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Unexpectedly low δ^{13}C in leaves, branches, stems and roots of three acacia species growing in hyper-arid environments

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Abstract

Aims In plant eco-physiology, less negative (enriched) carbon 13 (^{13}C) in the leaves indicates conditions of reducing leaf gas exchange through stomata, e.g. under drought. In addition, ^{13}C is expected to be less negative in non-photosynthetic tissues as compared with leaves. However, these relationships in δ^{13}C from leaves (photosynthetic organs) to branches, stems and roots (non-photosynthetic organs) are rarely tested across multiple closely related tree species, multiple compartments, or in trees growing under extreme heat and drought.

Methods We measured leaf-to-root ^{13}C in three closely related desert acacia species (*Acacia tortilis*, *A. raddiana* and *A. pachyceras*). We measured δ^{13}C in leaf tissues from mature trees in southern Israel. In parallel, a 7-year irrigation experiment with 0.5, 1.0 or 4.0 L day^{-1} was conducted in an experimental orchard. At the end of the experiment, growth parameters and δ^{13}C were measured in leaves, branches, stems and roots.

Important Findings The δ^{13}C in leaf tissues sampled from mature trees was ca. −27‰, far more depleted than expected from a desert tree growing in one of the Earth's driest and hottest environments. Across acacia species and compartments, δ^{13}C was not enriched at all irrigation levels (−28‰ to ca. −27‰), confirming our measurements in the mature trees. Among compartments, leaf δ^{13}C was unexpectedly similar to branch and root δ^{13}C, and surprisingly, even less negative than stem δ^{13}C. The highly depleted leaf δ^{13}C suggests that these trees have high stomatal gas exchange, despite growing in extremely dry habitats. The lack of δ^{13}C enrichment in non-photosynthetic tissues might be related to the seasonal coupling of growth of leaves and heterotrophic tissues.

Keywords stable isotope, δ^{13}C enrichment, desert, tree drought resistance, *Acacia raddiana*, *Acacia tortilis*

生长在超干旱环境下的3种相思树种表现出异常低的叶片、树枝、树干、根中δ^{13}C含量

摘要：在植物生理生态学中，叶片中碳13(^{13}C)含量负值较少(富集)，表明叶片处于通过气孔的气体交换减少，比如在干旱胁迫下。此外，与叶片相比，13C在非光合组织中的负值也较少。然而，对从叶片(光合器官)到树枝、树干和根(非光合器官)中的δ^{13}C数值的关系知之甚少，特别是缺少在关联密切的多个树种间或者不同器官间，以及对生长在极端高温和干旱胁迫下的树木中进行测定。本研究测定了3种近缘沙漠相思树种(*Acacia tortilis*、*A. raddiana*和*A. pachyceras*)从叶片到根的^{13}C含量。我们在以

色列南部成树的叶片组织中测定了δ^{13}C含量。与此同时，在试验果园进行了为期7年的3个水平的灌溉试验。在试验结束时，测定了叶片、树枝、树干和根的生长参数和δ^{13}C含量。研究结果表明，叶片组织中δ^{13}C含量约为–27‰，其同位素贫化程度远超过生长在地球上最干燥和最热环境中的沙漠树种的预期值。在不同的相思树种和不同器官中，所有灌溉水平处理中的δ^{13}C含量并没有富集(–28‰到ca. –27‰)，证实了在成熟相思树中的测定结果。在不同器官中，叶片δ^{13}C含量与树枝和根的δ^{13}C含量异常相似，甚至比树干的δ^{13}C含量负值更少。高度贫化的叶片δ^{13}C表明，尽管这些树木生长在极端干燥的生境中，但其气孔气体交换较高。非光合组织中缺乏δ^{13}C富集可能与叶片和异养组织生长的季节耦合有关。

关键词：稳定性同位素，^{13}C富集，沙漠，树木抗旱性，相思树属

INTRODUCTION

The genus *Acacia** inhabits some of the hottest and driest places on Earth, often found in deserts across Africa and the Middle East (Maslin *et al.* 2003; the genus name changed to *Vachellia* [The genus (*Acacia*) was recently split into two different genera, *Vachellia* and *Acacia*. While the original name (*Acacia* = 'thorn' in latin) was reserved for Australian (thornless) acacia trees, the new name, *Vachellia*, was reserved for acacias from the rest of the world, with thorns. Searching the web of science for scientifc papers published from 2015 to June 2016 showed that only 2.6% from the 346 acacia papers published during this periord chose to use the new name. A short survey among Israel's botanists also demsonstrates that it is preferable to continue to use the genus name *Acacia* (see e.g. Groner *et al.* 2017; Winters *et al.* 2015). For these reasons, we chose to use the genus name *Acacia*.]). In southern Israel, along the Arava valley, this genus includes three species: *Acacia tortilis* (Forssk.), *A. raddiana* (Savi) and *A. pachyceras* (O. Schwartz; formerly gerrardii [Benth.]/negevensis [Zohary]) (Ashkenazi 1995), which together encompass the main and dominant tree species in this region (Danin 1983). In these arid habitats, acacias are found mostly growing in the channels of ephemeral riverbeds. Several studies have shown that the survival of desert plants in general, and *Acacia* trees in particular, depends on a deep root system that reaches deep soil water reservoirs, which in turn are refilled by flashfloods once or twice a year (Do *et al.* 2008; Stave *et al.* 2005; Winters *et al.* 2018). The presence of *Acacia* trees in arid regions creates and maintains microclimatic conditions and ecological niches favorable to other organisms (Munzbergova and Ward 2002; Ward *et al.* 1993). These extraordinary functions and services, however, are at increasing risk, due to ongoing climatic, and anthropogenic changes (Andersen and Krzywinski 2007).

Climate change is likely to be a key contributing factor to the decline of acacia populations in the hyper-arid Arava valley of Israel, as well as in other arid regions across the Middle East and northern Africa (Stavi *et al.* 2014). Reduced water availability is thought to be the main determinant of this decline (Stavi *et al.* 2015). This is due to an ongoing warming, along with a precipitation decrease of about 20%–50% over the last few decades along the Arava valley (Ginat *et al.* 2011). Specifically, it has been suggested that acacia trees in the Arava directly rely on floodwater which refill the water reservoirs reached by the trees (Sher *et al.* 2010), and that the reduced frequency and magnitude of local flashfloods were predicted to diminish the access of trees to water (Shrestha *et al.* 2003). Although several studies have focused on the phenology and demography of acacia populations in the Arava valley (Stavi *et al.* 2015; Winters *et al.* 2018), a more thorough understanding of the effects of water depletion on local acacia trees is still lacking. This could be studied by directly monitoring the effect of water availability on a range of vegetative indices under semi-controlled conditions.

Isotopic fractionation of hydrogen (H), carbon (C), oxygen (O) and other elements has been a major tool in studying mechanisms in ecology and physiology (Bahn *et al.* 2009; Gat *et al.* 2007; Smith and Epstein 1971). In the field of plant eco-physiology, the natural abundance of carbon 13 (^{13}C) has been a useful tool for studying carbon allocation patterns (Bowling *et al.* 2008; Brüggemann *et al.* 2011; Gessler *et al.* 2007, 2008, 2009). In C_3 plants, ^{13}C (higher mass isotope) is discriminated against ^{12}C (lower mass isotope) during carbon fixation in the chloroplast, as long as carbon uptake is undisturbed.

δ^{13}C can range from less negative values (~−22‰) under drought conditions to very negative values (~−28‰) under well-watered conditions, due to leaf gas exchange through stomata (Ferrio *et al.* 2005; Lipp *et al.* 1996; Maseyk *et al.* 2011). For example, Klein *et al.* (2005) found that δ^{13}C in needles and stems of pine trees growing on the edge of the Negev desert in Israel were around −22‰ naturally, but dropped to −27‰ when the same trees were irrigated. Conversely, Bachar *et al.* (2020) found that stem δ^{13}C of trees in a riparian Mediterranean forest was around −25‰, and increased to −23‰ when their water source was pumped for human consumption (Bachar *et al.* 2020).

In arid environments, the strong limitation of water availability makes ^{13}C fractionation in plant tissues an ideal research tool (Ferrio *et al.* 2005; Winter *et al.* 1976). In the desert tree *Tamarix jordanis*, cellulose δ^{13}C changed from −28‰ in Mediterranean climate (mean annual precipitation, MAP = 900 mm) to −26‰ in semi-arid climate (MAP = 170 mm) and to −24‰ in hyper-arid climate (MAP = 20 mm; Lipp *et al.* 1996). Microhabitat differences also exert large effects on δ^{13}C within desert plant communities. For example, in a desert site in Arizona (MAP = 150 mm), δ^{13}C was <−26‰ in plants growing in the bottom of the valley where the soil is deep and soil water accumulates, whereas δ^{13}C was ca. −24‰ in plants growing on the slope (Ehleringer and Cooper 1988). Phenology is another important factor, which resulted in seasonal differences of up to 3‰ δ^{13}C in plants of the Arizona desert community (Ehleringer *et al.* 1992). Finally, lifespan affects δ^{13}C of desert plants, as seen in typically more negative δ^{13}C in short-lived opportunistic species (1–10 years in species such as *Eriogonum inflatum*, *Ambrosia eriocentra* and *Porophyllum graeile*, with a δ^{13}C of −28‰) compared with long-lived species (50+ years in species such as *Chilopsis linearis*, *Larrea divaricate* and *Cercidium floridum*, with a δ^{13}C of −24.6‰) (Ehleringer and Cooper 1988; Schuster *et al.* 1992).

Another scenario where tissue ^{13}C is generally enriched is in non-photosynthetic (heterotrophic) tissues as compared with photosynthetic leaves (autotrophic tissue) (Badeck *et al.* 2005; Bowling *et al.* 2008; Brüggemann *et al.* 2011; Cernusak *et al.* 2009; Gessler *et al.* 2014). In C_3 plants, this might happen as a result of six different, but not mutually exclusive, mechanisms: (i) variations in biochemical composition; (ii) seasonal decoupling in tissue growth; (iii) differential use of day *vs.* night sucrose in leaves and sink tissues; (iv) isotopic fractionation during dark respiration; (v) carbon fixation by phosphoenolpyruvate (PEP) carboxylase and (vi) developmental variation in photosynthetic discrimination against ^{13}C during leaf expansion. Overall, post-photosynthetic ^{13}C discrimination can be related to starch metabolism, which is transitory in the leaf, and can also occur in any sink tissue where starch is stored and remobilized (Gessler *et al.* 2014). Sucrose transport in the phloem involves additional discrimination, as well as mixing of different sugar pools. Finally, the fractionations in non-photosynthetic, sink tissues are related to respiration and PEP carboxylase activity. Tissue ^{13}C is generally enriched in non-photosynthetic (heterotrophic) tissues as compared with photosynthetic leaves (autotrophic tissue).

The aforementioned first mechanism driving ^{13}C enrichment in non-photosynthetic tissues is related to ratios of specific compounds, which diverge in their ^{13}C signature between source and sink tissues. Among the common compounds inside tree tissues (e.g. oils, gums, resins, tannins), starch is the most enriched (Bowling *et al.* 2008). Starch is the single most important form of carbon storage in plants (Hoch *et al.* 2003; Martínez-Vilalta *et al.* 2016). Considering the strong limitations to photosynthesis in hot and arid environments (heat, drought, photoinhibition), one would expect an efficient storage of photosynthetic carbon to be manifested in high starch content in woody tissues of desert plants. Indeed, in pine trees growing on the edge of the desert, branch and root starch levels were up to 4% and 7% of the dry weight (DW), respectively (Klein *et al.* 2014), higher than the global mean for these tissues (Martínez-Vilalta *et al.* 2016). In terms of divergence between leaf and woody tissues, drought-deciduous species tend to have only 2% DW starch in leaves, and as high as 6% DW starch in the stem (Martínez-Vilalta *et al.* 2016), supporting carbon storage in the form of starch in non-photosynthetic tissues.

In this study, we evaluated the δ^{13}C signals from leaves to branches, stem and roots in three desert acacia species, to estimate how the extremely dry habitat of these species influences their eco-physiology. Isotopic signals were measured in mature trees in the field (MAP = 20 mm). To further test the mechanisms behind ^{13}C fractionation in these unique trees and environment, isotopic signals were also tested in a field experiment, where young trees were subjected for 7 years to three levels of irrigation. Due to the fact that the root system of acacia trees can reach deep soil water, we considered two competing hypotheses: (i) ^{13}C discrimination would be very limited due to stomatal closure under the harsh conditions, but will increase when growing with improved irrigation; (ii) if seedlings can grow roots that access deep groundwater, than ^{13}C discrimination can be expected regardless of irrigation; (iii) ^{13}C would be enriched in sink tissues compared with source tissues, as observed globally. In addition, among species, we hypothesized that (iv) *A. tortilis* would respond better, in both growth and ^{13}C discrimination, than the other two species, to the conditions of the Southern Arava, where it is the dominant species of the three.

MATERIALS AND METHODS

Sites and climate

Our study was conducted in the Arava valley in southern Israel, in a field experiment near the village of Ketura (29°58′ N, 35°03′ E, 110 m a.s.l.) and in the nearby Evrona nature reserve (located 35 km south of Ketura; 29°40′48.82″ N, 35°0′4.53″ E, 42 m a.s.l.) (Supplementary Fig. S1). The Arava valley stretches from the Gulf of Aqaba in the south of Israel, northwards to the Dead Sea. The valley is characterized by extremely high temperatures (daily maximum 42 °C in August and 19 °C in January) and extremely low rainfall (15–70 mm year^{-1}, ca. 20 mm in our sites). Two acacia species, *A. raddiana* and *A. tortilis*, are prevalent throughout the region and are considered keystone species (Ashkenazi 1995). The third species, *A. pachyceras*, also grows in the Arava, yet is more prevalent in the Negev highlands to the West.

The Evrona nature reserve spans 40 km^2 of acacia savannah vegetation, along with other Sudanian vegetation species. Multiple individual trees of *A. raddiana* and *A. tortilis* are scattered throughout the plateau, but never forming a continuous canopy (Supplementary Fig. S1). Evrona and Ketura sites are very similar in terms of climate and soil, and both are part of the same Arava catchment and ecosystem. Meteorological data measured near the Evrona site showed that temperatures were almost identical to those in Ketura along the same period (data not shown).

Field irrigation experiment

In October 2011, an experimental orchard was established at the Arava Institute for Environmental Studies’ (AIES) experimental field site in Ketura (https://arava.org/arava-research-centers/arava-center-for-sustainable-agriculture/), located at the Southern Arava valley not far from the Evrona field site. A total of 135 seeds of *A. raddiana*, *A. tortilis* and *A. pachyceras* (45 seedlings per species) were germinated in November–December 2010. In order to simulate the natural environment, seeds were collected in nearby sites; none of the seeds were germinated using artificial methods; instead we depended on seeds within pods that were eaten by local desert herbivores including the Nubian Ibex (*Capra nubiana*), Arabian Oryx (*Oryx leucoryx*) and Wild Ass (*Equus asinus*) and exported as fecal samples (Tran *et al.* 2018). Once the seedlings were 1-year-old, they were taken from the AIES nursery and transplanted to the nearby experimental orchard. The orchard was arranged in a randomized block design along nine rows, with 15 trees per row, five of each species. Trees were planted 5 m apart from each other. The orchard was divided into three sections used to apply different irrigation regime, based on the experience from watering of acacia trees in landscaping projects across the region. We estimated that 4.0 L day^{-1} is a sufficient amount to promote seedling growth. We predicted that providing the trees with 25% and 12.5% of this amount could mimic the effect of medium and strong water deficiency on the trees. Accordingly, groups of three rows were set to be irrigated at a rate of 0.5, 1.0 or 4.0 L day^{-1}. The experiment was maintained for almost 7 years, until trees were harvested for analysis (October 2018; at the end of the dry season) at the age of 8 years. Prior to harvesting, the following morphological parameters were measured on all trees: tree height, bole (stem base) circumference, number of branches, canopy size and canopy greenness (assessed visually using a 1–5 scale; Groner *et al.* 2017). At harvest, leaf, branch, stem and root materials were sampled from 10 to 15 trees per species, 5 trees per treatment for ^{13}C analysis.

Mature acacias in Evrona nature reserve

In Evrona, leaf samples for ^{13}C analysis and shoot and root samples for nonstructural carbohydrates (NSCs) were sampled from mature trees (i.e. higher than 3 m) of *A. raddiana* (n = 7) and *A. tortilis* (n = 4) (23 November 2016). To account for differences derived from sunlight exposure, leaves from each of the 11 trees were collected separately from the South-East (SE) and North-West (NW) sides. Samples of shoot and root plant materials were sampled for NSC analysis from the same trees. Shoot pieces (2–3 mm ø, 10 cm long) were cut at 1–2 m height and fine roots (0.5–1.0 mm ø) were sampled at 10–50 cm depth. Roots were cleaned thoroughly with water shortly after sampling.

Tissue δ^{13}C analysis

All plant material sampled in the Evrona nature reserve and in the field irrigation experiment was oven-dried at 80 °C for 48 h and kept dehydrated until analysis. Samples were then ground with a ball mill (Retsch, Haan, Germany) in order to get homogenized samples. The samples (3 technical replicates per sample; 1 mg each) were mounted on a combustion module organic elemental analyzer equipped with autosampler (Costech ECS 4010, Costech Analytical, Valencia, CA, USA). The CO_2 gas product was analyzed with a δ^{13}C Picarro G2131-i isotope and gas concentration analyzer (Picarro, Santa Clara, CA, USA) directly interfaced to the combustion module. Results are expressed as parts per thousand (‰) deviations from the international carbon isotope standard. The analyzer was calibrated against δ^{13}C reference cellulose, and three technical replications from each sample were used. An internal standard (homogeneous Spirulina powder with a δ^{13}C of −29.20‰) was used in the sampling batch, once in nine samples.

NSCs in shoots and roots

All shoot and root plant materials were microwaved for three periods of 30 s each immediately after sampling and washing to stop further enzymatic activity, and then dried at 80 °C for 48 h. Samples were then ground carefully into fine powder (~2 min) using a ball mill (Retsch, Haan, Germany) at a frequency of 25 pulses per second. The analyses of the NSC samples were performed at the University of Basel, Switzerland, following the method by Wong (1990), modified as described in Hoch *et al.* (2002). The tissue powder was divided into two aliquots, one for soluble sugar quantification and the other for starch measurement. Part of the dried roots and shoot powder (12–16 mg) was extracted with 1.5 mL 80% ethanol in aqueous solution at 90 °C for 10 min. Ethanol was evaporated using a concentrator (Concentrator 5301; Eppendorf, Germany) in three sequential repeats. The extract was saturated with deionized water and invertase from baker's yeast (*S. cerevisiae*; Grade VII, ≥300 units mg^{-1} solid) was added in order to break sucrose into glucose and fructose. The rest of the tissue powder was used to extract starch, using enzymatic reaction involving α-amylase (from *Bacillus licheniformis*) and amyloglucosidase (from *Aspergillus niger*) in a sodium acetate buffer (25 mmol/L, pH 4.6). Glucose and fructose were converted into glucose-6-phosphate using glucose hexokinase and phosphoglucose isomerase (from baker's yeast). The total amount of formed glucose-6-phosphate was determined as the increase in NADH + H$^+$ using a photometer (HR 700; Hamilton, Reno, NE, USA). This reaction breaks starch into glucose. All concentrations were calculated on a % DW basis. All enzymes were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Analysis of ecological niche profile and auxiliary data

To identify the climatic conditions across the distribution of the three acacia species, a niche profile analysis was performed in BioGIS (2019), Israel Biodiversity Geographic Information System (http://www.biogis.huji.ac.il). The analysis was based on 101, 232 and 75 records of discrete location for *A. tortilis*, *A. raddiana* and *A. pachyceras*, respectively. Histograms were produced for each species, using the online application 'analysis tools', choosing the option 'Niche profile (Environmental data)'. Within the set of climate variables, we used the mean annual rainfall, maximum June temperature and mean January temperature, as proxies for the level of drought stress prevailing along the year at the native sites. We used data from existing datasets to calculate the overall mean lignin content of leaves and roots across woody species (Auger and Shipley 2013; Iversen *et al.* 2017). These data were used to test the contribution of variations in the lignin content to ^{13}C divergence among tissues. These auxiliary data were obtained from the plant trait database (TRY; Kattge *et al.* 2011; trait IDs 87 for leaf lignin and 88, 1784 and 2552 for root lignin).

Statistical analysis

The experiment tested saplings in their establishment stage in the natural habitat of acacia trees. Irrigation regimes of constant water availability do not reflect the actual dry conditions experienced by mature acacia trees in nature. We can however, still extrapolate from these results and learn about the differences between the species and the ^{13}C transport between the tree organs.

For the leaves sampled from the mature trees in Evrona, we performed a multifactorial analysis of variance (ANOVA) of the effects of acacia species

(*A. raddiana* and *A. tortilis*), and leaf direction (two sides) on the measured δ^{13}C values in the sampled leaves. Nonsignificant effects were removed from the analysis. For the results of the controlled experiment, we used a multifactorial ANOVA to analyze the effects of acacia species (*A. pachyceras*, *A. raddiana* and *A. tortilis*), irrigation treatment (as a continuous variable) and tissue type (4 tissues), on the measured δ^{13}C values, sequentially removing nonsignificant effects. We tested for significant deviation from normality using the Shapiro–Wilk test. To attain normal distribution of the data from the controlled experiment, we used data transformation that resulted in positive values of the data (by adding a constant minimum value to all results) and root-squared transformed these values. Differences among significant effects were *post hoc* tested with Tukey's HSD test, at significance level of 0.05. All the analyses were performed with JMP (SAS Co., Cary, NC, USA).

RESULTS

Four-compartment δ^{13}C in young acacia trees

In the field irrigation experiment, overall, δ^{13}C values fluctuated mostly between −26‰ and −28‰ across species, tissues and irrigation regimes (Fig. 1). Among the three species, *A. pachyceras* had the most consistent and most negative values, rarely above −27‰ and as low as −29‰, significantly more negative compared with *A. tortilis* and *A. raddiana* (Table 1). The δ^{13}C values of the two latter species were typically above −28‰. We found no significant effect of the irrigation treatments on δ^{13}C. However, δ^{13}C values were slightly lower (more negative) in the high irrigation treatment in the roots of *A. raddiana* (P = 0.0565). Among tissues, leaf and stem δ^{13}C were consistently more negative than branch and root δ^{13}C. The most notable differences among tissues were in *A. pachyceras* at high irrigation and in *A. tortilis* at mid-irrigation, in both cases due to relatively high δ^{13}C in roots.

Relationships between δ^{13}C of autotrophic and heterotrophic tissues

We plotted the δ^{13}C of each of the three heterotrophic tissues (branch, stem and root) against autotrophic (leaf) δ^{13}C (Fig. 2). Diagonal lines represent the 1:1 relationship, where all data points were expected above them, due to ^{13}C enrichment in heterotrophic tissues. Unexpectedly, we found data points distributed both above and below the 1:1 line across the tissues. In particular, stem δ^{13}C was rather similar with leaf δ^{13}C, with a significant regression for all species found mostly below the 1:1 line within the range of the results (Table 2). Branch δ^{13}C was slightly higher than leaf δ^{13}C especially in the low range of the results, with a significant linear relationship for all species. Root δ^{13}C was ~1‰ higher than leaf δ^{13}C in *A. tortilis*, whereas in *A. pachyceras* it was mostly the opposite situation, i.e. root δ^{13}C was depleted relative to leaf δ^{13}C.

Final size and greenness of young acacia trees of three species

Trees were measured at the end of the 8-year field experiment for height, bole circumference, number of branches, canopy size and greenness. In most parameters, interspecific differences were small, whereas the effect of the irrigation treatments was mostly found between the high irrigation and the two lower treatments (Fig. 3). In particular, tree height and canopy size responded significantly to the high irrigation, with 50%–150% and 50%–500% increase relative to the low irrigation, respectively (Fig. 3). Irrigation effects on the number of branches, bole circumference and canopy greenness were not significant and inconsistent for the latter two measures. Still, bole circumference was significantly higher in the high irrigation treatment in *A. raddiana*, and greenness was significantly higher with high irrigation in *A. pachyceras*.

Relationships between tissue-specific size parameters and tissue-specific δ^{13}C

Tissue-specific size parameters were considered as indicators for growth of their respective tree compartments, which could potentially be linked to their δ^{13}C enrichment patterns. The most significant correlation was at the stem level, where the three species showed R^2 of 0.35 or higher between stem height and stem δ^{13}C (Fig. 4a, significant for *A. raddiana* and *A. tortilis* but not for *A. pachyceras*; $R^2 = 0.35$, $P = 0.035$; $R^2 = 0.64$, $P = 0.0006$ and $R^2 = 0.38$, $P = 0.13$, respectively). The highest R^2 was for *A. tortilis*, this species had a significant, positive, correlation at the branch level too (R^2 = 0.42, P = 0.012), where the other two species also showed positive though nonsignificant relationships (Fig. 4b). Trees with large canopies also had less negative leaf δ^{13}C, although these relationships were not significant for the three species. Lacking a root growth parameter, we correlated between root δ^{13}C and bole circumference, but this did not yield any notable pattern.

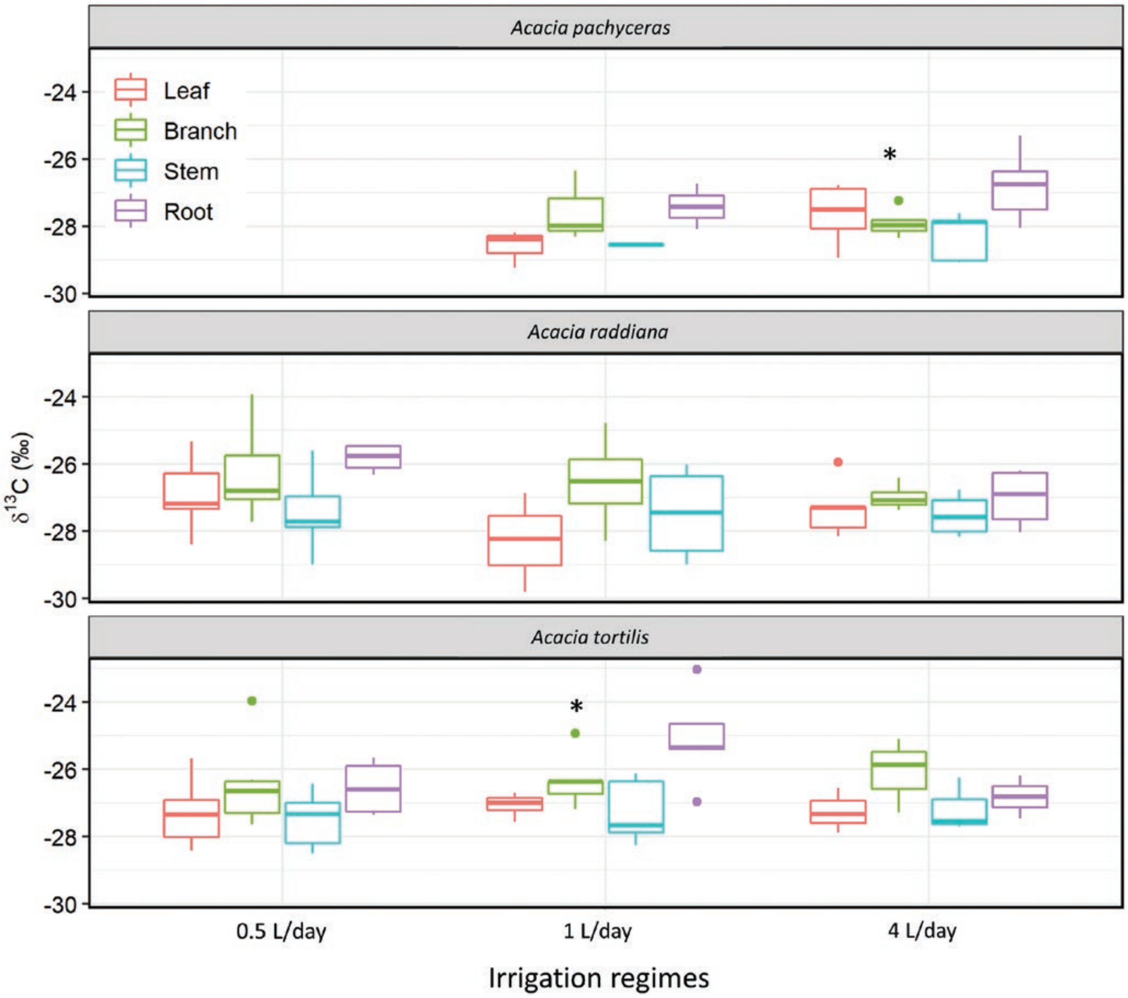

Figure 1: δ^{13}C in three hyper-arid acacia species (panels) across three irrigation regimes (groups) and in four tree tissues (colors). Data are missing for *Acacia pachyceras* at the low irrigation group due to low survival, and for *A. raddiana* roots at the medium irrigation group. Data are means ± SE (n = 3–5).

Table 1: Results of multifactorial ANOVA of the effects acacia species and plant tissue on δ^{13}C

Factor	df	Sum of squares	*F* ratio	Prob > *F*	R^2
Species	2	2.1487553	12.4353	<0.0001*	0.32
Tissue	3	2.9571101	11.4089	<0.0001*	

Nonsignificant effects were removed.

Leaf δ^{13}C of mature acacia trees

Leaves collected from mature *A. raddiana* and *A. tortilis* trees in Evrona nature reserve in late November 2016 had relatively lower δ^{13}C than expected from desert plants. Mean leaf δ^{13}C was *A. raddiana*: −27.043 ± 1.025 (SD) or 0.274 (SE) (n = 14); *A. tortilis*: −26.313 ± 0.615 (SD) or 0.217 (SE) (n = 8). We found no statistical differences between the leaf δ^{13}C values for the different sides within the trees (P = 0.202), and a marginally significant difference between the two species (P = 0.0786) with slightly less depleted/negative values in *A. tortilis*. The values were significantly more depleted in the stems and leaves compared with the roots in *A. tortilis*, for which we had samples from all three compartments (multifactorial ANOVA, df = 2, F = 11.582, P = 0.0011, n = 6, 8 and 3, respectively).

Shoot and root NSCs in mature acacia trees of two species

Tissues collected from mature *A. raddiana* and *A. tortilis* in Evrona nature reserve in late November 2016 had very low concentrations of NSCs. Starch content was essentially zero in *A. raddiana* shoots and roots; ranged 0.1%–1.1% DW in *A. tortilis*

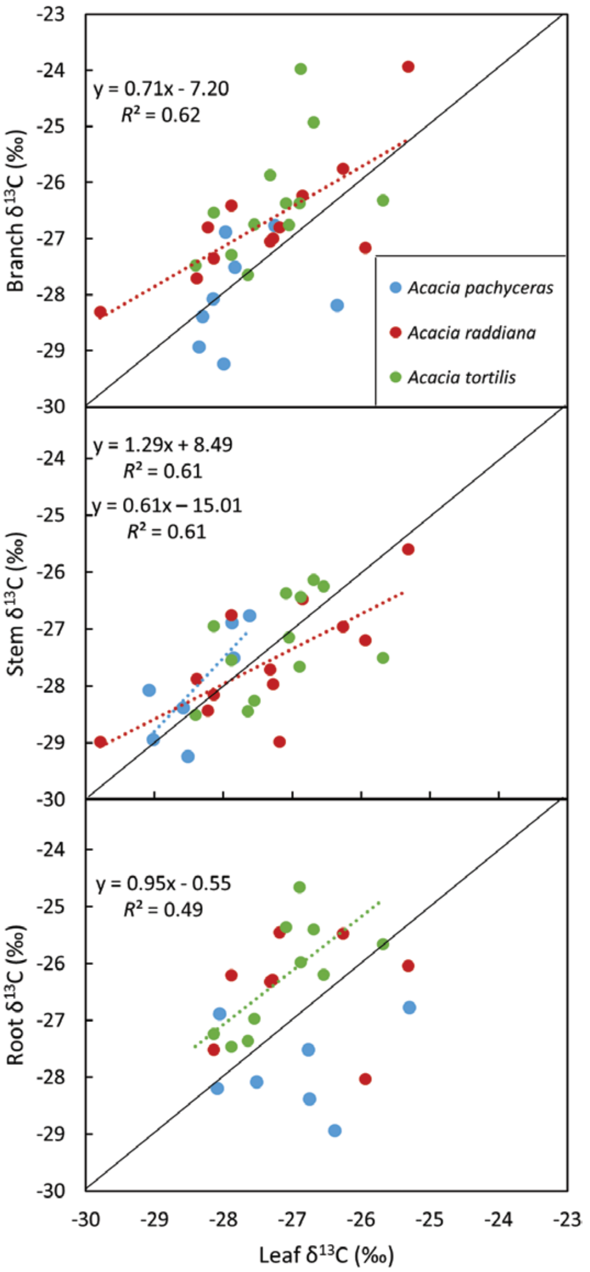

Figure 2: Relationships between $\delta^{13}C$ of non-photosynthetic tissues (branch, stem and roots) and $\delta^{13}C$ of photosynthetic tissues (leaf) in three hyper-arid acacia species (colors). Diagonal lines represent the 1:1 ratios. Line equations and R^2 are reported for linear fits that produced correlations with $R^2 > 0.4$. Data points are from the three irrigation regimes.

shoots; and was 3.8% DW in *A. tortilis* roots. Soluble sugar content ranged 0.9%–2.6% DW in *A. raddiana* shoots; zero in *A. raddiana* roots; 1.0%–1.7% DW in *A. tortilis* shoots and 0.9%–2.2% in *A. tortilis* roots. On average, tissue nonstructural content in acacia was 2.0% ± 0.7% DW.

Table 2: Regression analyses of heterotrophic to autotrophic $\delta^{13}C$ in three acacia species in a controlled irrigation experiment

Variable	Intercept	Slope	R^2	*n*	*P*
Branch $\delta^{13}C$					
All species	−6.723	0.732	0.42	32	<0.0001
A. raddiana	−7.197	0.712	0.61	12	0.0024
Stem $\delta^{13}C$					
All species	−9.982	0.643	0.47	31	<0.0001
A. pachyceras	−15.012	0.477	0.61	7	0.0372
Root $\delta^{13}C$					
All species	−14.749	0.431	0.15	25	0.0538
A. tortilis	−0.548	0.947	0.49	10	0.0234

Only significant regressions are shown for each species separately.

Niche profile analysis of three hyper-arid *Acacia* species

We found a large overlap in the climatic niche profiles of the three acacia species of Israel's dry and hot desert. Most trees grow in sites with 30–50 mm annual rainfall, and maximum and minimum temperatures of 38–40 and 11–14 °C, respectively (Fig. 5). Nevertheless, the low elevation species *A. tortilis* and *A. raddiana* occupied sites with 3 °C higher winter temperature than the higher-elevation *A. pachyceras*. Between the two low elevation species, *A. tortilis* was more confined to hot and dry sites (36–41 °C in summer, 11–15 °C in winter and 20–100 mm annual rainfall) than *A. raddiana*. In contrast, the latter species had populations at wetter and cooler sites, i.e. as wet as 300 mm rainfall and as cool as 30 °C in summer and 9 °C in winter.

DISCUSSION

We present the first study of leaf-to-root ^{13}C composition in hyper-arid tree species. While numerous studies have tested the ^{13}C composition of one or two tree tissues, there is a lack of knowledge in understanding the ^{13}C pathway along the tree system, let alone in marginal tree populations as studied here. Specifically, the field irrigation experiment helped characterize the different sensitivity of each species to water availability. Very negative $\delta^{13}C$ values disproved our first hypothesis, that under the harsh conditions

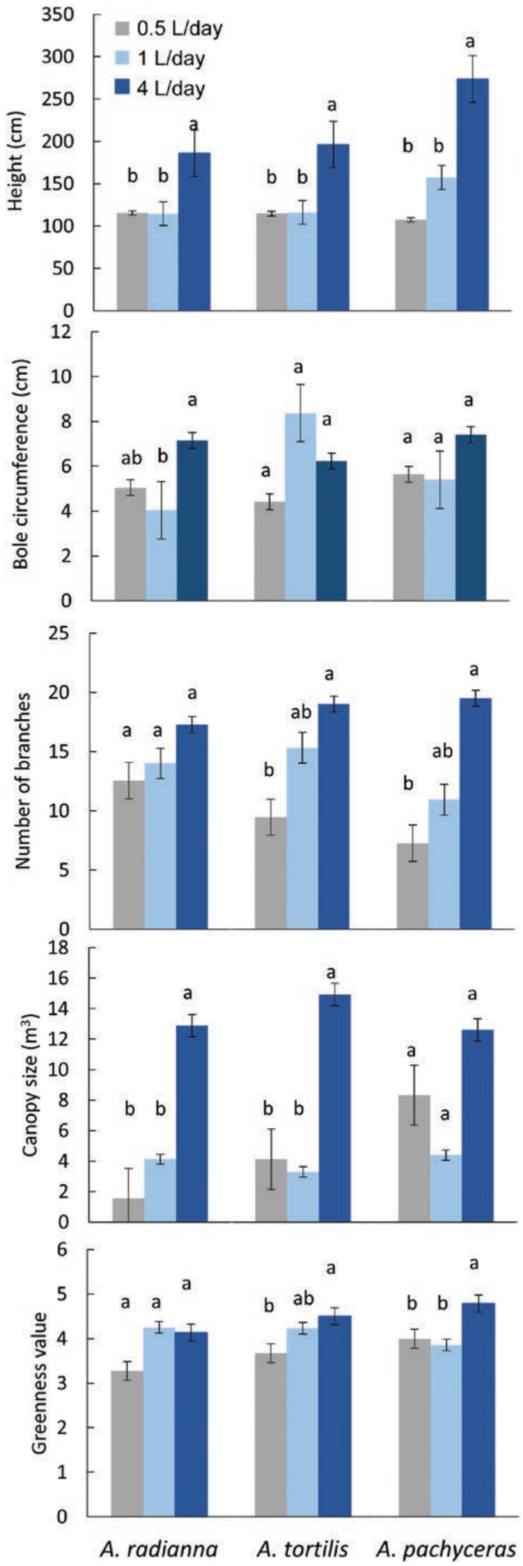

Figure 3: Final size and greenness parameters of three hyper-arid acacia species across three irrigation regimes (groups) and in four tree tissues (colors). Data are means ± SE (n = 12). Different letters denote significant differences between irrigation levels, separately for each species.

^{13}C discrimination would be limited but will increase when growing with improved irrigation. Instead, we accept our second hypothesis, that ^{13}C discrimination occurred regardless of irrigation, probably due to root access to groundwater. Leaf δ^{13}C was not enriched, and instead it was strongly depleted in mature acacia trees in the field, as well as in planted young trees. The δ^{13}C of non-photosynthetic tissues was also depleted, with no sign of the expected enrichment. Finally, we found no differences in morphological parameters (tree height, bole circumference, number of branches, canopy size and canopy greenness) under low irrigation of *A. tortilis* over the other two species. The experiment tested saplings in their establishment stage in the natural habitat of acacia trees, and although the irrigation treatments did not yield differences in δ^{13}C, there is valuable information we can deduce about the differences between the species and the ^{13}C transport between the tree organs.

High ^{13}C discrimination in *Acacia* growing in a hyper-arid environment

The ^{13}C composition in acacia leaves was far more negative than expected. This is noteworthy, especially considering the long lifespan of these species (Ehleringer and Cooper 1988; Schuster *et al.* 1992), and the hyper-arid environment they inhabit, drier than that of any other species of acacia for which δ^{13}C was measured (Cook and Dawes-Gromadzki 2005). Discrimination against ^{13}C during carbon fixation in the chloroplast is usually limited by stomatal closure, and hence in arid environments leaf δ^{13}C tends to shift to less negative values, around −24.0‰ and up to −21.5‰ (Klein *et al.* 2005) compared with −25‰ and down to −31‰ in trees growing without water stress (Bachar *et al.* 2020; Gessler *et al.* 2008, 2009; Klein *et al.* 2005). The negative values found for acacias in our study sites suggest that in most cases these trees are not suffering from lack of water, at least not in a way that influences stomatal closure. A possible explanation could be the ability of the acacia trees to develop a wide and deep root system that allows them to reach deep soil water reservoirs (Do *et al.* 2008; Sher *et al.* 2010; Stave *et al.* 2005; Winters *et al.* 2018). Sher *et al.* (2010) have also suggested that *Acacia* trees used an alternate source to the aquafer, most likely surface water. Studies have yet to determine the root length of *Acacia* trees in our region. However, based on a previous study on seedlings of *A. raddiana* and *A. tortilis*, the

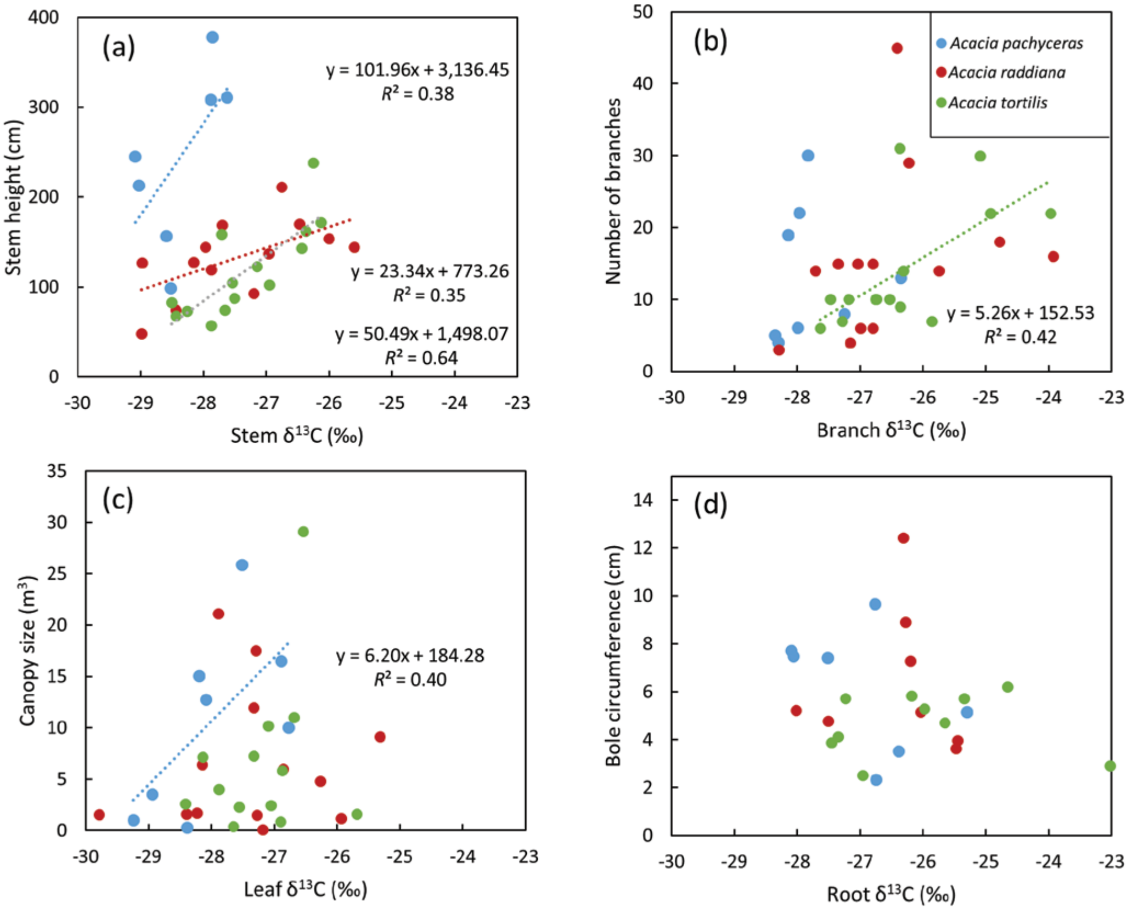

Figure 4: Relationships between tissue-specific size parameters and tissue-specific δ^{13}C in three hyper-arid acacia species (colors). Line equations and R^2 are reported for linear fits that produced correlations with $R^2 > 0.35$. Data points are from the three irrigation regimes.

vertical root elongation rate of these species was 1.8 and 1.6 cm day^{-1}, respectively (Tran *et al.* 2018). Our study site is characterized by alluvial soil, mostly composed by coarse sand with high infiltration rate (Stavi and Rosenzweig 2020). In a nearby study site in the Arava, a perched aquifer exists at depth of 7–10 m (Winters *et al.* 2015). We calculated that with the estimated rate of root elongation, it would take seedlings 470 days to reach water at depth of 8 m. Therefore, we suggest, in the first 1.5 year of the seedling's life there was a temporary advantage to the trees, which received 4.0 L day^{-1}. This advantage is shown by height and canopy size of these trees, nevertheless after this period of time, it is estimated that all trees could access water. By this mechanism, gas exchange was not limited by water, and therefore δ^{13}C values were very negative (−26‰ to −28‰) in all irrigation regimes (Fig. 1). The fact that there were no differences in δ^{13}C between irrigation treatments indicates that the advantage of high irrigation manifested mostly in taller trees with higher leaf area (Fig. 3), in turn increasing carbon uptake at the whole plant scale and higher biomass, while the gas exchange activity of single leaves remained the same at 0.5, 1.0 or 4.0 L day^{-1} (Fig. 1).

In a previous study of *A. raddiana* in the Arava, δ^{13}C was around −25.5‰ (Shrestha *et al.* 2003), yet still higher than the values around −26.5‰ measured here in mature trees. *Acacia tortilis* leaves growing in an African Savanna had δ^{13}C of −29‰ to −30‰ (MAP = 1100 mm; 65 m a.s.l. Cramer *et al.* 2010), close to our young, irrigated, trees (Fig. 1), and suggesting very high discrimination. In needles of mature pine (*Pinus halepensis*) growing on the northern edge of the Arava valley (in the semi-arid Yatir forest, MAP = 280 mm; 650 m a.s.l.), δ^{13}C was below −25.0‰ only under supplemented irrigation (Klein *et al.* 2005). Our acacia δ^{13}C was also lower than that of *Prosopis tamarugo* growing in the Atacama Desert with access to groundwater at ~7 m depth (Garrido *et al.* 2016). The more negative, although not significant, δ^{13}C in the

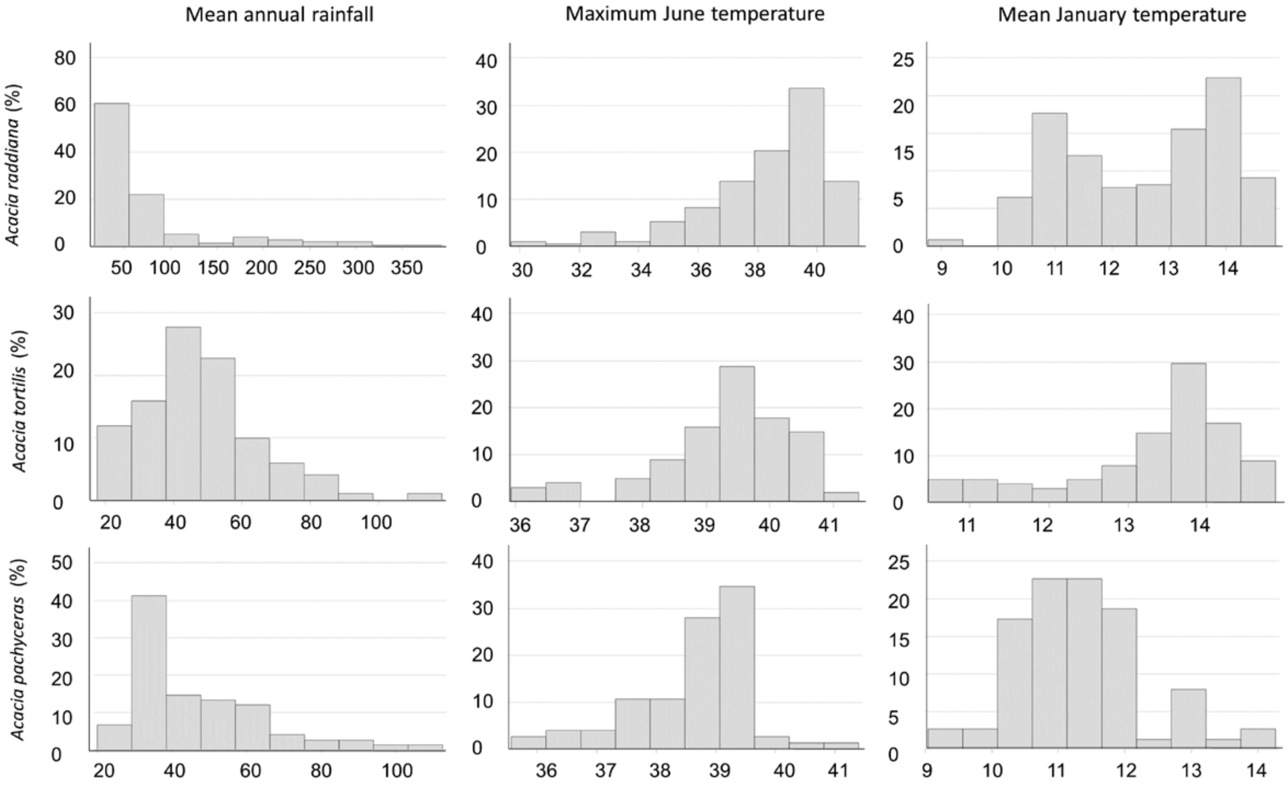

Figure 5: Niche profile analysis histograms for *Acacia raddiana*, *A. tortilis* and *A. pachyceras*, based on the mean annual rainfall (left), June temperature (middle) and January temperature (right) in their native locations in Israel. Data are from BioGIS (2019), Israel Biodiversity Information System.

NW *vs.* the more exposed SE side of *A. raddiana* provides further support to the lack of a water stress signature: higher stomatal conductance which can increase photosynthetic rates in leaves receiving more sunlight (SE *vs.* NW) means faster reduction in CO_2 concentration inside the leaf, and hence the observed $\delta^{13}C$ increase in sun-exposed leaves compared with more shaded leaves.

Our isotopic records indicate that these trees were fully active in their photosynthesis despite the heat and dryness. Indeed, previous study in another site in the Arava valley showed that mature trees of *A. raddiana* and *A. tortilis* were actively growing and transporting water during the hot and dry summer (Winters *et al.* 2018). Counter-intuitively, these trees are not typically water-limited, and seem to benefit from a steady underground source of water, allowing for high carbon fixation, and, as a result, high ^{13}C discrimination. Studies that looked for the water source for acacia trees *in situ* have used multiple approaches and tools. Analyses of *A. raddiana* shoot water potential dynamics across sites and seasons suggested that flash floods are the major water source for acacia populations in the Arava (Shrestha *et al.* 2003). Other studies exposed root systems (Peled 1988), compared the $^{18}O/^{16}O$ isotopic ratios in water samples extracted from acacia twigs and from nearby water resources (Sher *et al.* 2010), and mapped subsurface water reservoirs using electrical resistivity tomography (Winters *et al.* 2015). Still, none of these studies determined the maximum rooting depth of the hyper-arid *Acacia* species and the dynamics of their water budget in this desert.

Lack of enrichment in non-photosynthetic tissues *Acacia* growing in a hyper-arid environment

While high stomatal conductance can explain the very negative leaf $\delta^{13}C$, it cannot explain why this signal carried on to non-photosynthetic tissues (Fig. 2). We expected stem and root tissues to be more enriched in ^{13}C than leaves, due to one or more of six mechanisms as listed in the introduction section (Cernusak *et al.* 2009). Our empirical evidence calls for either inexistence of these mechanisms in our trees, or, to the existence of stronger depletion mechanisms, acting simultaneously. Testing each of these mechanisms is beyond the scope of this study, however we can provide some important hints based on our analyses.

Our previous work in the Arava supports the lack of a seasonal decoupling mechanism, showing that stem and leaf growth in mature *A. tortilis* and *A. raddiana* are highly coupled, peaking in March and November (Winters *et al.* 2018). Moreover, the fact that these tree species flush twice a year, can be another reason for their high ^{13}C discrimination, characteristic of expanding *vs.* mature leaves, as proposed by developmental variation in ^{13}C discrimination during leaf expansion. Here, the highest ^{13}C discrimination was limited to small canopies (Fig. 4c), which probably contained younger leaves.

In general, our field irrigation experimental results showed that trees which had higher stems and more branches, had also more enriched δ^{13}C (Fig. 4). Taller stems and more branches could also result in increased hydraulic resistance along the flow path from the roots to the leaves and, in consequence, to reduced stomatal conductance, resulting in less negative δ^{13}C (Becker *et al.* 2000; Petit *et al.* 2008; Ryan and Yoder 1997). It is hence possible, that as the size of non-photosynthetic tissue increased, the effects of dark respiration and PEP carboxylase carbon fixation also increased, respectively. We report here tissue concentrations of NSCs of about 2% DW. Such concentrations are among the lowest recorded for trees (and especially for roots) in any environment (Hoch and Körner 2012; Hoch *et al.* 2003; Landhäusser and Lieffers 2012) including drought-treated spruce saplings (Hartmann *et al.* 2015) and drought-stressed pines growing on the edge of the desert (Klein *et al.* 2014). Specifically, the very low, almost negligible, concentrations of starch in shoots and roots of mature acacia trees support a mechanism of differential use of day *vs.* night sucrose between leaves and sink tissues. If our acacia trees produce very little starch, then it can be expected that diel variations in δ^{13}C of sucrose would be small, δ^{13}C of the phloem sap quite constant, and the isotopic signal preserved throughout the plant.

Lastly, considering that these acacia species are capable of symbiotically associating with dinitrogen fixing bacteria (Assefa and Kleiner 1998; Sprent 1995), they are considered as nitrogen fixers plants. Recent work in our region has found that total leaf N concentration in nitrogen fixers plants is 2.54% on average, while nonfixers plants contain an average of 1.77% (Dovrat *et al.* 2020). Despite the fact we did not measure the N content of the specific leaves, we hypothesized that total leaf N concentration in acacia improves rubisco content, and therefore can be related to the lack of ^{13}C enrichment. However, measurements in the desert plant *Nitraria tangutorum* in China showed that higher nitrogen content in heterotrophic organs correlated with higher ^{13}C enrichment (Zhang *et al.* 2014), opposite to our observations. The very low starch content measured here is supportive of the biochemical composition effect on δ^{13}C of the sink tissues. Among the common compounds inside tree tissues, starch is one of the most enriched (Bowling *et al.* 2008). Therefore, lack of starch can explain the lack of ^{13}C enrichment. We did not measure other compounds, however such data exist in the literature. Specifically, lignin is among the most depleted compounds, and its variations among tissues can affect their ^{13}C content. Among six African *Acacia* species, including *A. tortilis*, leaf lignin was 11% DW (Abdulrazak *et al.* 2000). For comparison, wood lignin in six *Acacia* species, including *A. tortilis* and *A. pachyceras* (*gerrardii*), was 32% DW (Abdel-Sayed Nasser and Aref 2014). Indeed, it is well known that non-photosynthetic tissues are richer in lignin than leaves: a meta-analysis of global datasets of hundreds of records, covering dozens of woody species, showed mean lignin contents of 11.4% DW in leaves (Auger and Shipley 2013) and 19.5% DW in roots (Iversen *et al.* 2017). For example, wood lignin content in subtropical *Acacia* species in China was 19%–23% DW (Yao *et al.* 2010). This means that our hyper-arid *Acacia* species are richer in lignin than most tree species, consequently shifting their ^{13}C content to the more depleted side.

Interspecific differences between *Acacia* species growing in a hyper-arid environment

Differences among the three species studied here were mostly small. Still, *A. pachyceras* had the most negative δ^{13}C values (Fig. 1), and, when grown with high irrigation, saplings of this species were higher, with more branches, and greener canopies compared with the two southern species *A. raddiana* and *A. tortilis* (Fig. 3). Niche partitioning analysis showed that *A. tortilis* was more confined to extreme desert sites than *A. raddiana*, while *A. pachyceras* was found in cooler sites than the other two (Fig. 5). The success of *A. pachyceras* in our field experiment was surprising, as, unlike the other two species, it is more common in highlands with cool winters and nights than in the hot Arava. As climate in the region changes, species' distributions might change as well. Among the three species, it is possible that *A. tortilis* is a slower growing species, as part of its acclimation to the very hot and dry edge of life on Earth.

CONCLUSIONS

A negative leaf δ^{13}C in three desert *Acacia* species indicates low stomatal sensitivity to drought and high activity during the hot and dry conditions of the extreme desert. This phenomenon requires further research which is already in progress. The fact that stem and root tissues of the three desert *Acacia* species were not more enriched in ^{13}C than leaves as expected (Cernusak *et al.* 2009) can be explained by their (i) seasonal coupling of growth of leaves and heterotrophic tissues; (ii) high lignin content and (iii) low starch content. Among the three species, *A. pachyceras* had the most consistent and most negative values, significantly less negative compared with *A. tortilis* and *A. raddiana*. *Acacia pachyceras* had the largest non-photosynthetic biomass, which can affect the dark respiration and PEP carboxylase carbon fixation, and therefore more enriched δ^{13}C.

Supplementary Material

Supplementary material is available at *Journal of Plant Ecology* online.

Figure S1: Location of the study within the Middle East (**a**) and within the regional context, with Ketura in the North and Evrona in the South (**b**).

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Conflict of interest statement. The authors declare that they have no conflict of interest.

Authors' Contributions

E.G. initiated the field experiment, coordinated by A.H. and S.J. E.S. was in charge of the orchard. D.U. and T.K. initiated the isotopic study, and D.U. performed the isotopic analyses together with I.R. G.W. and E.S. coordinated the field site, where Y.W. took samples for nonstructural carbohydrates. D.U. and E.S. performed data analyses. T.K. and D.U. wrote the paper, with contributions from all coauthors.

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