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Research

Long-term nitrogen isotope dynamics in *Encelia farinosa* reflect plant demographics and climate

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Summary

- While plant $\delta^{15}N$ values have been applied to understand nitrogen (N) dynamics, uncertainties regarding intraspecific and temporal variability currently limit their application. We used a 28 yr record of $\delta^{15}N$ values from two Mojave Desert populations of *Encelia farinosa* to clarify sources of population-level variability.
- We leveraged > 3500 foliar $\delta^{15}N$ observations collected alongside structural, physiological, and climatic data to identify plant and environmental contributors to $\delta^{15}N$ values. Additional sampling of soils, roots, stems, and leaves enabled assessment of the distribution of soil N content and $\delta^{15}N$, intra-plant fractionations, and relationships between soil and plant $\delta^{15}N$ values.
- We observed extensive within-population variability in foliar $\delta^{15}N$ values and found plant age and foliar %N to be the strongest predictors of individual $\delta^{15}N$ values. There were consistent differences between root, stem, and leaf $\delta^{15}N$ values (spanning *c.* 3‰), but plant and bulk soil $\delta^{15}N$ values were unrelated.
- Plant-level variables played a strong role in influencing foliar $\delta^{15}N$ values, and interannual relationships between climate and $\delta^{15}N$ values were counter to previously recognized spatial patterns. This long-term record provides insights regarding the interpretation of $\delta^{15}N$ values that were not available from previous large-scale syntheses, broadly enabling more effective application of foliar $\delta^{15}N$ values.

Introduction

Nitrogen isotope ratios ($\delta^{15}N$) serve as an integrated indicator of complex nitrogen (N) cycling processes and are commonly used to understand soil N transformations, N fixation, and plant N uptake (Handley & Raven, 1992; Högberg, 1997; Evans, 2001; Robinson, 2001; Hobbie & Ouimette, 2009; Craine *et al.*, 2015a). Foliar $\delta^{15}N$ values reflect both the isotopic composition of the N source to the plant as well as fractionations that occur during N uptake, assimilation, and reallocation. Although foliar N isotope ratios are easy to measure, interpreting the drivers of $\delta^{15}N$ values is often not straightforward.

Broadly, soil $\delta^{15}N$ values are thought to represent the 'openness' of the N cycle, or the relative dominance of within-system N cycling as opposed to inputs and outputs (Austin & Vitousek, 1998; Martinelli *et al.*, 1999). Soil $\delta^{15}N$ values tend to increase with increasing temperature and decrease with increasing precipitation across spatial gradients (Craine *et al.*, 2015b). However, global relationships between soil $\delta^{15}N$ values and climate are largely indirect and can be influenced a number of other variables, including soil depth (Natelhoffer & Fry, 1988; Jobbágy & Jackson, 2001; Hobbie & Ouimette, 2009), soil age (Brenner *et al.*, 2001), elevation (Wang *et al.*, 2019), dominant plant species (Templer *et al.*, 2007), and clay and carbon concentrations (Craine *et al.*, 2015b). Soil pH is an important underlying driver of broad spatial patterns in soil $\delta^{15}N$ values, as arid land soils tend to be alkaline and therefore susceptible to gaseous losses of NH_3 (McCalley & Sparks, 2009).

Although trends in foliar $\delta^{15}N$ values tend to broadly parallel those in soils, they have also been associated with other factors, including mycorrhizal type (Evans, 2001; Craine *et al.*, 2009; Hobbie & Högberg, 2012; Schweiger, 2016), photosynthetic pathway (Luo *et al.*, 2018), relative uptake of nitrate vs ammonium (Evans, 2001), CO_2 concentration (Billings *et al.*, 2002a; Koyama *et al.*, 2019), and soil N availability (Craine *et al.*, 2009, 2015a). Given the large number of potential drivers of leaf $\delta^{15}N$ and our relatively limited understanding of the mechanisms underlying large-scale patterns, effective ecological interpretation of leaf $\delta^{15}N$ values remains challenging.

Fractionations that occur during and after N uptake from soil further complicate relationships among environmental drivers, soil $\delta^{15}N$ and foliar $\delta^{15}N$. Large fractionations are known to occur during uptake of nitrate from soils with high N availability and during assimilation of nitrate (Evans, 2001). Although fractionation has not been observed during reabsorption of N during leaf senescence, reallocation of stored N has been identified as a fractionating process (Kolb & Evans, 2002). Additionally, leaf–atmosphere exchange of gaseous NH_3 occurs during photosynthesis, resulting in further fractionation in leaves and photosynthetic stems (Farquhar *et al.*, 1983; Johnson & Berry, 2013).

Substantial variation in $\delta^{15}N$ values among roots, stems, and leaves of individual plants has also been widely documented (Evans, 2001; Kolb & Evans, 2002; Kalcsits *et al.*, 2015) and is thought to be primarily associated with the site of N assimilation or with intra-plant reallocation. Because of the potential for differences in $\delta^{15}N$ values among plant tissues, several studies have suggested that foliar $\delta^{15}N$ values should not be used as a direct proxy for whole-plant $\delta^{15}N$ values (Evans, 2001; Kolb & Evans, 2002; Kalcsits *et al.*, 2014). Within-plant fractionations in N isotopes are often assumed to be constant across individuals within a given species. However, it is clear that there is intraspecific variability in some of these potentially fractionating processes, such as N storage potential and photosynthetic rate, which may contribute to intraspecific variability in plant $\delta^{15}N$ values in addition to source effects. This underexplored source of variability has important ramifications for conclusions about ecosystem N cycling that are drawn from plant $\delta^{15}N$ data.

Uncertainties regarding the interpretation of $\delta^{15}N$ values are exacerbated in ecosystems where N cycling is variable across space and time. Deserts in particular are ideal for studying variability in plant $\delta^{15}N$, as the rates of biotic and abiotic N processing in desert soils are highly heterogenous and contingent on the presence of biocrusts, location relative to vegetation (i.e. under a plant canopy or between canopies), and the timing and intensity of precipitation events and high temperatures (Evans & Ehleringer, 1994; Schlesinger *et al.*, 1996; Gebauer & Ehleringer, 2000; Schade & Hobbie, 2005; James & Richards, 2007; McCalley & Sparks, 2008, 2009; Koyama *et al.*, 2019). As a result of these heterogeneous nutrient inputs and losses, areas of relatively high nutrient concentration ('nutrient islands' or 'islands of fertility') tend to form beneath desert perennials amidst very low nutrient availability in plant interspaces (Charley & West, 1975; Schlesinger & Pilmanis, 1998; Whitford, 2002; Ridolfi *et al.*, 2008). While substantial work has been done to understand plant–soil interactions and their role in N cycling in arid ecosystems, measurements of foliar N isotope ratios in deserts are far less abundant relative to other ecosystems (Wang *et al.*, 2014). Additionally, the majority of ecological studies that use natural abundance $\delta^{15}N$ values rely on data collected from a limited subset of plants at a single point in time and often fail to quantify intraspecific or intrasite variability in $\delta^{15}N$ values. These limitations hinder a broader understanding of the drivers of variability in $\delta^{15}N$ values operating over time and within populations.

Here, we use a three-decade record of annual foliar $\delta^{15}N$ values, survey data, and additional measurements of soil, root, stem, and leaf $\delta^{15}N$ values from two populations of *Encelia farinosa*, a common perennial shrub in the Mojave Desert, to address four questions regarding interpretation of foliar $\delta^{15}N$ values:

(**1**) To what extent does interannual variation in climate influence foliar $\delta^{15}N$ values?
(**2**) What plant-level factors other than the $\delta^{15}N$ value of the N source drive intraspecific variability in foliar $\delta^{15}N$ values?
(**3**) How well do foliar $\delta^{15}N$ values reflect whole-plant $\delta^{15}N$ values?
(**4**) How strongly is foliar $\delta^{15}N$ driven by soil $\delta^{15}N$ at small spatial scales?

Materials and Methods

Site descriptions and annual surveys

Two near-monospecific stands of *Encelia farinosa* A. Gray ex Torr. were identified in the early 1980s, and rectangular plots were marked for continuous, long-term monitoring. Sites with adequate *E. farinosa* abundance were selected to represent the northern and southern Mojave Desert based on their likelihood of long-term preservation (see Supporting Information Fig. S1 for a map of site locations). The first plot is located *c.* 21 km southwest of Shoshone, CA, covering *c.* 450 m^2 of a south-facing footslope in Death Valley National Park (referred to as the 'Death Valley' site). The second plot is *c.* 315 m^2 and is located on a southwest-facing footslope *c.* 8 km southwest of Oatman, AZ (referred to as the 'Oatman' site). Mean annual precipitation was 169 mm at the Oatman site and 131 mm at the Death Valley site (1981–2010 PRISM normals), with late-summer monsoonal rains slightly more common at the Oatman site. Mean annual temperature was 21.1°C at the Oatman site and 19.4°C at the Death Valley site (1981–2010 PRISM normals). Soils at the Oatman site were a colluvium derived from volcanic rock with a sandy loam texture, a pH of 7.9, and 0.5% organic matter (derived from the Soil Survey Geographic Database, Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture, 2019). Soils data from the Death Valley site were not available in the Soil Survey Geographic Database, but data from a research station in the northern Mojave Desert with comparable climate and vegetation structure (*c.* 107 km away from the site) indicate clay aridisols derived from calcareous alluvium with a loamy sand texture and a pH of 8.3 (Titus *et al.*, 2002).

The sites were surveyed annually during the last 2 wk of March, beginning in 1981 at the Death Valley site and 1982 at the Oatman site. *Encelia farinosa* shrubs in this area typically leaf out with precipitation in late fall, undergo several rounds of leaf turnover over the growing season, and abscise their leaves in mid-to-late spring at the onset of the summer drought period (Ehleringer & Sandquist, 2018). The March sampling date occurs towards the end of the growing season, allowing for measurement of current-season growth and reproductive status and for collection of leaves that primarily reflect carbon assimilated in the current growing season rather than stored carbon (Comstock & Ehleringer, 1986; Van Der Heyden & Stock, 1996).

In the first survey year, all plants aged 1 yr or older (as indicated by the presence of a woody basal stem) were tagged and their coordinates on an *X*, *Y* grid were recorded. Each year thereafter, new individuals were tagged and added to the record if they were 1 yr old. Plants were not considered to be dead until they were entirely leafless for three consecutive years, at which point tags were removed and no further data were recorded. During annual surveys, we measured plant height and width at the widest and perpendicular widths, recorded any signs of flowering (including the presence of flowers, buds, or seed heads), and estimated leaf cover on a 1–4 scale (with a value of 1 corresponding to leaf cover of 0–25% of the canopy area and a value of 4 corresponding to leaf cover of 76–

100%). *Encelia farinosa* shrub canopies have a hemispherical shape with leaf growth concentrated toward the ends of branches (Ehleringer & Sandquist, 2018); we calculated projected hemispherical surface area (HSA) as an indicator of total plant size using the mean of plant height, half of the width along the widest axis, and half of the width along the perpendicular axis as the plant radius. In the Death Valley population, 1503 unique plants were surveyed for a total of 9150 sets of annual observations over 39 yr. In the Oatman population, 1341 unique plants were surveyed for a total of 9126 sets of annual observations over 38 yr. The Oatman population has experienced complete turnover since the initiation of the study, and three individuals from the original Death Valley population were still alive as of the 2021 survey. In addition to survey measurements, five to 10 of the most recently produced, mature sun leaves were collected from each plant for analysis of carbon and N concentrations and stable isotope ratios. Leaf collection began in 1989 at the Oatman population and in 1991 at the Death Valley population. Leaves were not collected from plants that would have been damaged by leaf collection, and not all plants produced leaves in every year (especially during extreme drought years).

Destructive sampling of soils, roots, stems, and leaves

In January 2021, we conducted additional sampling from an area *c.* 5–15 m outside of the plot boundary at the Oatman site. We collected leaf, stem, and root samples from 20 individuals to assess variability in $\delta^{15}N$ and %N values within and among plant tissues. Before sampling, we measured the height, width, and perpendicular width of each shrub, recorded any signs of flowering in the current or previous year, and recorded current leaf cover on a 1–4 scale. From each individual, we collected three sets of leaf samples from different locations on the plant, each comprising five leaves from a single branch. We also collected three, 3 cm stem segments of varying diameters, including one green, small-diameter segment, one segment from a woody branch, and one segment from the main stem of the plant. Green stems were non-woody, photosynthetically active, and supported the current year's leaves. We excavated soil directly under the plant base and obtained three root samples within 10 cm of the soil surface, including one sample of the primary root, one sample of a second- or third-order coarse root, and one sample of several fine (< 2 mm) roots. No fine roots were identifiable for two of the sampled shrubs; in these cases, a second coarse root sample was collected. The diameter of each stem and root sample was measured before grinding for analysis. We avoided destructive sampling inside of the long-term monitoring plot, and therefore did not know the exact ages of the sampled plants. In an effort to capture a variety of plant ages, we collected samples from plants ranging in HSA from 0.067 to 2.61 m^2. Long-term survey data from this population indicate a moderately strong correlation between plant HSA and age ($y = 6.89x + 2.82$, $r^2 = 0.56$, $P < 0.0001$), suggesting that the destructively sampled plants could have ranged in age from *c.* 3 to 21 yr.

We collected shallow surface soils at two depths (0–2 and 3–5 cm) underneath the canopy of each of the sampled plants, as well as in 20 interspaces located at least 1.5 m from the nearest plant. Coarse leaf litter was minimal and removed before collecting shallow surface soil samples. We also excavated four soil pits to assess trends in $\delta^{15}N$ and %N throughout the depth profile. Two pits were located directly underneath large, well established shrubs and two were located in interspaces at least 2 m from the nearest plant. Two pits (one interspace and one subcanopy pit) were excavated to a depth of 50 cm, with three soil samples collected at each of the following depths: 0–2, 3–5, 10–15, 20–25 and 45–50 cm. An impenetrable caliche rock layer was reached at 25 cm in the second subcanopy pit and at 35 cm in the second interspace pit, so the final soil sample was collected from the deepest 5 cm of these two pits.

Isotope analyses

Plant samples were dried upon returning from the field and stored in a cool, dark, dry place until analysis was conducted. Selected samples were ground and loaded into tin capsules for analysis of carbon and N isotope ratios and concentrations using a Carlo-Erba EA-1110 elemental analyzer coupled to a Finnigan Mat Delta+ IRMS via a continuous flow interface (Conflo III; ThermoFinnigan, Bremen, Germany). Laboratory reference materials were calibrated using international standards USGS40 ($\delta^{13}C = -26.24‰$ and $\delta^{15}N = -4.5‰$) and USGS41 ($\delta^{13}C = 37.76‰$ and $\delta^{15}N = 47.6‰$). Results were reported in delta notation relative to VPDB for carbon and atmospheric N_2 for N. Long-term measurement uncertainty for quality control materials was ±0.2‰ for $\delta^{13}C$, ±0.2‰ for $\delta^{15}N$, and ±0.03% for N concentration. A total of 2114 leaf samples from 591 plants over 26 yr from the Death Valley population and 1753 leaf samples from 578 plants over 28 yr from the Oatman population were analyzed. Additional soil and plant samples collected in January 2021 were analyzed using the same methods, with a total of 48 root, 46 stem, 48 leaf, and 137 soil samples.

Data acquisition

We acquired daily data on total precipitation, maximum vapor pressure deficit (VPD), and minimum and maximum temperatures for each site from the PRISM Climate Group datasets (4 km resolution; http://www.prism.oregonstate.edu/; accessed 15 September 2020). For the Death Valley population, we used climate data from the grid cell containing Shoshone, CA, because the presence of a weather station in Shoshone improved the reliability of the data. For the Oatman population, we used the grid cell containing the site coordinates. We also accessed the 5 month March Standardized Precipitation-Evapotranspiration Index (SPEI) at each site to better capture drought stress with a single variable (Beguería *et al.*, 2014; accessed 22 March 2021). We identified the period from November, corresponding to initial typical leaf-out, through March, when surveys were conducted, as the climatic window most relevant for plant growth and leaf isotope composition. Thus, climate data included throughout are averages over the November–March growing season unless otherwise specified.

We obtained data on the monthly average concentration of atmospheric CO_2 in Wendover, UT, from the Earth System Research Laboratory Global Monitoring Division of the National Oceanic and Atmospheric Administration (NOAA ESRL; Dlugokencky *et al.*, 2021). Although other NOAA ESRL sites have longer-term datasets, we used data from the Wendover site as it is similar to the study sites in latitude, aridity, vegetation, and proximity to urban areas. CO_2 concentration data were available for 1993–2019, and values for missing years were extrapolated using the linear relationship between year and CO_2 concentration at Wendover, UT ($r^2 = 0.996$, $y = 2.108x - 3846$).

Data analysis

All data analyses were conducted in R v.3.6.1 (R Core Team, Vienna, Austria). We graphically assessed data for normality and equal variance across groups using histograms when parametric tests for comparisons of means were used. Residuals of ordinary least-squares (OLS) regressions were graphically examined for normality and homoscedasticity using residual and quantile-quantile plots. Data were transformed to meet parametric assumptions if needed. We compared the annual mean $\delta^{15}N$ and %N values between populations using *t*-tests paired by year. We analyzed temporal trends in annual population mean $\delta^{15}N$ and %N values using generalized least-squares (GLS) regression with a first-order autoregressive structure to account for temporal autocorrelation. Results of GLS regressions were not qualitatively different from results of OLS regression. To identify climatic drivers of interannual variations, we conducted simple linear regressions of annual mean $\delta^{15}N$ values by total growing season precipitation, mean maximum temperature, and mean maximum VPD for each individual population and across both populations. We also used linear regressions to test for effects of annual climate parameters on $\delta^{15}N$ values, including mean minimum and maximum temperatures, total precipitation, and mean maximum VPD. Three additional variables, including the number of days with > 1 mm precipitation, total June–September precipitation, and the number of days reaching > 35°C, were selected for regressions because they have been suggested to strongly influence ammonia losses in desert soils (McCalley & Sparks, 2008), a process that causes large fractionations that may ultimately be reflected in foliar $\delta^{15}N$ values.

We fit a linear mixed-effects model of $\delta^{15}N$ values (using package LME4; Bates *et al.*, 2019) to understand the drivers of individual-scale $\delta^{15}N$ values. Shrub ID was included as a random intercept, and all measured environmental and plant-level variables likely to affect N cycling were included as fixed effects in the full model. Continuous variables were converted to *z*-scores (centered and scaled) before inclusion in the model. For plants with an unknown age that germinated before the beginning of the survey period (184 individuals corresponding to 784 observations), we conservatively assumed that the plant was established in the year before the first survey. The model with the lowest Akaike information criterion was selected by stepwise deletion of each fixed effect. *P*-values for fixed effects were estimated using the Satterthwaite's degrees of freedom method implemented through the package LMERTEST (Kuznetsova *et al.*, 2017).

We tested for differences in $\delta^{15}N$ and %N between root, green stem, woody stem, and leaf tissues using one-way ANOVAs followed by Tukey's *post hoc* test of honest significant differences (HSD). We used linear regressions to test for relationships between $\delta^{15}N$ and %N values and plant HSA for roots, green stems, woody stems, and leaves, and between tissue diameter and $\delta^{15}N$ and %N for roots, woody stems, and green stems. For shallow surface soils, we used *t*-tests to assess differences in $\delta^{15}N$ and %N between subcanopy and interspace soils and paired *t*-tests to assess differences between the 0–2 and 3–5 cm depths. We used linear regressions to test for the relationship between log-transformed %N and $\delta^{15}N$ for roots, woody stems, green stems, leaves, surface soils, and depth profile soils separately, as well as for all plant tissues and all soil samples grouped together. Finally, we used linear regressions to test for relationships between $\delta^{15}N$ and %N in subcanopy shallow surface soil samples (average of the 0–2 and 3–5 cm depths) and all tissues from the overlying shrub.

Results

Interannual fluctuations and climatic drivers of population mean $\delta^{15}N$ and %N values

Over the study period, $\delta^{15}N$ values of the Death Valley population (mean = 3.22‰) were significantly higher than those of the Oatman population (mean = 0.61‰, $P < 0.0001$). Measured $\delta^{15}N$ values spanned wide ranges of 7.49‰ in the Death Valley population (range: −0.73–6.76‰) and 9.32‰ in the Oatman population (range: −4.12–5.20‰). Variance in $\delta^{15}N$ values was large among individuals within a given year (1.20‰ on average in Death Valley and 1.47‰ in Oatman) relative to interannual variance in population mean $\delta^{15}N$ values (0.53‰ in Death Valley and 0.56‰ in Oatman; Fig. 1). Although slight fluctuations in $\delta^{15}N$ values occurred from year to year, population mean $\delta^{15}N$ values did not significantly increase or decrease over the study period ($P = 0.25$ for Death Valley, $P = 0.83$ for Oatman) and were statistically unrelated to CO_2 concentration ($P = 0.25$ for Death Valley, $P = 0.73$ for Oatman). Annual average foliar N concentration (%N) was similar for the two populations (Death Valley mean = 2.64%, Oatman mean = 2.54%; $P = 0.87$). %N values fluctuated from year to year (Fig. 2; interannual variance = 0.70% in Death Valley and 0.55% in Oatman), but they also did not exhibit any temporal trends over the study period ($P = 0.55$ for Death Valley, $P = 0.46$ for Oatman) and were statistically unrelated to CO_2 concentration ($P = 0.56$ for Death Valley, $P = 0.17$ for Oatman).

In the Death Valley population, interannual variations in population mean leaf $\delta^{15}N$ values were significantly and positively related to growing season precipitation ($r^2 = 0.15$, $P = 0.027$; Fig. 3a) and significantly and negatively related to growing season VPD ($r^2 = 0.15$, $P = 0.027$; Fig. 3c), but not significantly related to growing season maximum temperature ($r^2 = 0.077$, $P = 0.091$; Fig. 3b). In the Oatman population, mean $\delta^{15}N$ values were

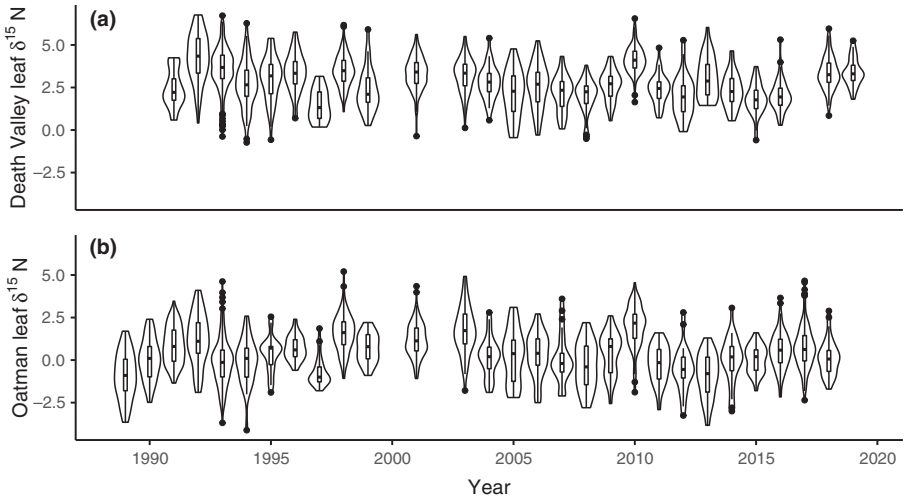

Fig. 1 Violin plots of measured leaf $\delta^{15}N$ values from *Encelia farinosa* from the Death Valley population (a) and Oatman population (b) over the study period. Inlaid boxes indicate the first quartile (Q1), median, and third quartile (Q3), and whiskers indicate Q3 and Q1 ± 1.5 × interquartile range, respectively.

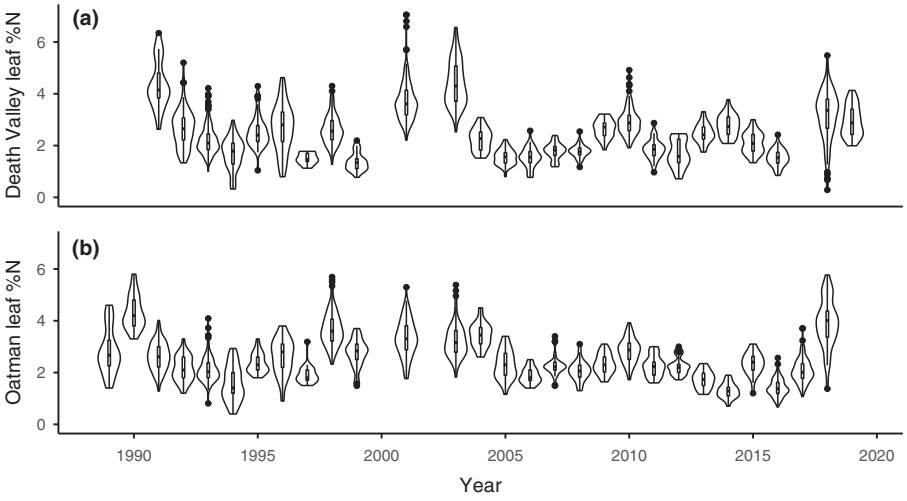

Fig. 2 Violin plots of measured leaf %N values of *Encelia farinosa* from the Death Valley population (a) and Oatman population (b) over the study period. Inlaid boxes indicate the first quartile (Q1), median, and third quartile (Q3), and whiskers indicate Q3 and Q1 ± 1.5 × interquartile range, respectively.

marginally positively related to growing season precipitation ($r^2 = 0.09$, $P = 0.065$; Fig. 3a), marginally negatively related to growing season VPD ($r^2 = 0.06$, $P = 0.10$; Fig. 3c), and unrelated to maximum temperature ($P = 0.33$; Fig. 3b). Temporal trends in site climate are shown in Fig. S2. Within each population, $\delta^{15}N$ values were not significantly related to total annual precipitation, total summer precipitation (June–September), the number of days on which > 1 mm of precipitation fell, annual mean maximum temperature, annual or growing season mean minimum temperature, annual mean maximum VPD, or the number of days that reached 35°C in either population ($P > 0.05$ for all; full results in Table S1). Foliar %N values were not significantly related to any of the evaluated climate variables in either population.

Plant-level drivers of $\delta^{15}N$ values

We integrated plant and environmental parameters into a mixed-effects model of foliar $\delta^{15}N$ values to better understand the dominant drivers of foliar $\delta^{15}N$ values in individual shrubs. The full model included a random intercept term for plant ID. Population, SPEI, CO_2 concentration, leaf $\delta^{13}C$ value, leaf %N, flowering status, plant growth rate, plant age, and plant HSA were included as fixed effects in the full model. All fixed effects, except for leaf $\delta^{13}C$ value, significantly improved the fit of the model and were retained in the final model ($n = 3543$ observations from 1050 plants, marginal $R^2 = 0.59$, conditional $R^2 = 0.70$). Foliar $\delta^{15}N$ values were negatively related to plant age and positively related to SPEI, CO_2 concentration, leaf N concentration, and plant surface area and growth rate (Fig. 4). Additionally, plants in the Oatman population had significantly lower leaf $\delta^{15}N$ values than plants in the Death Valley population if all other variables are held constant ($\beta = -1.57$, 95% CI: −1.64 to −1.49), and flowering plants had significantly higher leaf $\delta^{15}N$ values than plants that were not flowering ($\beta = 0.143$, 95% CI: 0.0961–0.191). The relationship between observed and predicted $\delta^{15}N$ values is shown in Fig. S3.

Intra-plant variation in $\delta^{15}N$ and %N values

We sampled plant roots, stems, and leaves at the Oatman site to better understand how $\delta^{15}N$ values varied among and within plant tissues. $\delta^{15}N$ values varied significantly among tissue types (ANOVA, df = 3, $F = 33.84$, $P < 0.0001$; Fig. 5a). Roots had the

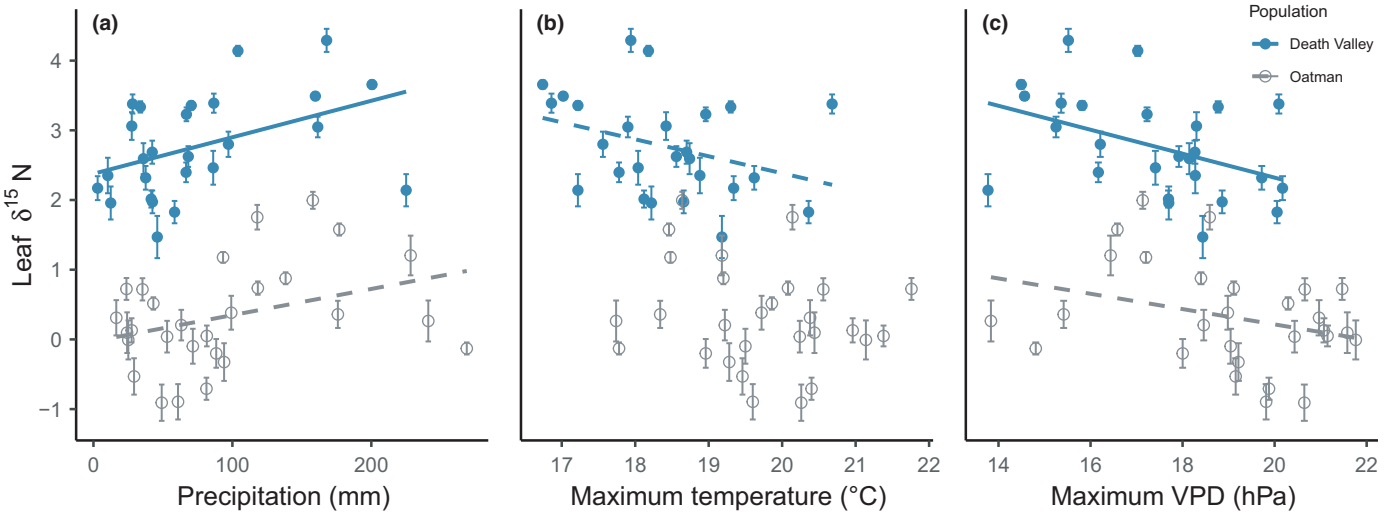

Fig. 3 Relationships between foliar $\delta^{15}N$ values of *Encelia farinosa* and total growing season precipitation (a), mean growing season daily maximum temperature (b), and mean growing season daily maximum vapor pressure deficit (VPD) (c). Blue solid circles represent annual mean $\delta^{15}N$ values from the Death Valley population and open gray circles represent annual mean $\delta^{15}N$ values from the Oatman population. Error bars represent ±1 SE. Solid lines indicate significant linear regressions ($P < 0.05$), and dashed lines represent marginally significant linear regressions ($P < 0.1$).

lowest $\delta^{15}N$ values, with a mean of −2.76‰ and a range of −6.30‰ to −0.01‰, followed by woody stems (mean $\delta^{15}N$ = −2.07‰, range: −4.86–1.72‰), then green stems (mean $\delta^{15}N$ = −0.36‰, range: −3.41–1.01‰), and finally leaves (mean $\delta^{15}N$ = 0.36‰, range: −3.10–3.23‰). Differences between leaves and green stems were not significant ($P = 0.38$), nor were differences between woody stems and leaves ($P = 0.29$). N concentration also varied significantly among tissues (Fig. 5b; ANOVA, df = 2, $F = 52.18$, $P < 0.0001$) and exhibited a trend similar to $\delta^{15}N$ values. %N values were lowest among woody stems (mean = 0.49%, range: 0.18–0.95%) and roots (mean = 0.61%, range: 0.34–1.17%), followed by green stems (mean = 1.25%, range: 0.45–2.38%), and then leaves (mean = 1.72%, range: 0.51–3.09%). The difference in %N values between roots and woody stems was not significant (Tukey's HSD, $P = 0.74$). Overall, woody stems tended to have $\delta^{15}N$ and %N values comparable to those of roots, whereas green stems tended to have $\delta^{15}N$ and %N values comparable to those of leaves.

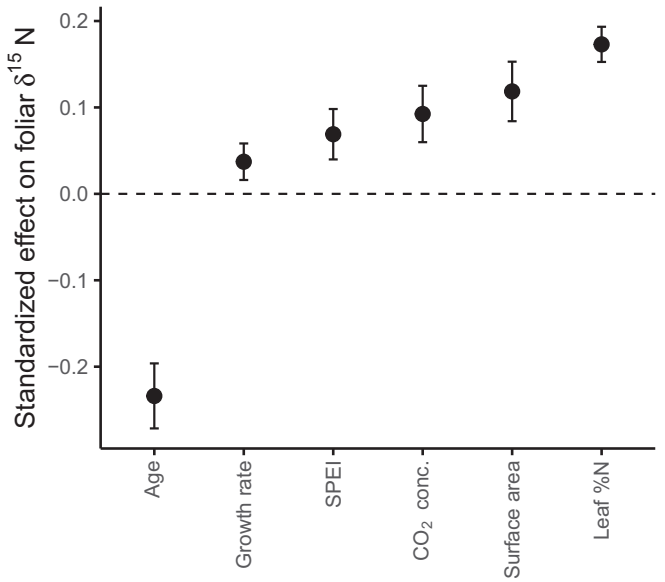

Fig. 4 Standardized coefficients from the optimized mixed-effects model of foliar $\delta^{15}N$ values in *Encelia farinosa* ($n = 3543$ observations from 1050 plants), representing the change in $\delta^{15}N$ value in SDs associated with a 1 SD change in the predictor variable. The dashed horizontal line indicates no effect on $\delta^{15}N$ values, and error bars represent the 95% confidence interval for each coefficient estimate. $P < 0.0001$ for all variables except growth rate ($P = 0.00057$).

Offsets between tissues were fairly consistent across individuals, and we observed significant, positive linear relationships between $\delta^{15}N$ values for all pairs of tissues (Fig. 6). With the exception of the relationship between green stems and leaves (Fig. 6f), the slopes of these linear regressions did not significantly differ from 1. There were no significant negative relationships between $\delta^{15}N$ value and tissue diameter for green stems, woody stems, or roots. There was a significant negative relationship between %N and diameter for roots ($r^2 = 0.14$, $P = 0.0052$), but not for woody or green stems. Finally, plant HSA did not significantly influence the $\delta^{15}N$ values of roots, woody or green stems, or leaves among these destructively sampled shrubs. There was a negative relationship between %N values and plant HSA for woody stems ($r^2 = 0.16$, $P = 0.019$), but not for roots, green stems, or leaves.

Patterns in soil $\delta^{15}N$ and %N

The presence of a shrub greatly influenced both soil N isotope ratios and N content, creating a highly heterogenous landscape of 'islands of fertility' over small spatial scales. $\delta^{15}N$ values for shallow surface layers (0–5 cm) ranged from −0.45‰ to 5.92‰ and soil %N ranged from 0.017% to 0.33%, and we found large differences in $\delta^{15}N$ and %N values between shallow surface soil

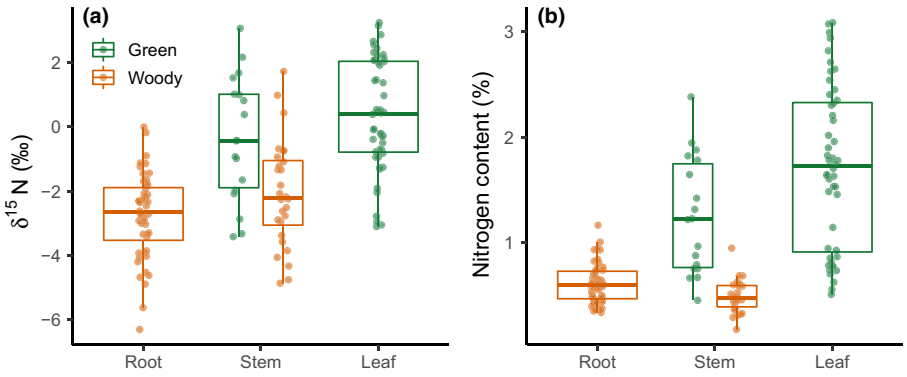

Fig. 5 δ^{15}N values (a) and %N values (b) from *Encelia farinosa* root, stem, and leaf samples. Green boxes and circles represent nonwoody, green tissues, and orange boxes and circles indicate woody tissues. Both young, photosynthetic stems and large-diameter, woody stems were collected from each plant sampled. Boxes indicate the first quartile (Q1), median, and third quartile (Q3), and whiskers indicate Q3 and Q1 ± 1.5 × interquartile range, respectively.

samples collected under shrub canopies and interspaces between shrubs. Specifically, interspace soils had a mean δ^{15}N value of 3.76‰ and a mean %N value of 0.046%, whereas subcanopy soils had a mean δ^{15}N value of 2.59‰ and a mean %N value of 0.12% (*t*-test, $P = 0.0001$ for δ^{15}N and $P < 0.0001$ for %N). Subcanopy soils also exhibited a higher variance in δ^{15}N values (2.12‰) and %N values (0.0058%) relative to interspace soils (variance in δ^{15}N = 1.20‰; variance in %N = 0.00043%). δ^{15}N values for soils collected at 0–2 cm were significantly lower than those collected at 3–5 cm under shrubs ($P = 0.00048$, means = 2.23‰ and 2.94‰, respectively), although δ^{15}N values did not differ between depths in interspaces ($P = 0.18$). %N values were higher in the 0–2 cm layer than in the 3–5 cm layer for both subcanopy soils ($P < 0.0001$, means = 0.15% and 0.085%, respectively) and interspace soils ($P < 0.0001$, means = 0.058% and 0.035%, respectively). There were no significant relationships between the HSA of the overlying shrub and subcanopy soil δ^{15}N or %N values at either depth ($P > 0.2$ for all).

Along soil depth profiles (down to 50 cm), δ^{15}N values tended to increase with depth (Fig. 7a), although this pattern was more pronounced in the subcanopy soil pits than in interspaces. Subcanopy %N values from depth profiles were substantially higher under shrubs than in interspaces for samples collected at and above 10–15 cm, but declined rapidly by a depth of 20–25 cm, at which point subcanopy %N values were only slightly higher than interspace %N values (Fig. 7b).

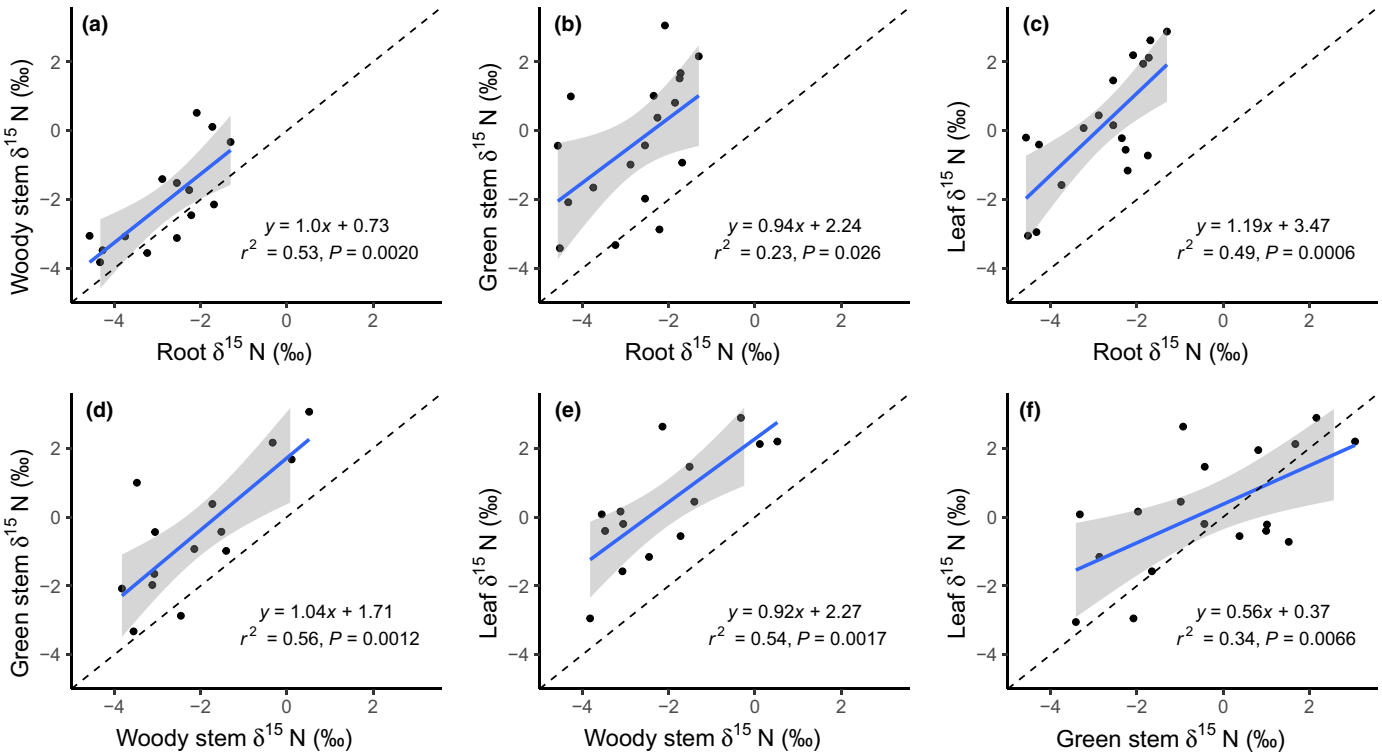

Fig. 6 Relationships between the δ^{15}N values of the four different tissue types collected from individual *Encelia farinosa*. Blue lines indicate significant linear regressions, gray bands indicate the 95% confidence interval of the regression, and the dashed line indicates a 1 : 1 relationship. On-plot statistics correspond to linear regressions.

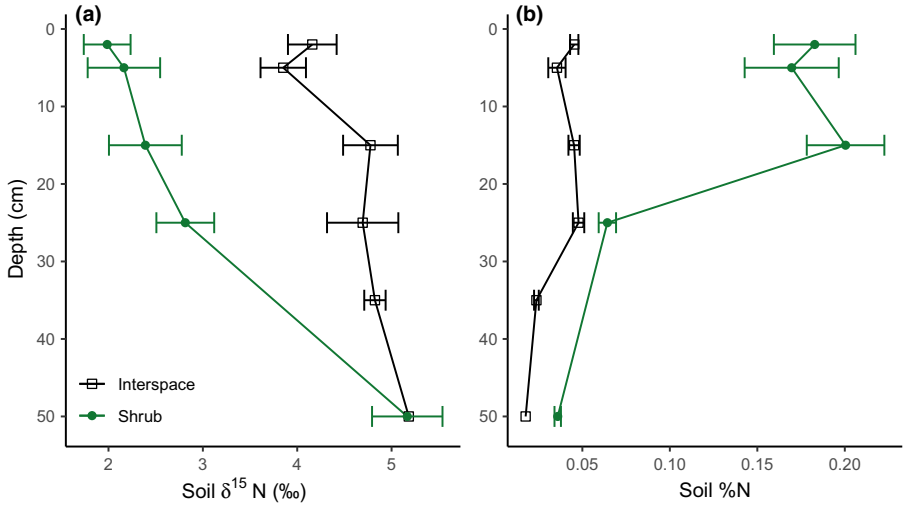

Fig. 7 Relationships between soil depth and bulk soil $\delta^{15}N$ values (a) and %N values (b) at the Oatman site. Each data point represents the mean value from two soil pits, and error bars represent the standard error of all samples collected at each depth. Two soil pits were located in shrub interspaces (black open squares), and two were located under large *Encelia farinosa* canopies (green filled circles).

Relationships between plant and soil N status

Surprisingly, we did not observe any significant correlations between the average $\delta^{15}N$ values of shallow subcanopy surface soils (0–5 cm) and the $\delta^{15}N$ values of corresponding shrub roots, woody stems, green stems, or leaves (Fig. S4; $P > 0.05$ for all), although the relationship between green stem $\delta^{15}N$ values and soil $\delta^{15}N$ values was marginally significant ($P = 0.060$). Similarly, there were no significant relationships between average soil %N values and corresponding shrub root, woody stem, green stem, or leaf %N values.

$\delta^{15}N$ values were strongly related to %N values in plant tissues ($^{15}N = 2.57 \times \log(\%N) - 0.82$, $P < 0.0001$, $r^2 = 0.61$; Fig. 8a). The $\delta^{15}N$ vs %N relationship was relatively weak among root samples ($r^2 = 0.081$, $P = 0.028$), moderately strong among woody stem samples ($r^2 = 0.31$, $P = 0.0012$), and strong among green stem samples ($r^2 = 0.68$, $P < 0.0001$) and leaf samples ($r^2 = 0.57$, $P < 0.0001$). In soils, %N values were negatively related to $\delta^{15}N$ values ($\delta^{15}N = -1.13 \times \log(\%N) - 0.23$, $r^2 = 0.34$, $P < 0.0001$; Fig. 8b). This trend persisted for shallow surface soils ($r^2 = 0.26$, $P < 0.0001$) as well as soils samples collected from depths of 10–50 cm ($r^2 = 0.48$, $P < 0.0001$).

Discussion

Resolving the environmental and physiological drivers of plant N isotope ratios remains a hurdle to effectively leveraging $\delta^{15}N$ values for understanding ecosystem N cycling. We identified a high degree of variability in $\delta^{15}N$ values within two populations of *E. farinosa* (spanning ranges of 7.5‰ and 9.3‰), much of which occurred within individual years. A previous meta-analysis found that 95% of foliar $\delta^{15}N$ values globally fall into a 15.5‰ range (Craine *et al.*, 2015a), indicating that the observed intraspecific variability encompassed across these two populations represents *c.* 70% of global variability in $\delta^{15}N$ values. Some of our key findings call into question common assumptions about plant $\delta^{15}N$ values, including the assumptions that physiological processes influencing $\delta^{15}N$ values are consistent within a species and that foliar $\delta^{15}N$ values can be used as a tracer of source $\delta^{15}N$ values. We identified limited population-level effects of interannual climatic variability on foliar $\delta^{15}N$ values; strong effects of plant-level structural and physiological factors on $\delta^{15}N$ values; consistent differences in $\delta^{15}N$ values between plant leaves, stems, and roots; and no relationship between plant and bulk soil $\delta^{15}N$ values at small spatial scales.

Environmental drivers of foliar $\delta^{15}N$

Precipitation, temperature, and CO_2 concentration have been identified as potential drivers of global patterns in foliar $\delta^{15}N$ values (Amundson *et al.*, 2003; Craine *et al.*, 2009). However, current understanding of relationships between climate and foliar $\delta^{15}N$ values rests primarily on observations made across spatial, rather than temporal, gradients in climate. It remains unclear if climate influences $\delta^{15}N$ values directly or whether it is simply a covariate with variables such as soil type and species composition (Craine *et al.*, 2009; Elmore *et al.*, 2017). At our study sites, VPD and temperature have substantially increased over the study period, and precipitation has decreased at the Death Valley site. These climatic trends have coincided with a steady increase in CO_2 concentration (of *c.* 60 ppm between 1990 and 2019), which together triggered a large increase in leaf intrinsic water-use efficiency (Driscoll *et al.*, 2020). Additionally, the average age of both populations has increased since the onset of the study. Interestingly, there were no significant temporal trends in $\delta^{15}N$ or %N values in either population, suggesting that prevailing environmental and demographic trends either have had little effect on N cycling in this ecosystem thus far, or that they have had contrasting and net-neutral effects on $\delta^{15}N$ values. This finding runs counter to a recent global synthesis that suggested a broad decline in foliar $\delta^{15}N$ and %N values since 1980, which was attributed to increased N demand relative to supply (Craine *et al.*, 2018, although see Hiltbrunner *et al.*, 2019 for counter-arguments). The discrepancy between these results highlights the value of long-term observations collected at a single site, as variation across species and sites complicates interpretation of temporal trends.

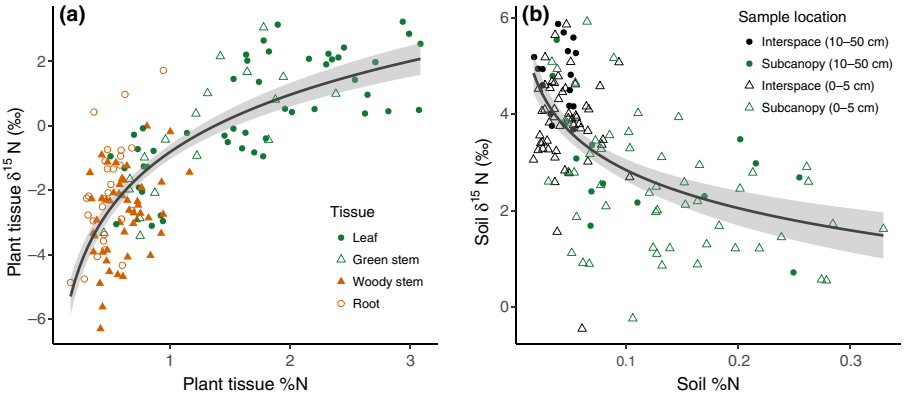

Fig. 8 Relationships between %N and $\delta^{15}N$ values for *Encelia farinosa* plant samples (a) and soil samples (b). Regression lines represent significant relationships between $\delta^{15}N$ and log(%N), and gray bands indicate the standard error of the regression. Point shape corresponds to plant tissue type (a) and soil sample depth (b), and point color indicates if the plant tissue is green (photosynthetic) or woody (a) and if the soil sample was collected under a shrub or in an interspace (b).

Population mean $\delta^{15}N$ values tended to increase with increasing precipitation and decrease with increasing VPD, although these trends were only significant in the Death Valley population. Notably, these relationships were different from those commonly observed over spatial climate gradients, where leaf $\delta^{15}N$ values tend to decrease with increasing precipitation and increase with increasing temperatures (Craine *et al.*, 2009; Murphy & Bowman, 2009; Ma *et al.*, 2012). However, they align with the positive relationship between precipitation and soil $\delta^{15}N$ that has been observed in arid ecosystems with alkaline soils (Wang *et al.*, 2014; Sperber *et al.*, 2017). At the individual level, SPEI was positively related to $\delta^{15}N$ values after controlling for other plant and environmental variables. However, a 1 unit increase in SPEI corresponded to an increase in foliar $\delta^{15}N$ value of only *c.* 0.1‰. These findings suggest that climatic effects on $\delta^{15}N$ values operate differently across spatial and temporal gradients, and that space-for-time substitutions may be inadequate for understanding temporal trends in $\delta^{15}N$.

The reported effects of atmospheric CO_2 concentration on $\delta^{15}N$ values have also been variable. We did not identify any significant effects of CO_2 concentration on population mean $\delta^{15}N$ values, but we did observe a positive effect of CO_2 on individual $\delta^{15}N$ values after controlling for other variables, with a CO_2 increase of 50 ppm corresponding to an increase in foliar $\delta^{15}N$ of *c.* 0.45‰. Several studies conducted over a broad range of ecosystems have shown decreases in foliar $\delta^{15}N$ associated with increasing CO_2, attributing the effect to changes in physiological fractionations within plants or declines in N supply relative to demand (Constable *et al.*, 2001; BassiriRad *et al.*, 2003; Craine *et al.*, 2018). Other free-air CO_2 enrichment experiments, including one in the Mojave Desert with comparable alkaline soils, have reported increases in foliar $\delta^{15}N$ values under enriched CO_2 that the authors attributed to increased soil microbial activity and consumption of N causing enrichment of the remaining plant-available soil N pool (Högberg, 1997; Billings *et al.*, 2002a,b; McCalley *et al.*, 2011).

Plant-level drivers of $\delta^{15}N$ values

Plant characteristics appeared to drive a large portion of the variability in foliar $\delta^{15}N$ values and mediated relationships between environmental drivers and $\delta^{15}N$ values. We found that plant age, growth rate, surface area, flowering status, and foliar %N were significant drivers of $\delta^{15}N$ values at the individual level after controlling for SPEI, CO_2 concentration, and population.

Relationships between $\delta^{15}N$ values and plant age have not previously been documented and have particular relevance for both experimental studies relying on young plants and field studies relying on samples from a subset of plants in a population. Although we are unable to pinpoint the exact mechanism underlying the negative relationship between plant $\delta^{15}N$ and age (Fig. 4), several processes are potential contributors. First, soil N availability may increase as the plant ages, resulting in increasing discrimination during N uptake with age and thus lower $\delta^{15}N$ values (Evans, 2001; Yoneyama *et al.*, 2001). Nutrient availability in deserts is characterized by 'islands of fertility', or areas of particularly high nutrient availability that form under shrub canopies (Schlesinger & Pilmanis, 1998; Walker *et al.*, 2001; Titus *et al.*, 2002). Consistent with this pattern, our soil data indicated that $\delta^{15}N$ values were much more negative and %N values were much higher in soils under shrubs than in interspaces (Fig. 7). These $\delta^{15}N$ and %N trends could be a result of elevated rates of free-living microbial N fixation beneath well-established shrubs. Second, transformation of N associated with reallocation of N from storage has been identified as a discriminating process in *E. farinosa* (Kolb & Evans, 2002), and therefore increased internal recycling of N in older plants could underlie the negative relationship between age and $\delta^{15}N$ values. Third, changes in the depth of N uptake as a plant ages may influence foliar $\delta^{15}N$ values. Although we observed increases in bulk soil $\delta^{15}N$ with increasing depth, the $\delta^{15}N$ value of plant-available N may exhibit a different trend.

Transfer of N from mycorrhizae to the host plant discriminates against ^{15}N (Hobbie *et al.*, 2000; Hobbie & Ouimette, 2009; Hobbie & Högberg, 2012), leading to lower $\delta^{15}N$ values in the plant than in the source. If plants rely more heavily on mycorrhizal uptake of N as they age, this could also contribute to the negative relationship between $\delta^{15}N$ values and age. More broadly, we observed a strong positive relationship between foliar %N and $\delta^{15}N$ values. Similar trends have previously been reported in leaf tissues in other ecosystems and have traditionally been interpreted as an indication of mycorrhizal reliance (Hobbie *et al.*, 2000; Hobbie & Högberg, 2012). However, we observed a relationship between $\delta^{15}N$ and %N across plant root, stem, and

leaf tissues both within and among individuals (Fig. 8). While the trends across individuals could be explained by differing reliance on mycorrhizal uptake of N, within-individual trends suggest that there may be an additional mechanism at play. Mycorrhizal associations are not well understood in this ecosystem and offer a potentially informative avenue for further study of N isotope dynamics in desert plants.

Intra-plant fractionations and the decoupling of soil and plant $\delta^{15}N$ values

In addition to between-plant variability in $\delta^{15}N$ values, we also observed significant differences in $\delta^{15}N$ values among plant tissues, which has previously been documented in a variety of species (Handley & Raven, 1992; Comstock, 2001; Evans, 2001; Kolb & Evans, 2002; Kalcsits *et al.*, 2014). Patterns of intra-plant fractionation are inconsistent between species, and therefore cross-species comparison of foliar $\delta^{15}N$ values is potentially misleading. However, we found a remarkably consistent pattern of fractionation among plant tissues, with moderately strong correlations among roots, woody stems, and leaves that had slopes statistically indistinguishable from 1. Although the foliar $\delta^{15}N$ value was not identical to the stem or root $\delta^{15}N$ value, the consistent offset among tissues indicates that foliar $\delta^{15}N$ values are representative of whole-plant $\delta^{15}N$ values within this population. We suggest that a standard enrichment pattern across tissues is present in *E. farinosa*: the $\delta^{15}N$ values of woody stems are *c.* 0.73‰ heavier than those of roots, and the $\delta^{15}N$ values of leaves are *c.* 2.27‰ heavier than those of older, woody stems.

This span of intra-plant variability (*c.* 3‰) is comparable to that identified in previous experimental work on *E. farinosa*, as is the pattern of enrichment from roots to woody stems to leaves (Kolb & Evans, 2002). Intra-plant variability in $\delta^{15}N$ values has been commonly attributed to the site of nitrate assimilation, which can occur in roots, stems, or leaves. Nitrate reductase strongly discriminates against ^{15}N, and therefore if nitrate assimilation occurs in the roots, an enriched pool of N is transported for assimilation in the stems, and a further enriched pool to the leaves. In addition, gaseous exchange with atmospheric N, including losses (Farquhar *et al.*, 1983; Johnson & Berry, 2013) and uptake (Vallano & Sparks, 2013) through photosynthetic tissues, could cause $\delta^{15}N$ values in photosynthetic tissues to approach 0 (through equilibrium processes or uptake) or become enriched (through gaseous loss from the plant). Any of these exchange processes could contribute to the observed higher $\delta^{15}N$ values in leaves and photosynthetic stems relative to woody tissues.

Many studies have cautioned against interpreting foliar $\delta^{15}N$ values as tracers of soil $\delta^{15}N$ values (Handley & Scrimgeour, 1997; Evans, 2001; BassiriRad *et al.*, 2003), and our findings support the notion that the link between foliar $\delta^{15}N$ values and soil $\delta^{15}N$ values is complex and variable. We found that plant $\delta^{15}N$ values were statistically unrelated to the $\delta^{15}N$ values of shallow surface soils directly beneath the canopy. The absence of a relationship between soil and plant $\delta^{15}N$ values could have occurred if the $\delta^{15}N$ signal of the bulk soil was simply not reflective of the $\delta^{15}N$ signal of the plant-available N pool, as a result of either a temporal mismatch in plant tissue and soil sampling or low concentrations of plant-available N vs unavailable N. Alternatively, the relative contributions of fractionations associated with N uptake, assimilation, transformation, and storage in the plant could have been large relative to the contribution of the $\delta^{15}N$ value of the absorbed inorganic N, implying that variability in $\delta^{15}N$ at small spatial scales in this ecosystem is more strongly determined by plant physiology than source $\delta^{15}N$ values.

It remains unclear precisely which processes underlie many of the observed patterns in plant $\delta^{15}N$ values. The extensive intraspecific variability in $\delta^{15}N$ values and the strong effects of plant physiology and structure suggest that the effects of plant-level fractionations may be commonly underestimated in interpretations of plant $\delta^{15}N$ values, highlighting the need for careful quantification of population-level variability and representative sampling. However, these data also underscore the utility of foliar $\delta^{15}N$ values as integrated indicators of environmental and physiological determinants of N cycling.

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Author contributions

JRE designed and led the long-term surveys, AWD and SAK designed and performed additional field sampling, AWD analyzed the data and wrote the initial manuscript draft, and all authors contributed to data interpretation and subsequent manuscript drafts.

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Data availability

The data that support the findings of this study are available from the corresponding author upon request.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Map of study sites in the US southwest.

Fig. S2 Temporal trends in precipitation, vapor pressure deficit, and temperature at the study sites.

Fig. S3 Relationship between observed and predicted $\delta^{15}N$ values of individuals using the mixed effects model presented in Fig. 4.

Fig. S4 Relationships between soil and plant $\delta^{15}N$ and %N values.

Table S1 Results of regressions between population mean $\delta^{15}N$ values and alternative climate parameters.

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