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Growth in two common gardens reveals species by environment interaction in carbon isotope discrimination of *Eucalyptus*

NEIL C. TURNER,[1,2,3] ERNST-DETLEF SCHULZE,[4] DEAN NICOLLE[5] and IRIS KUHLMANN[4]

[1] *Centre for Legumes in Mediterranean Agriculture, M080, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia*

[2] *Institute of Agriculture, M082, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia*

[3] *Corresponding author (ncturner@clima.uwa.edu.au)*

[4] *Max-Planck Institute for Biogeochemistry, Box 100164, 07701 Jena, Germany*

[5] *Currency Creek Arboretum—Eucalyptus Research, PO Box 808, Melrose Park, SA 5039, Australia*

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Summary One-year-old sun leaves of 60 species of *Eucalyptus* were collected in August 2005 at an arboretum in South Australia with a mean annual rainfall of 427 mm, and 14 of the same species were sampled at an arboretum in Western Australia with a mean annual rainfall of 216 mm. We determined the genetic and phenotypic variation in carbon isotope composition ($\delta^{13}C$), specific leaf area (SLA) and nitrogen content per unit area of the species at each site. There were very significant ($P < 0.001$) differences in $\delta^{13}C$ among the species at both sites. The mean $\delta^{13}C$ of the 60 species at the wetter site was −27.6‰ (from −25.8‰ in *Eucalyptus youngiana* to −29.9‰ in *Eucalyptus salicola*) and of the 14 species at the drier site was −25.3‰ (from −23.7‰ in *Eucalyptus ravida* to −27.3‰ in *Eucalyptus ewartiana*). Of the 14 species common to both sites, four species had similar values of $\delta^{13}C$ at the two sites despite the differences in rainfall, whereas in others the values of $\delta^{13}C$ were significantly ($P < 0.001$) lower (more negative) at the wet than at the dry site. The SLA and nitrogen content per unit leaf area also differed significantly among the species ($P < 0.001$), but there was not a common relationship between $\delta^{13}C$ and SLA or between $\delta^{13}C$ and nitrogen content at the two sites. The strong species by environment interaction resulted from some species demonstrating phenotypic plasticity for $\delta^{13}C$, while others were inherently stable across environments.

Keywords: Corymbia, *leaf nitrogen content*, *phenotypic plasticity*, *rainfall*, *species by environment interaction*, *specific leaf area*, *water-use efficiency.*

Introduction

The carbon isotope discrimination by a leaf during photosynthesis is a function of the concentration of carbon dioxide in the air at the surface of the leaf and that in the intercellular spaces of the leaf (Farquhar et al. 1982) and, when integrated over time, has often been taken as a measure of the stomatal response to water shortage (Farquhar et al. 1982), the integral of water-use efficiency from the time of leaf development to time of sampling (e.g., Read and Farquhar 1991, Anderson et al. 1996, Warren et al. 2006) and a measure of the adaptation to aridity. Studies along aridity gradients have shown either a negative relationship between the ecosystem mean of $\delta^{13}C$ or the $\delta^{13}C$ of a particular species and rainfall (Stewart et al. 1995, Macfarlane et al. 2004), or that the $\delta^{13}C$ of an individual species did not vary with rainfall, but species in wetter regions had lower (more negative) values of $\delta^{13}C$ than species in drier regions (Schulze et al. 1998, Miller et al. 2001). In order to partition the variation in $\delta^{13}C$ arising from the environmental conditions along an aridity gradient from the inherent or genetic differences in $\delta^{13}C$ from adaptation to a particular environment, species have been compared in a common garden or under similar environmental conditions in a controlled-environment chamber or greenhouse (Read and Farquhar 1991, Anderson et al. 1996, 2000, Warren et al. 2005, 2006). Some of these studies suggest that the variation in $\delta^{13}C$ is associated with rainfall, or rainfall distribution, at the place of origin of the seed, whereas Warren et al. (2006) observed no relationship between $\delta^{13}C$ and rainfall at the place of origin. Indeed, Warren et al. (2006) concluded that for *Eucalyptus* there is no simple relationship between water-use efficiency in a common garden as measured by $\delta^{13}C$ and rainfall at the place of origin of the species because long-term rainfall measurement may not be a good indication of water availability, or rainfall may not be the sole climatic determinant of species' distribution.

Analysis of the $\delta^{13}C$ of *Eucalyptus* species along an aridity gradient showed that, as the annual rainfall decreased from 300 to 125 mm, the values of $\delta^{13}C$ of 1-year-old leaves could increase, decrease or were unchanged (Schulze et al. 2006*a*).

Sampling 1-year-old leaves of the same species and trees along the same transect after a wetter year showed only a modest lowering of δ^{13}C and did not clarify the influence of rainfall on the values of leaf δ^{13}C (Turner et al. 2008). Likewise, when the species were grown in a common garden with an annual rainfall similar to the average of the annual rainfall along the previously studied aridity gradient, the 1-year-old leaves of species differed in their δ^{13}C in the common garden to as great an extent as across the entire aridity gradient (Schulze et al. 2006*b*). From these studies, it was not clear whether the values of leaf δ^{13}C of same-aged leaves of *Eucalyptus* species were genetically stable across rainfall environments or varied with environmental conditions in the year of growth.

What was suggested from all our three studies was that rainfall did not directly determine the value of leaf δ^{13}C. Path analysis (Grace and Pugesek 1998) of rainfall, δ^{13}C, specific leaf area (SLA) and nitrogen content of the leaves suggested that mean annual rainfall did not directly affect the δ^{13}C of the species, but affected δ^{13}C through the effect of rainfall on SLA and nitrogen content of the leaves (Schulze et al. 2006*a*, 2006*b*, Turner et al. 2008). Species that grow in dry environments often reduce water loss by producing smaller and thicker leaves, resulting in a decrease in SLA (Cunningham et al. 1999), a lower nitrogen concentration but higher nitrogen content per unit area (Farquhar et al. 2002, Schulze et al. 2005). When 29 *Eucalyptus* species were compared in two common gardens, one at a wet fertile site and one at a dry infertile site, SLA and nitrogen concentration were higher at the wet site than at the dry site, while leaf area, leaf shape and δ^{13}C were not different at the two sites (Warren et al. 2006). This suggests that rainfall and/or fertility did not determine the δ^{13}C of the species and questions the conclusion that rainfall affects δ^{13}C indirectly through changes in SLA and nitrogen concentration of the leaves.

To try and clarify whether leaf δ^{13}C is inherently stable across environments or varies with environment, particularly rainfall, we measured the leaf δ^{13}C of a number of *Eucalyptus* species in two common gardens varying in rainfall. To clarify the role of leaf morphology and chemistry on δ^{13}C, the SLA and nitrogen content of the leaves of the species were also measured in both common gardens. To minimize variation in δ^{13}C due to leaf development, leaves of similar age (1-year-old) and similar canopy position were sampled in the same month at the two sites. The aims of the study were (i) to determine whether all the species responded in the same way to the different amounts of annual rainfall at the two sites, or whether *Eucalyptus* showed a strong species by environment interaction in δ^{13}C, SLA or nitrogen content, and (ii) to establish whether δ^{13}C was uniquely related to SLA and leaf nitrogen content in both environments. If there is an indirect effect of rainfall on δ^{13}C through SLA and nitrogen content, we would expect this to be similar in both environments.

Materials and methods

In August 2005, 1-year-old sun leaves from the upper third of the canopy of 60 species of *Eucalyptus* and the closely related *Corymbia* species were collected at the Currency Creek Arboretum [35° 26′ S, 138° 46′ E; 100 m above sea level (m.a.s.l.), 427 mm mean annual rainfall (MAR), texture-contrast soil of sand over clay] near Adelaide in South Australia (Nicolle 2003) and similar-aged leaves of 14 of the same species at the Coolgardie Arboretum (30° 57′ S, 121° 09′ E; 417 m.a.s.l., 216 mm MAR, clay soil) adjacent to the township of Coolgardie in Western Australia. At Coolgardie, 10–25 leaves, depending on size, were collected from four replicate trees of each species, whereas at Currency

Table 1. *Eucalyptus* species sampled at both the Coolgardie Arboretum and the Currency Creek Arboretum. The number (No) of trees sampled, year the trees were planted and the mean height in August 2005 are given.

Species	Coolgardie			Currency Creek		
	No	Year planted	Height (m)	No	Year planted	Height (m)
E. yilgarnensis (Maiden) Brooker	4	1974	6.5	4	1996	2.5
Eucalyptus loxophleba Benth. *gratiae* Brooker	4	1978	8	7	1994	6
Eucalyptus jutsonii Maiden	4	1978	3	3	1994	3
E. ravida Johnson & Hill	4	1976	7.5	4	1993	4
Eucalyptus celastroides Turcz.	4	1976	5	2	1994	3.5
E. calycogona Turcz.	4	1976	7	7	1994	4
Eucalyptus lesouefii Maiden	4	1979	9	3	1994	5
E. youngiana F. Muell.	5	1980	6	5	1994	3
Eucalyptus intertexta R. Baker	4	1978	5	3	1994	3
E. kingsmillii (Maiden) Maiden & Blakely	4	1976	3.5	2	1994	2.5
Eucalyptus urna Nicolle	4	1976	8	6	1997	4
E. leptopoda Benth.	4	NA	5	5	1993	3
E. ewartiana Maiden	4	1979	4.5	4	1993	2.5
Eucalyptus transcontinentalis Maiden	4	1975	10	2	1994	5

NA, not known as trees were growing in native habitat.

Creek a similar number of leaves of similar age were sampled from two to seven replicate trees. At the Currency Creek Arboretum, four trees grown from seed from the same mother tree are grown along with trees from several other mother trees. For 54 of the 60 species, 10–25 leaves were taken from each of up to four of the trees grown from the same mother tree. In addition, 10–25 leaves were also collected from each tree that originated from other mother trees in the collection. The trees at Currency Creek were planted from 1993 to 1997 in a 5-m grid pattern, while the trees at Coolgardie were planted between 1974 and 1980 also in a 5-m grid pattern. At Coolgardie, one species, *Eucalyptus leptopoda*, was collected from a bush site (30° 57′ S, 121° 09′ E, 406 m.a.s.l.) 1 km from the arboretum. In August 2005, the trees at Currency Creek were from 2.5 to 6 m (see photograph in Schulze et al. 2006*b*) and at Coolgardie were 3–10 m in height (Table 1). At both sites, the species were spaced such that the crowns of the individual trees did not touch.

After sampling and measurement of tree height, the leaves were transferred to the laboratory where the area of leaves was measured with a LI-3100 (LiCor Inc., Lincoln, NE, USA) area meter and the dry weight determined after drying at 70 °C for 48 h in a forced-draft oven. SLA was calculated as area per unit mass and the leaves finely ground to a powder for the analysis of nitrogen content with a Vario EL II analyser (Elementar, Hanau, Germany) and the carbon isotope ratio (δ^{13}C) determined after combustion using a ratio mass spectrometer (Finnigan Delta+ XL, Bremen, Germany) with a routine precision of better than 0.1‰. The δ^{13}C was referenced against Vienna Peedee Belemnite on a scale normalized by assigning consensus values of −46.6‰ to L-SVEC lithium carbonate and +1.95‰ to NBS 19 calcium carbonate (Coplen et al. 2006). Using this referencing system, the δ^{13}C of NBS 22 oil is −30.03‰ compared with a value of −29.78‰ used in previous studies (Farquhar et al. 1989). The isotopic ratios are expressed as delta values in parts per thousand and not as carbon isotope discrimination (Farquhar and Richards 1984) because the isotopic composition of carbon dioxide at the sites was not measured.

The results at one site were analysed by one-way ANOVA and at the two sites by two-way ANOVA using Genstat (Release 9.2). Regressions were fitted and analysed in SigmaPlot (version 10.0).

Results

Carbon isotope discrimination (δ^{13}C)

There was a highly significant ($P < 0.001$) difference among the 60 species of *Eucalyptus*, including the closely related *Corymbia* species, sampled at the Currency Creek Arboretum (all data can be accessed at http://www.bgc-jena.mpg.de/bgc-processes/publdata/australia2005.html). The mean δ^{13}C of all species was −27.6‰ and varied from −25.8‰ (range −25.7 to −25.8‰) in *Eucalyptus youngiana* to −29.9‰ (range −29.2 to −30.4‰) in *Eucalyptus salicola.* Of the 54 species that had half-siblings from the same mother tree, no significant difference among the siblings was found, indicating that the differences among the species did not arise from their location or localized environmental conditions within the arboretum. There was also a highly significant ($P < 0.001$) difference among the 14 species at Coolgardie. The mean δ^{13}C of all species was −25.3‰ and varied from −23.7‰ (range −23.1 to −24.6‰) in *Eucalyptus ravida* to −27.3‰ (range −26.4 to −28.9‰) in *Eucalyptus ewartiana.*

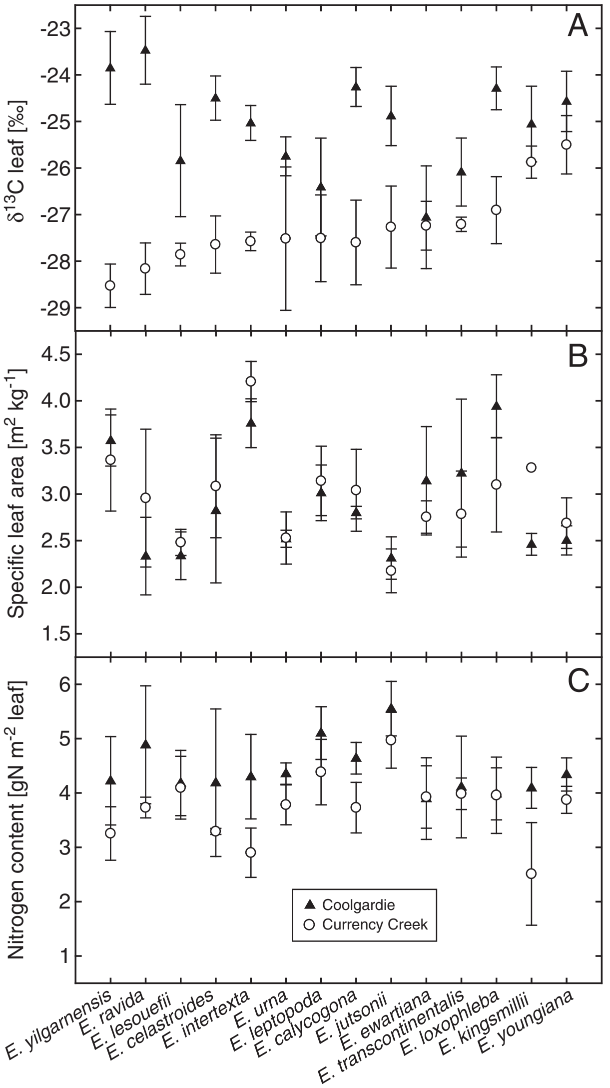

Figure 1. (A) Leaf carbon isotope discrimination (δ^{13}C), (B) SLA and (C) nitrogen content per unit leaf area in 14 *Eucalyptus* species measured at two arboreta, Currency Creek (open circles) and Coolgardie (filled triangles), in August 2005. Species are arranged in ascending values of δ^{13}C at Currency Creek. Values are means ± 1 SD (n = 2–7, see Table 1).

When the 14 species of *Eucalyptus* common to both Coolgardie and Currency Creek (Table 1) were compared, the ANOVA showed that not only did the species differ significantly in δ^{13}C (P <0.001) but there was a significant interaction in δ^{13}C ($P < 0.001$) with species identity at the two sites.

Table 2. *Eucalyptus* species sampled at both the Coolgardie Arboretum and the Currency Creek Arboretum and the soil type on which the mother trees of the species were collected. The species are ranked on the basis of the difference in carbon isotope discrimination ($\delta^{13}C$) between the Coolgardie Arboretum and Currency Creek Arboretum. Additionally, the values of carbon isotope discrimination for seven *Eucalyptus* species collected in their native habitat in Western Australia in 2004 (Turner et al. 2008) and the means of the same species collected from the Coolgardie and Currency Creek arboreta in 2005 are presented. Values of $\delta^{13}C$ (based on NBS 22 oil = −30.03‰) are means ± 1 SD. Note that *E. leptopoda* is not listed as it was collected from a natural sandy site and not from the Coolgardie Arboretum.

Species	Usual soil and habitat of naturally occurring populations	$\delta^{13}C$ (‰) Coolgardie	$\delta^{13}C$ (‰) Currency Creek	$\delta^{13}C$ (‰) difference	$\delta^{13}C$ (‰) native habitat	$\delta^{13}C$ (‰) Mean of two arboreta
E. ravida	Reddish clay loams on plains	−23.72 ± 0.73	−28.41 ± 0.55	4.69	−25.72 ± 0.36	−26.06 ± 0.64
E. calycogona	Reddish clay loams to loams on plains	−24.51 ± 0.42	−27.85 ± 0.91	3.34		
E. yilgarnensis	Reddish clay loams on plains	−24.10 ± 0.78	−27.27 ± 0.46	3.17	−26.86 ± 0.80	−25.68 ± 0.62
E. celastroides	Reddish clay loams on plains	−24.75 ± 0.48	−27.89 ± 0.61	3.14	−26.53 ± 0.76	−26.32 ± 0.54
E. loxophleba	Red-brown loams on plains	−24.54 ± 0.46	−27.15 ± 0.72	2.61	−26.54 ± 1.07	−25.84 ± 0.59
E. intertexta	Sandy ephemeral water courses in arid regions	−25.28 ± 0.38	−27.83 ± 0.20	2.55		
E. jutsonii	Deep sand in dune formations	−25.13 ± 0.64	−27.52 ± 0.88	2.39		
E. lesouefii	Gravelly clay loams, often on greenstone rises	−26.09 ± 1.20	−28.11 ± 0.24	2.02	−26.08 ± 0.56	−27.10 ± 0.72
E. urna	Reddish clay loams on plains	−26.00 ± 0.42	−27.77 ± 1.54	1.77	−26.58 ± 0.92	−26.88 ± 0.98
E. transcontinentalis	Reddish clay loams on plains	−26.34 ± 0.73	−27.46 ± 0.15	1.12	−27.05 ± 0.79	−26.90 ± 0.44
E. youngiana	Desert sands on plains or dune formations	−24.82 ± 0.65	−25.75 ± 0.63	0.93		
E. kingsmillii	Desert sands on plains or rocky/gravelly ridges	−25.31 ± 0.81	−26.12 ± 0.34	0.81		
E. ewartiana	Gravelly sandy loam on plains or rocky/gravelly ridges	−27.31 ± 1.10	−27.49 ± 0.52	0.18		

Four species had similar values of $\delta^{13}C$ at the two sites despite the differences in rainfall, whereas in others the values of $\delta^{13}C$ were significantly ($P < 0.001$) lower (more negative) at the wet site than at the dry site. This can clearly be seen in Figure 1A in which *Eucalyptus yilgarnensis* and *E. ravida*, for example, had significantly more negative values of $\delta^{13}C$ at the wetter Currency Creek site (−27.3 and −28.4‰, respectively) than at the drier Coolgardie site (−24.1 and −23.7‰, respectively). In contrast, *E. youngiana* and *Eucalyptus kingsmillii*, for example, had similar values of $\delta^{13}C$ of −25.8 and −26.1‰ at Currency Creek and 24.8 and −25.3‰ at Coolgardie, respectively. Seven of the species in the two arboreta were also observed in their native habitat along an aridity gradient (Turner et al. 2008). The values of $\delta^{13}C$ from the aridity gradient were always intermediate between those at the Currency Creek and Coolgardie arboreta (Table 2) and were not significantly different from the mean of the values of $\delta^{13}C$ from the two sites (overall mean of the seven species at the two common gardens was −26.40‰, while along the transect it was −26.48‰), even when the values of $\delta^{13}C$ at the two sites differed significantly.

Specific leaf area, leaf nitrogen content and tree height

Both the SLA and nitrogen content per unit leaf area differed significantly ($P < 0.001$) among the 14 species at the two sites. While the SLA of the species differed ($P < 0.001$) depending on the site from 2.18 to 4.46 $m^2 kg^{-1}$ at Currency Creek and 2.31–3.94 $m^2 kg^{-1}$ at Coolgardie (Figure 1B), the nitrogen content did not vary significantly between sites (2.51–4.97 $g m^{-2}$ at Currency Creek and 3.90–5.55 $g m^{-2}$ at Coolgardie) and there was no interaction with species identity at the two sites ($P = 0.110$) (Figure 1C).

At both sites, $\delta^{13}C$ did not change with an increase in SLA (for Coolgardie: $\delta^{13}C = -25.1 - 0.01$ SLA, $r^2 = 0.00$; for Currency Creek: $\delta^{13}C = -26.7 - 0.19$ SLA, $r^2 = 0.01$) or increase in nitrogen (N) content (for Coolgardie: $\delta^{13}C = -26.7 + 0.37$ N, $r^2 = 0.03$; for Currency Creek: $\delta^{13}C = -26.8 - 0.14$ N, $r^2 = 0.01$) on a leaf area basis (Figure 2). However, the relationship with $\delta^{13}C$ differed with location such that the values of $\delta^{13}C$ were significantly more negative ($P < 0.001$) at the Currency Creek site than at the Coolgardie site for SLA (Figure 2A) and nitrogen content (Figure 2B). When the data for the same species were linked (Figure 2), it was clear that $\delta^{13}C$ was higher at Currency Creek than Coolgardie in all species, but this was not correlated with any consistent change in SLA (Figure 2A), while the nitrogen content per unit area as well as $\delta^{13}C$ increased at Coolgardie compared with Currency Creek, but not to the same extent (Figure 2B). Although the nitrogen content for a particular SLA tended to be higher at Coolgardie than at Currency Creek, there was no statistically significant difference in the relationship between SLA and nitrogen content between sites, with nitrogen content on a leaf area basis decreasing as SLA increased (Figure 3) and nitrogen content on a leaf dry weight basis increasing as SLA increased (data not shown).

The trees were planted about 20 years earlier at the Coolgardie site than at the Currency Creek site, which resulted in differ-

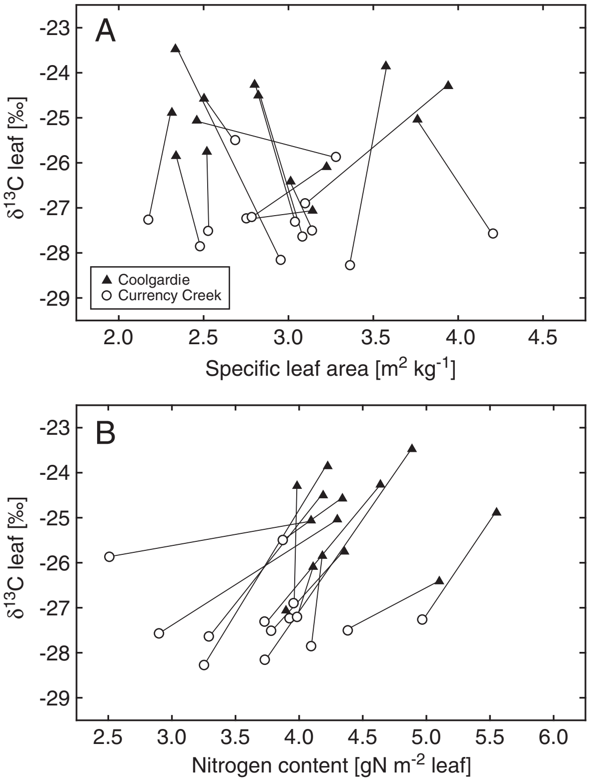

Figure 2. Relationship between leaf carbon isotope discrimination (δ^{13}C) and SLA (A) and nitrogen content per unit leaf area (B) for 14 *Eucalyptus* species measured at two arboreta, Currency Creek (open circles) and Coolgardie (filled triangles), in August 2005. Values are means (n = 2–7, see Table 1) with lines joining the same species in the two arboreta.

ences in tree height (Table 1), which might be taken as a proxy for crown size and leaf area. Generally, we would expect that δ^{13}C increases with tree height due to a larger water demand. At both sites, δ^{13}C did not change significantly with tree height (H) (for Coolgardie: $\delta^{13}\text{C} = -27.0 + 0.29$ H, $r^2 = 0.00$; for Currency Creek: $\delta^{13}\text{C} = -27.0 - 0.01$ H, $r^2 = 0.01$), even though Currency Creek operated at lower δ^{13}C than Coolgardie (Figure 4). The lack of a height response is taken as an indication that larger trees also reach deeper soil horizons and thus tap additional water at this dry site. The variation with tree size does not explain the conservative response of some species, especially *E. ewartiana*, *E. kingsmillii* and *E. youngiana*, in which the δ^{13}C value remained constant between sites (Figure 4).

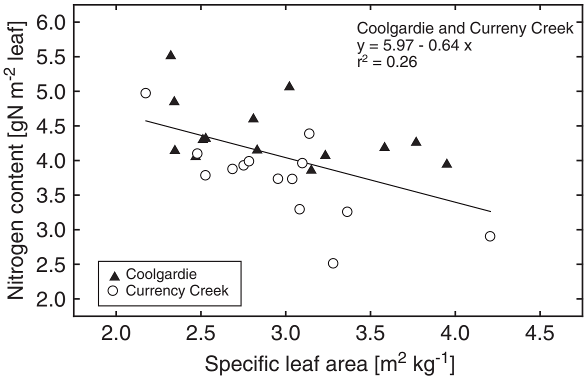

Figure 3. The relationship between leaf nitrogen content per unit leaf area and SLA for 14 *Eucalyptus* species measured at two arboreta, Currency Creek (open circles) and Coolgardie (filled triangles), in August 2005. Values are means of 2–7 samples (see Table 1). The single regression for the two sites is given as the individual site regressions were not significantly different.

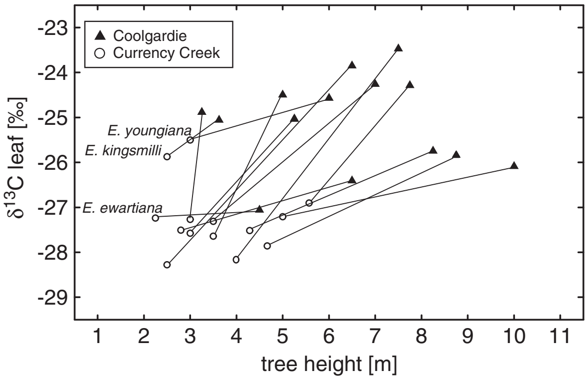

Figure 4. The relationship between leaf carbon isotope discrimination (δ^{13}C) and tree height for 14 *Eucalyptus* species measured at two arboreta, Currency Creek (open circles) and Coolgardie (filled triangles), in August 2005. Values are means (n = 2–7, see Table 1) with lines joining the same species in the two arboreta.

Discussion

The statistically significant differences in δ^{13}C for some species growing in the two common gardens have clearly shown that there are genetic differences among species of *Eucalyptus* in their δ^{13}C signature. Inherent differences or ecotypic adaptation in δ^{13}C among species of *Eucalyptus* have been observed previously (Anderson et al. 1996, Warren et al. 2006), but the species by environment interaction in δ^{13}C observed in the present study has not been previously reported. While the values of δ^{13}C were, as expected, higher at the drier Coolgardie site than at the wetter Currency Creek site in some species, the values were not higher in all species. *E. ewartiana*, *E. youngiana*, *E. kingsmillii* and *E. leptopoda* had similar values of δ^{13}C whether the plants were growing at Currency Creek (427 mm) or Coolgardie (216 mm), while *E. yilgarnensis*, *E. ravida*, *Eucalyptus calycogona* and the remaining species showed variation in δ^{13}C with the species having significantly lower (more negative) values of δ^{13}C at the wetter Currency Creek site than at the drier Coolgardie site. This indicates that some *Eucalyptus* species have genetically stable values of δ^{13}C under variable environmental conditions, whilst other species demonstrated phenotypic plasticity with the values of δ^{13}C varying with site, as observed previously for *Eucalyptus globulus* (Macfarlane et al. 2004).

Differences in δ^{13}C can arise from differences in stomatal conductance, photosynthetic capacity or both. Generally, an increase in δ^{13}C with decreasing rainfall has been assumed to indicate more conservative water use by stomatal closure (e.g., Anderson et al. 2000), but several studies have shown that SLA decreases and nitrogen content per unit area increases with decreasing rainfall, suggesting that photosynthetic capacity may also be influenced by aridity through changes in leaf morphology and chemistry (Anderson et al. 2000, Schulze et al. 2006*a*, 2006*b*, Warren et al. 2006, Turner et al. 2008). The present study suggests that species such as *E. yilgarnensis*, *E. ravida* and *E. calycogona* that showed phenotypic plasticity in δ^{13}C, and hence the internal to external carbon dioxide concentrations, likely showed phenotypic plasticity in stomatal conductance or photosynthetic capacity, while species such as *E. ewartiana*, *E. youngiana* and *E. kingsmillii* with inherently stable values of δ^{13}C likely had inherently stable stomatal conductance and/or photosynthetic capacity across environments. We recognize that both stomatal conductance and photosynthesis may each show variation across rainfall environments in such a way that the internal to external carbon dioxide concentration and δ^{13}C may remain constant.

Whether the species was phenotypically plastic or genetically stable was not related to the rainfall in the region of origin as all the species came from the 200–300-mm MAR zone and had similar values of δ^{13}C in their native habitat (Schulze et al. 2006*a*, Turner et al. 2008). Of the seven species measured in the two common gardens and in their native habitat in 2004 (Turner et al. 2008), the values of δ^{13}C along the aridity gradient were intermediate between the values of δ^{13}C observed in the common gardens in the species that were phenotypically plastic and the same as those that showed genetic stability (Table 2). The rainfall in the 12 months prior to the 2004 sampling was between 290 and 340 mm at the sites where the species were collected, intermediate between the annual rainfall at the two common garden sites, so that the phenotypically plastic species showed an approximately linear response between δ^{13}C and rainfall. Thus, the species by environment interaction observed in this study helps to explain the variation in δ^{13}C with rainfall in some species along an aridity gradient and the lack of any relationship between δ^{13}C and rainfall in other species (Schulze et al. 2006*a*, Turner et al. 2008).

We suggest that whether the species have genetically stable or phenotypically plastic δ^{13}C values may depend on the edaphic conditions to which they are adapted. The species that occur naturally on sites with clay soils were the ones that showed phenotypic plasticity in δ^{13}C when grown in different rainfall environments, whereas the species that are found on well-drained sandy and gravelly soils had less variation in δ^{13}C when grown at a wet or dry site (Table 2). The species that grow naturally on sandy and gravelly soils likely have deep roots and take advantage of water when it is available to prevent it from draining below the root zone compared with those that are adapted to clay soils with shallower water penetration and root depth that need to conserve water when plentiful for times when it is less plentiful.

Previously, we have suggested that rainfall indirectly influenced δ^{13}C through its influence on the morphology and chemistry of the leaves, namely the SLA and nitrogen content of the leaf (Schulze et al. 2006*a*, 2006*b*, Turner et al. 2008). Both SLA and the nitrogen content of the leaf varied significantly among the species, and the relationships between δ^{13}C and SLA and δ^{13}C and the nitrogen content per unit area were similar to those observed previously (Warren et al. 2005, Schulze et al. 2006*a*, 2006*b*, Turner et al. 2008). However, the values of δ^{13}C were always significantly lower at the Currency Creek site than at the Coolgardie site at the same values of SLA or nitrogen content. The lack of a unique relationship between δ^{13}C and leaf morphology or chemistry that encompasses these two sites that differ in rainfall does not negate our previous conclusion that rainfall indirectly determines δ^{13}C through changes in leaf morphology and chemistry, but does suggest that differences other than rainfall, such as soil type, nutrition, tree size and age, or mean vapour pressure deficit may also be affecting the relationship between δ^{13}C, SLA and leaf nitrogen content. For example, higher temperatures and greater vapour pressure deficits at the inland Coolgardie site (ca. 315 km from the nearest coast) than the more maritime climate at the Currency Creek site (ca. 10 km from the nearest coast) could lead to greater stomatal closure and a higher (less negative) values of δ^{13}C.

The study shows a higher δ^{13}C with decreasing rainfall, which confirms the common understanding that stomata close with aridity, which in turn affects the value of δ^{13}C. However, the species by environment interaction has shown that some species respond to the environment (phenotypic plasticity), while others are inherently stable in both environments studied. The analysis of SLA and nitrogen content of the leaves at the two sites confirms that δ^{13}C of a species at a particular site is influenced by the leaf morphology (SLA) and chemistry (nitrogen content), but that between sites other factors in addition to rainfall were influencing the values of δ^{13}C observed (cf. Warren et al. 2005, 2006). The variation in response of the *Eucalyptus* species at the two common gardens has helped to explain the variation in δ^{13}C among species along an aridity gradient, with some species showing phenotypic variation and others showing consistent values of δ^{13}C irrespective of the rainfall. This variation in response to rainfall within a species that grows along an aridity gradient shows the range of adaptation that has evolved within the *Eucalyptus* species and that a single adaptive mechanism is unlikely to enable a species to survive the rigours of a dry environment.

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