>4</sub> transition zone (C<sub>3</sub> and C<sub>4</sub> cover ~50 %) where the relationship between % C<sub>4</sub> cover and soil δ<sup>13</sup>C began to change from the expected positive relationship to more variable and even negative (Fig. 4). Wet sites with C<sub>4</sub> coverage above 50 %, were found to have soil δ<sup>13</sup>C values around −23.5 ‰. Sites with C<sub>4</sub> coverage above 90 % were also found to have low soil δ<sup>13</sup>C values ranging from −18 to −24 ‰; these values are much lower than would be expected for high % C<sub>4</sub> cover and do not correspond with typical C<sub>4</sub> endmembers (Cerling et al. 1997).

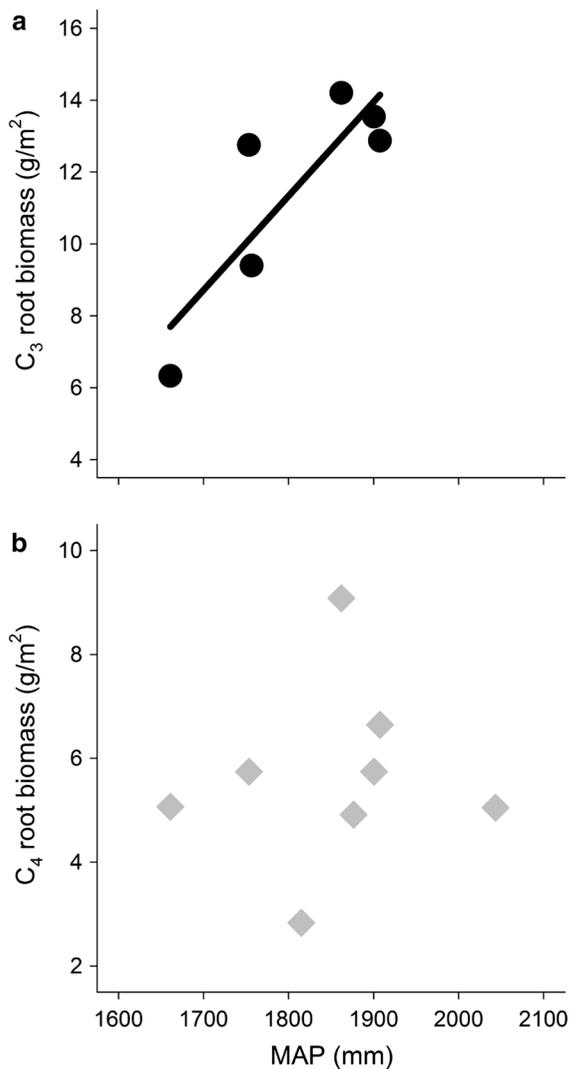
C<sub>3</sub> root biomass increased with increasing MAP ( $P = 0.03$ ,  $r = 0.85$ ,  $n = 6$ ; Fig. 5a). Individual C<sub>3</sub> species root biomass follows C<sub>3</sub> root biomass patterns with increasing MAP (Online Resource 1, Fig. OR1). Thus, at the wetter sites, where soil δ<sup>13</sup>C values appeared more depleted than % C<sub>4</sub> cover indicated, C<sub>3</sub> species were allocating more to roots than at drier



**Fig. 4** Percent C<sub>4</sub> cover and soil δ<sup>13</sup>C (per mil) along precipitation gradient. **a** Percent C<sub>4</sub> cover transitions from 0 to 100 % along climatic gradient (transition zone = area where C<sub>3</sub> and C<sub>4</sub> cover ~50 %) ( $n = 45$ ). **b** From 1800 to 1900 mm MAP, % C<sub>4</sub> cover ranges from 0 to 100 %; however, beyond 1876 mm MAP, soil δ<sup>13</sup>C values become more variable ( $n = 45$ )

sites. The correlation between C<sub>3</sub> root biomass and MAT was not significant ( $P = 0.07$ ,  $r = 0.78$ ,  $n = 6$ ). There were no significant correlations between C<sub>4</sub> root biomass and MAP or MAT ( $P = 0.78$ ,  $r = 0.01$ ,  $n = 8$ , Fig. 5b and  $P = 0.86$ ,  $r = -0.07$ ,  $n = 8$ ). Individual C<sub>4</sub> species root biomass follows C<sub>4</sub> root biomass patterns with increasing MAP (Online Resource 1, Fig. OR2).

Soil carbon content did not contribute to low soil δ<sup>13</sup>C values ( $P = 0.26$ ,  $r = -0.42$ ,  $n = 9$ ; Online Resource 1, Fig. OR3a) and did not vary across our



**Fig. 5** The relationship between mean annual precipitation (MAP) mm and C<sub>3</sub> and C<sub>4</sub> root biomass (g/m<sup>2</sup>). **a** C<sub>3</sub> root biomass (black dots) significantly increases at wetter sites compared with drier sites ( $P = 0.03$ ;  $r = 0.85$ ;  $n = 6$ ), and **b** C<sub>4</sub> root biomass (gray diamonds) shows no relationship with MAP ( $P = 0.78$ ;  $r = 0.01$ ;  $n = 8$ )

precipitation gradient ( $P = 0.89$ ;  $r = 0.05$ ,  $n = 9$ ; Online Resource 1, Fig. OR3b). In addition, low aboveground % C<sub>3</sub> woody cover was found at low C<sub>4</sub>-dominated sites (Online Resource 1, Fig. OR4). C<sub>3</sub> grasses did not have a larger litter contribution at wet sites [based on plant height (cm) and leaf area (cm<sup>2</sup>)], and remained relatively consistent across our gradient. Plant heights for C<sub>3</sub> and C<sub>4</sub> species did not vary across sites ( $P = 0.13$ ,  $r = -0.63$  and  $P = 0.20$ ,  $r = 0.47$ , respectively; Online Resource 1, Fig. OR5). Plant leaf

areas for C<sub>3</sub> and C<sub>4</sub> species did not vary across sites ( $P = 0.45$ ;  $r = 0.34$  and  $P = 0.18$ ,  $r = 0.53$ , respectively; Online Resource 1, Fig. OR6).

Substrate age ranged from intermediate (>140 to <2500 years) to old age (>2500 years) (Wolfe and Morris 1996), and soil  $\delta^{13}\text{C}$  values were not different across substrate age ( $P = 0.59$ ;  $r = 0.21$ ,  $n = 9$ ; Online Resource 1, Fig. OR7a). Foliar  $\delta^{13}\text{C}$  values for C<sub>3</sub> and C<sub>4</sub> grasses also did not vary with substrate age ( $P = 0.31$ ,  $r = -0.51$ ,  $n = 6$ ;  $P = 0.48$ ,  $r = 0.29$ ,  $n = 8$ ; respectively; see also Online Resource 1, Figs. OR7b, c). We saw a minimal difference in foliar  $\delta^{13}\text{C}$  values for C<sub>3</sub> and C<sub>4</sub> species with changes of 0.4 and 0.2 ‰, respectively, every 100 mm in precipitation. The overall changes in foliar  $\delta^{13}\text{C}$  across our gradient from dry to wet sites were 0.98 and 0.76 ‰ for C<sub>3</sub> and C<sub>4</sub> grasses, respectively. These differences in foliar  $\delta^{13}\text{C}$  were not significant along our precipitation gradient ( $P = 0.37$ ,  $r = -0.44$ ,  $n = 6$ ,  $\beta = -0.004$ ; and  $P = 0.36$ ,  $r = 0.37$ ,  $n = 8$ ,  $\beta = 0.002$ ; respectively). Thus, iWUEs were not significantly different across substrates or our precipitation gradient and did not contribute to lower soil  $\delta^{13}\text{C}$  values at wet C<sub>4</sub>-dominated sites.

## Discussion

Our first objective was to determine if soil  $\delta^{13}\text{C}$  values are proportional to the percent cover of C<sub>4</sub> grasses (i.e., the relative abundance of C<sub>4</sub>/C<sub>3</sub> plants). In our study, % C<sub>4</sub> cover tended to contribute less to soil  $\delta^{13}\text{C}$  than C<sub>3</sub> plants in wetter locations, despite an increase in aboveground cover. For example, sites with greater than 50 % C<sub>4</sub> cover had relatively depleted soil  $\delta^{13}\text{C}$  values (circa  $-23.5$  ‰), and even sites with C<sub>4</sub> coverage above 90 % were found to have low soil  $\delta^{13}\text{C}$  values (i.e.,  $-18$  to  $-24$  ‰) in the same range as that of mixed C<sub>3</sub>–C<sub>4</sub> sites. The low soil  $\delta^{13}\text{C}$  values at sites with >90 % C<sub>4</sub> coverage are considerably more depleted than widely used C<sub>4</sub> endmembers, which are known to be around  $-12.5 \pm 1.1$  ‰ (Cerling et al. 1997). We also found larger variability in soil  $\delta^{13}\text{C}$  values at C<sub>4</sub>-dominated sites compared with sites dominated by C<sub>3</sub> grasses. This suggests that aboveground % C<sub>4</sub> cover does not always indicate a proportional contribution to belowground soil carbon pools. At our sites, using soil  $\delta^{13}\text{C}$  to infer % C<sub>4</sub> cover would result in an underestimate of C<sub>4</sub> dominance in

regions that receive greater than  $\sim 1876$  mm MAP. Our results are in contrast to other regional-scale studies that found soil  $\delta^{13}\text{C}$  values represented above-ground cover of  $\text{C}_4$  grasses with high fidelity (Tieszen et al. 1997; Sage et al. 1999b; von Fischer et al. 2008). Differences in our results may be due to our sites receiving greater MAP compared with studies conducted in temperate grasslands. First, the wetter sites in our case indeed exhibited the largest deviation from typically used  $\text{C}_4$  endmembers. Second, at the regional-scale, the relationship between %  $\text{C}_4$ , soil  $\delta^{13}\text{C}$ , and climate could be too coarse to detect changes associated with mixed  $\text{C}_3$ – $\text{C}_4$  grasslands. Here our data were collected at the plot level and encompassed a transition from  $\text{C}_3$  to  $\text{C}_4$  grasses, allowing for a more mechanistic understanding of soil  $\delta^{13}\text{C}$  values at smaller scales. Studies conducted at fine scales where  $\text{C}_3$  and  $\text{C}_4$  species coexist will be the most informative to answer questions about resource partitioning and help us determine if aboveground and belowground patterns are comparable when competition is occurring. These results can help refine our use of soil  $\delta^{13}\text{C}$  to determine  $\text{C}_4$  dominance across space or time.

Our second objective was to determine if PFT root biomass changes in response to climate variation.  $\text{C}_3$  root biomass was found to have greater plasticity in response to climate compared with  $\text{C}_4$  root biomass.  $\text{C}_3$  root biomass at wetter sites appears to be contributing more to soil  $\delta^{13}\text{C}$  than is explained simply by aboveground  $\text{C}_3$ – $\text{C}_4$  patterns. Temperature and precipitation have been shown to influence root distributional patterns at different spatial and temporal scales (Hui and Jackson 2006; Mokany et al. 2006; Luo et al. 2013); however, little research has been done in tropical areas (Wilson 2014). Our data showed that  $\text{C}_3$  root biomass increased with increasing MAP, while  $\text{C}_4$  root biomass did not change with MAP.  $\text{C}_3$  root allocation has typically been found to be the greatest in drier habitats compared with wetter regions (Mokany et al. 2006; Nippert and Knapp 2007a, b; Luo et al. 2013), while relatively consistent  $\text{C}_4$  root patterns across gradients of water availability have been previously observed (Nippert and Knapp 2007a, b; Nippert et al. 2012). Furthermore, Hui and Jackson (2006) found no general patterns with respect to belowground primary productivity and climate (MAT and MAP) in tropical systems dominated by  $\text{C}_4$  grass species. These results suggest that the disproportionate allocation of  $\text{C}_3$ – $\text{C}_4$  root biomass is, at least in part,

contributing to more negative soil  $\delta^{13}\text{C}$  values at sites with substantial %  $\text{C}_4$  cover.

To reduce competition between  $\text{C}_3$  and  $\text{C}_4$  plants at the same location, different belowground allocation patterns have been demonstrated (Fargione and Tilman 2005; Nippert and Knapp 2007a). Nippert and Knapp (2007a) suggest that increased competition for water may occur when it is not limiting. Luo et al. (2013) also found a trade-off between aboveground and belowground growth patterns in both  $\text{C}_3$  and  $\text{C}_4$  grass genera in response to variations in climate; however, PFT responses to climate were not the same and may be specific to each PFT. Moreover, changes in allocation patterns for  $\text{C}_3$  species were more influenced by precipitation, whereas  $\text{C}_4$  root biomass was more controlled by temperature (Luo et al. 2013). Similarly, our results revealed that  $\text{C}_3$  root biomass was more sensitive to changes in precipitation in comparison with  $\text{C}_4$  root biomass.

The amount of woody tissue present can affect the relationship between soil  $\delta^{13}\text{C}$  and %  $\text{C}_4$  cover because lignin-rich woody tissue is more depleted in the  $^{13}\text{C}$  isotope and may be selectively preserved in SOM compared with grass tissues (Benner et al. 1987; Bird et al. 1994). Although all our sites had an open canopy, there were occasional woody species in our plots (they were included in our %  $\text{C}_4$  cover—see “Methods” section) (Online Resource 1, Fig. OR4). Sites with high %  $\text{C}_4$  cover have low aboveground woody cover, but have depleted soil  $\delta^{13}\text{C}$  values, suggesting that woody root biomass could in part be contributing to our observed soil patterns.

Other mechanisms in addition to differences in  $\text{C}_3$  root biomass may contribute to the changing relationship between %  $\text{C}_4$  cover and soil  $\delta^{13}\text{C}$  in our data. The amount of total soil carbon content can influence soil  $\delta^{13}\text{C}$  values (Balesdent et al. 1993; Bird and Pousai 1997; Ehleringer et al. 2000). Bird and Pousai (1997) showed a negative relationship between soil  $\delta^{13}\text{C}$  and carbon content across a variety of biomes in Australia. However, our data did not show that soil  $\delta^{13}\text{C}$  decreased with increasing soil carbon content (Online Resource 1, Fig. OR3a), and importantly soil carbon content did not increase at wetter sites (Online Resource 1, Fig. OR3b). Thus, there is no evidence that differences in soil carbon content explain the depleted soil  $\delta^{13}\text{C}$  values in our wetter sites.

Unequal decomposition rates of  $\text{C}_3$ - and  $\text{C}_4$ -derived carbon could result in the depleted soil  $\delta^{13}\text{C}$  values in

our wet C<sub>4</sub>-dominated sites. Wynn and Bird (2007) similarly showed that soil  $\delta^{13}\text{C}$  values across environmental gradients throughout Australia were relatively depleted and not representative of C<sub>4</sub>-dominated sites. They attributed this pattern to the faster rate of decomposition of C<sub>4</sub>-derived carbon compared with C<sub>3</sub>-derived carbon in incubation experiments. Bulk soil organic carbon from mixed C<sub>3</sub>–C<sub>4</sub> vegetation exhibited a negative shift of  $\delta^{13}\text{C}$  in the range of 1–3 ‰, which may partially contribute to our observed patterns. However, our data show a negative shift in  $\delta^{13}\text{C}$  by as much as 5–11 ‰. Even sites with minimal C<sub>3</sub> presence (<50 %) had very depleted  $\delta^{13}\text{C}$  values relative to expected endmembers.

Another important consideration is that  $\delta^{13}\text{C}$  values of C<sub>3</sub> plants may be lower (more negative) in wet sites compared with dry sites because of changes in their iWUE (Murphy and Bowman 2009; Cernusak et al. 2013). The isotopic  $^{13}\text{C}/^{12}\text{C}$  ratio is determined at the leaf-level in C<sub>3</sub> plants from the relationship between  $c_i/c_a$  (internal CO<sub>2</sub> concentrations in relation to atmospheric CO<sub>2</sub> concentrations), and this relationship provides an estimate of a plant's iWUE. A reduction in stomatal conductance under water-stress can change the relationship between  $c_i/c_a$ ; thus, the heavier  $^{13}\text{C}$  isotope is discriminated against less in drier sites, resulting in more enriched  $\delta^{13}\text{C}$  values and higher iWUEs (Farquhar et al. 1989). However, we found that the change in foliar  $\delta^{13}\text{C}$  values for C<sub>3</sub> and C<sub>4</sub> were not significantly different across our precipitation gradient. C<sub>3</sub> and C<sub>4</sub> foliar  $\delta^{13}\text{C}$  values only changed by 0.98 and 0.76 ‰, respectively, from dry to wet sites.

Most of our C<sub>4</sub>-dominated sites appear to occur in regions that have been C<sub>4</sub>-dominated for at least the last 45 years. C<sub>3</sub> and C<sub>4</sub> grass distributional patterns along our transect have been surveyed multiple times in the last 45 years (Newell 1968; Angelo and Daehler 2013, 2015). Rundel (1980) identified the transition point for C<sub>3</sub>–C<sub>4</sub> grass turnover to be at ~1400 m using historical data collected by Newell (1968). However, Angelo and Daehler (2013) identified the historical transition point at ~1200 m using a logistic regression. Present-day estimates for the transition point between C<sub>3</sub>–C<sub>4</sub> dominance are in the range of around 1440–1480 m (Angelo and Daehler 2013, 2015). Based on the modeled transition point of 1200 m, four of our nine sites were in an area that

potentially experienced changes in C<sub>3</sub>–C<sub>4</sub> abundance over the last 45 years, however; only two of them show an irregular relationship between soil  $\delta^{13}\text{C}$  values and % C<sub>4</sub> cover. In addition, Townsend et al. (1995) found that 75 % of carbon in the top 20 cm of soil has a residence time of 30 years or less in Hawai'i, suggesting that our soils are accurately reflecting the current vegetation. Other studies have additionally found carbon turnover times to be faster in tropical low-elevation regions compared with temperate low-elevation regions (Bird et al. 1996). Nonetheless, at two of our sites, we cannot rule out an invasion of C<sub>4</sub> grasses into a previously C<sub>3</sub>-dominated site as a potential explanation for the depleted soil  $\delta^{13}\text{C}$  values we observed.

Tropical grasslands are known to have a higher rate of annual root turnover compared with other biomes with rates increasing at higher temperatures (Gill and Jackson 2000). In C<sub>4</sub>-dominated tropical grasslands, interannual variability in belowground productivity has also been found to be greater compared with temperate regions (Hui and Jackson 2006). Our C<sub>3</sub> and C<sub>4</sub> root biomass differences from the dry season may not reflect total annual production or interannual variability in root biomass. Similarly, our results showed greater variability in C<sub>3</sub> root biomass compared with C<sub>4</sub> root biomass in response to changes in precipitation. If these spatial patterns are robust across temporal gradients of precipitation, C<sub>4</sub> root biomass would not vary substantially across seasons. For the effect of greater C<sub>3</sub> root biomass in the dry season to be unimportant (i.e., to have no effect at all) in the depleted  $\delta^{13}\text{C}$  values we document, C<sub>4</sub> root biomass would need to substantially increase and C<sub>3</sub> root biomass would need to decrease at C<sub>4</sub>-dominated sites during the wet season. Although there is no evidence that C<sub>3</sub> and C<sub>4</sub> root biomass show complete reversals across seasons, further studies with belowground sampling during different seasons would be valuable.

## Conclusion

Little is known about the belowground components of C<sub>3</sub> and C<sub>4</sub> grasslands (Mokany et al. 2006), especially in the tropics, despite substantial research on aboveground distributional patterns. Here we show that soil  $\delta^{13}\text{C}$  may underestimate C<sub>4</sub> dominance in wetter tropical grasslands in part due to C<sub>3</sub> and C<sub>4</sub> grasses

allocating disproportionately belowground. Although it is unclear if our results are applicable to drier grasslands, such as in warm temperate zones, similar patterns of  $C_3$  root plasticity in response to changes in water availability have emerged in both areas (Nippert and Knapp 2007a, b). Our results show that widely observed patterns of  $C_3$ – $C_4$  dominance may be very different when considering belowground ecosystem function.

The potential shifts in soil  $\delta^{13}C$  due to the differences in  $C_3$  and  $C_4$  decomposition (1–3 ‰) (Wynn and Bird 2007) and foliar  $\delta^{13}C$  values (<1 ‰), may also contribute; however, these effects are considerably smaller than the 5–11 ‰ shift we show at  $C_4$ -dominated sites compared with commonly used  $C_4$  endmembers (Cerling et al. 1997). At two of our sites, we cannot rule out the potential recent invasion of  $C_4$  grasses into previously  $C_3$ -dominated sites that may in part explain the depleted  $\delta^{13}C$  values observed; however, soil carbon turnover is typically less than 30 years in our study site region; thus, our soil  $\delta^{13}C$  values likely reflect current vegetation.

Our findings have important implications considering that  $C_3$ – $C_4$  grasslands are predicted to shift in response to future climate and atmospheric  $CO_2$  changes. Using statistical modeling, Epstein et al. (2002) have shown that  $C_4$  grasses may increase with climate change in North and South America compared with  $C_3$  grasses based on temperature, while experimentally based studies have found increases in biomass for both  $C_3$  (Ainsworth and Long 2005) and  $C_4$  grasses with elevated  $CO_2$  concentrations (Ward et al. 1999; Morgan et al. 2011). Furthermore, understanding current patterns of belowground dominance is imperative for our ability to predict future ecosystem level impacts with shifting  $C_3$  and  $C_4$  grass distributions. Carbon isotopes are widely used to indicate  $C_4$  dominance across space and time in modern and paleoecological models of vegetation and climate dynamics, and future studies should consider belowground ecological dominance.

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