Leaf and wood carbon isotope ratios, specific leaf areas and wood growth of *Eucalyptus* species across a rainfall gradient in Australia[†]

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Summary   Leaves and samples of recent wood of *Eucalyptus* species were collected along a rainfall gradient parallel to the coast of Western Australia between Perth in the north and Walpole in the south and along a southwest to northeast transect from Walpole in southwestern Australia, to near Mount Olga in central Australia. The collection included 65 species of *Eucalyptus* sampled at 73 sites and many of the species were collected at several sites along the rainfall gradient. Specific leaf area (SLA) and isotopic ratio of ^{13}C to ^{12}C ($\delta^{13}C$) of leaves that grew in 2002, and tree ring growth and $\delta^{13}C$ of individual cell layers of the wood were measured. Rainfall data were obtained from the Australian Bureau of Meteorology for 29 locations that represented one or a few closely located collection sites.

Site-averaged data and species-specific values of $\delta^{13}C$ decreased with decreasing annual rainfall between 1200 and 300 mm at a rate of 1.63‰ per 1000 mm decrease in rainfall. Responses became variable in the low rainfall region (< 300 mm), with some species showing decreasing $\delta^{13}C$ with rainfall, whereas $\delta^{13}C$ increased or remained constant in other species. The range of $\delta^{13}C$ values in the low rainfall region was as large as the range observed at sites receiving > 300 mm of annual rainfall.

Specific leaf area varied between 2 and 6 $m^2\,kg^{-1}$ and tended to increase with decreasing annual rainfall in some species, but not all, whereas $\delta^{13}C$ decreased with SLA. The relationship between $\delta^{13}C$ and SLA was highly species and soil-type specific. Leaf-area-based nitrogen (N) content varied between 2 and almost 6 $g\,m^{-2}$ and decreased with rainfall. Thus, thicker leaves were associated with higher N content and this compensated for the effect of drought on $\delta^{13}C$. Nitrogen content was also related to soil type and species identity. Based on a linear mixed model, statistical analysis of the whole data set showed that 27% of the variation in $\delta^{13}C$ was associated with changes in SLA, 16% with soil type and only 1% with rainfall. Additionally, 21% was associated with species identity. For a subset of sites with > 300 mm rainfall, 43% of the variation was explained by SLA, 13% by soil type and only 3% by rainfall. The species effect decreased to 9% because there were fewer species in the subset of sites. The small effect of rainfall on $\delta^{13}C$ was further supported by a path analysis that yielded a standardized path coefficient of 0.38 for the effect of rainfall on SLA and –0.50 for the effect of SLA on $\delta^{13}C$, but an insignificantly low standardized path coefficient of –0.05 for the direct effect of rainfall on $\delta^{13}C$. Thus, in contrast to our hypothesis that $\delta^{13}C$ decreases with rainfall independent of soil type and species, we detected no statistically significant relationship between rainfall and $\delta^{13}C$ in leaves of trees growing at sites receiving < 300 mm of rainfall annually. Rainfall affected $\delta^{13}C$ indirectly through soil type (a surrogate for water-holding capacity) across the rainfall gradient.

Annual tree rings are not clearly visible in evergreen *Eucalyptus* species, even in the seasonally cool climate of SW Australia. Generally, visible density transitions in the wood are related not to a strict annual cycle but to periods of growth associated mainly with rainfall. The relationship between $\delta^{13}C$ of leaves and the width of these stem increments was not statistically significant. Analysis of stem growth periods showed that $\delta^{13}C$ in wood responded to rainfall events, but carbohydrate storage and reallocation also affected the isotopic signature. Although $\delta^{13}C$ in wood of any one species varied over a range of 2 to 4‰, there was a general relationship between $\delta^{13}C$ of the leaves and the annual range of $\delta^{13}C$ in wood. We conclude that species-specific traits are important in understanding the response of *Eucalyptus* to rainfall and that the diversity of the genus may reflect its response to the large climatic gradient in Australia and to the large annual and inter-annual variations in rainfall at any one location.

Keywords: *aridity gradient, biodiversity, $\delta^{13}C$ in leaves and wood, drought, soil type, tree rings.*

Introduction

Plants have developed a range of mechanisms for adapting to water shortage (Walter 1964, Evenari et al. 1971, Turner and Kramer 1980, Schulze et al. 2005). Two important leaf-level mechanisms are stomatal closure and a reduction in leaf area—which is usually associated with increases in leaf thickness and dry mass resulting in a decrease in specific leaf area (SLA)—both of which reduce water loss from the canopy and may also affect assimilation. It is unclear which of these mechanisms of adaptation is more important in trees growing along an aridity gradient. An integrated measure of the stomatal response to water shortage is the isotopic ratio of ^{13}C to ^{12}C ($\delta^{13}C$) of leaves (Farquhar et al. 1982), because the diffusion of ^{13}C through partially closed stomata is slower than the diffusion of ^{12}C. However, in nature, different relationships between $\delta^{13}C$ and rainfall have been observed. Stewart et al. (1995) showed that there is a linear relationship between ecosystem means of $\delta^{13}C$ and rainfall, whereas Schulze et al. (1998) and Miller et al. (2001) observed that the $\delta^{13}C$ of individual species did not change over a broad range of rainfall amounts, but total leaf area and SLA changed in response to a rainfall gradient along a transect from the wet north to the dry south of the Northern Territory of Australia. Constancy of $\delta^{13}C$ along a rainfall gradient was also observed in plants in the Namib Desert (Schulze et al. 1976, 1991a, 1991b, 1996). In addition to the interaction between SLA and stomatal closure, Farquhar et al. (2002) showed in a modeling study that nitrogen (N) content should increase with aridity and that changes in stomatal conductance are therefore less than if leaf area re-

mained constant. The work of Schulze et al. (1998) also indicated that the change in SLA is highly species specific. Thus, the initial hypothesis of our study was that $\delta^{13}C$ decreases with decreasing rainfall, independent of soil type and species.

To test this hypothesis, we measured the responses of SLA, N content and $\delta^{13}C$ of leaves, as well as growth and $\delta^{13}C$ of recent tree rings of *Eucalyptus* species to changes in rainfall and soil type along a rainfall gradient extending from the southwest of Western Australia with an annual rainfall of > 1000 mm to central Australia with an mean annual rainfall of < 200 mm. Sixty-five species of *Eucalyptus* were sampled along this gradient, each species gradually replacing another as rainfall decreases. Thus, the interaction between species diversity and response to aridity were investigated and correlations were established between plant parameters and rainfall. The relationship between $\delta^{13}C$ of wood and $\delta^{13}C$ of leaves was also investigated. Additionally, the importance of speciation and the different ecophysiological responses of individual species to rainfall along the aridity gradient were explored.

Materials and methods

In September 2003, leaves and wood of *Eucalyptus* species (and the close relative *Corymbia*) were sampled from trees growing along the coast of Western Australia from Perth in the north to Walpole in the south and from there along a southwest to northeast transect to the center of Australia near Mount Olga (Figure 1). Wherever possible, several species were sampled at one site and species were sampled at several sites along
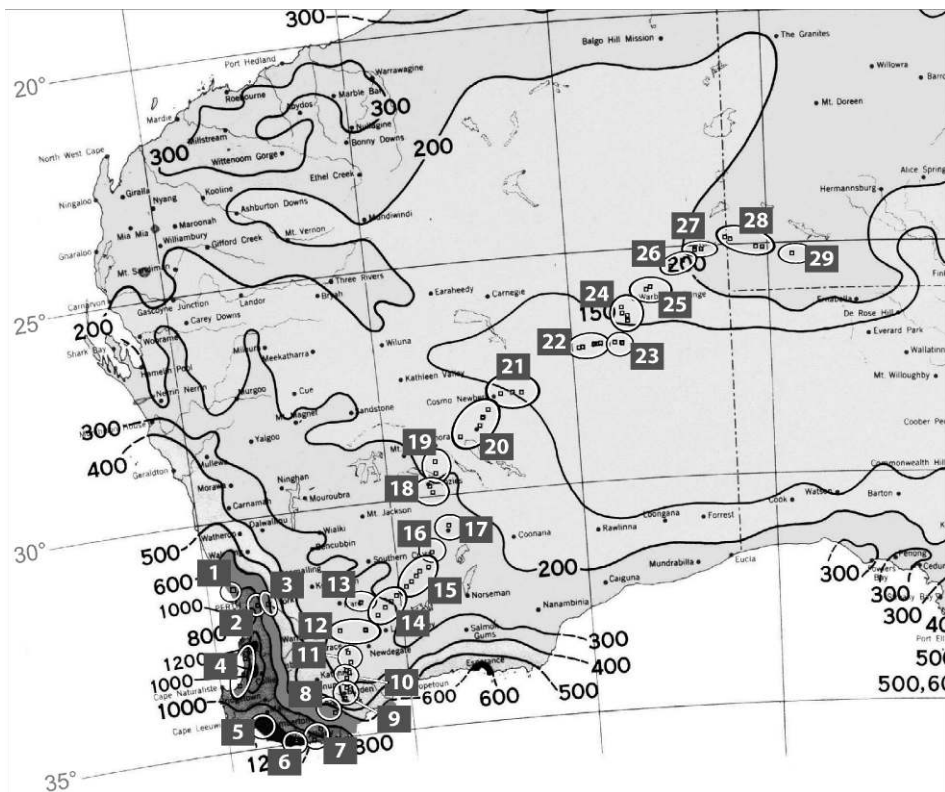

Figure 1. Long-term annual rainfall (with isohyets at 100-mm intervals) for southwestern Australia showing the individual *Eucalyptus* species sampling sites. The large numbers in squares indicate the locations for which rainfall records were obtained from the Australian Bureau of Meteorology. The locations are identified in Table 1. Individual sites included at each location are circled.

Table 1. Rainfall measurement locations and sample collection sites (with local names) and numbers of samples. Identities of the numbered species are given in Table 2. For geographic coordinates of the sampling positions see Nicolle et al. (2006).

Location	Site no.	Site name	Samples	No. of species	Collected species
1	1	Yanchep	1 to 3	1	1
2	2,3	Mundaring	4 to 15	4	2,3,4,5
3	4	York	16 to 24	3	2,3,5
4	5,6	Myalup, Ludlow	25 to 30	1	1
5	7,8	Warren	31 to 42	4	3,6,2,3
6	9,10	Frankland, Walpole	43 to 46	2	7,8
7	11,12	Denmark, Mt. Baker	47 to 52	2	6,5
8	13	Stirling Range	53 to 61	3	2,3,5
9	14,15	Amelup, Bordon	62 to 67	2	9,1
10	16–18	Nanicup, Pingrup, Chinocup	68 to 85	6	9,10,11,12,13,14
11	19,20	Lake Grace, Grace Camp	86 to 103	5	10,15,16,17,18
12	21–23	Jilakin, Dragon Rocks	104 to 121	6	2,10,11,13,16,19,65
13	24	Twin Rock	122 to 127	6	5,12,20,21,22,23
14	25–29	Bushfire Rock, Holland Track South	128 to 165	17	9,12,14,15,16,17,18,21,24, 25,26,27,28,29,30,31,32
15	30–34	Holland Track N; Thursday Rock	166 to 192	13	12,13,15,19,24,33,34,35,36, 37,38,39,40
16	35	Queen Victoria Rock	193 to 198	2	16,14
17	36	Kalgoorlie	199 to 202	2	12,41
18	37,38	Menzies	203 to 215	7	8,19,24,28,37,42,43,57
19	39,40	Lake Ballard, Bore Breakaway	216 to 222	5	15,43,44,45,46
20	41–45	Mt. Morrains, Warburton Camp	223 to 248	9	15,19,28,44,47,48,49,50,51
21	46–48	Cosmo Newberry, Lake Throssel	249 to 259	4	24,28,52,53
22	49–55	Tjukayiria Roadhouse, Parallel Road West	260 to 285	7	24,42,44,47,54,55,56
23	56–57	Parellel Road East	286 to 297	4	44,49,50,57
24	58–62	ConnieSue Highway	298 to 333	8	28,44,49,51,53,58,59,60
25	63,64	Warburton	334 to 342	3	58,61,62
26	65,66	Warakurna	343 to 357	5	54,55,58,61,60
27	67,68	Giles	358 to 368	3	54,56,63
28	69–72	Docker River, Peterman Ranges, Irving River	370 to 398	5	49,58,59,61,64
29	73	Near Mt. Olga	399 to 401	1	54

the rainfall gradient. The soil type, location in the landscape and other species present were recorded at each site.

We sampled 401 individuals of 65 species at 73 sites (Tables 1 and 2). At most sites, all *Eucalyptus* species growing at the site were sampled in triplicate, except for sites near Perth and in the Lake Grace region, where only selected species were sampled and at a few sites where only one individual of a species was present. Some sites had up to 10 species. Several closely located sites were combined to represent one of 29 locations for which rainfall and temperature data were obtained from the Australian Bureau of Meteorology, Melbourne, Victoria. The plots where 1–3 specimens per species were collected will hereafter be referred to as "sites," whereas "locations" refers to the collection of sites in a region for which rainfall and temperature data were obtained.

Rainfall and temperature were obtained from the Australian Bureau of Meteorology using the SILO Data Drill service, which supplies long-term data (1980–2003) of monthly rainfall and temperature for specific geographic locations. Rainfall and temperatures are interpolated data from the sampling net-

work of the Bureau. Figure 2 shows the annual rainfall from 1980 to 2003 for the 29 locations along the transect. Trees sampled were from three rainfall regions: (1) the region with high winter rainfall in southwestern Australia, (2) the west coast regions with winter-dominant rainfall between 400 and 800 mm and (3) the dry interior with intermittent annual rainfall between 100 and 400 mm. The transect had an over-representation of dry sites because the rainfall gradient away from the coast is very steep and because we were particularly interested in the response of *Eucalyptus* to dry conditions. In the high rainfall region of southwestern Australia, years 2001 and 2002 were about 10% drier than the long-term mean. In the agricultural region, rainfall was average in 2002 and 15% less than average in 2001. In the dry interior, 2002 was a dry year (40% below average) following the very wet years of 2000 and 2001.

At each site, leaf samples from the crown were obtained by shooting, by throwing a line over a branch or by cutting a stem of the short shrub-type multi-stemmed mallee species. The samples were then placed in paper bags to allow for rapid dry-

Table 2. Species collected.

| Species collected	No.	Species collected	No.
Corymbia calophylla (Lindl.) K.D. Hill & L.A.S. Johnson	3	*E. latens* Brooker	65
C. chippendalei (D.J. Carr & S.G.M. Carr) K.D. Hill & L.A.S. Johnson	60	*E. leptopoda* Benth.	24
		E. lesouefii Maiden	41
C. opaca (D.J. Carr & S.G.M. Carr) K.D. Hill & L.A.S. Johnson	54	*E. livida* Brooker & Hopper	26
Eucalyptus aequioperta Brooker & Hopper	35	*E. longicornis* (F. Muell.) F. Muell. ex Maiden	9
E. alipes (L. Johnson & K. Hill) Nicolle & Brooker	31	*E. loxophleba* Benth. *gratiae* Brooker	13
C. aparrerinja K.D. Hill & L.A.S. Johnson	63	*E. lucasii* Blakely	47
E. aspratilis L. Johnson & K. Hill	40	*E. mannensis* Boomsma *mannensis*	59
E. astringens (Maiden) Maiden	11	*E. marginata* Donn ex Smith	2
E. calygogona Turcz. *calycogona*	21	*E. oldfieldii* F. Muell.	42
E. capillosa Brooker & Hopper *polyclada* Brooker & Hopper	23	*E. oxymitra* Blakely	61
E. carnei C. Gardner	45	*E. patens* Benth.	4
E. carnei × *E. salubris* F. Muell.	46	*E. phaenophylla* Brooker & Hopper	10
E. concinna Maiden & Blakely	28	*E. pileata* Blakely	17
E. dendrosheath Nicolle ms	32	*E. incrassata* Labill.	39
E. diversicolor F. Muell.	6	*E. polita* Brooker & Hopper	27
C. eremaea (D.J. Carr & S.G.M. Carr) K.D. Hill & L.A.S. Johnson *eremaea*	64	*E. rigidula* Maiden	19
		E. salicola Brooker	30
E. eremicola Boomsma *peeneri* (Blakely) Nicolle	53	*E. salmonophloia* F. Muell.	12
E. ewartiana Maiden	52	*E. salubris* F. Muell.	15
E. exigua Brooker & Hopper	29	*E. sheathiana* Maiden	22
E. flocktoniae (Maiden) Maiden *flocktoniae*	16	*E. socialis* F. Muell. ex Miq.	51
E. gamophylla F. Muell.	58	*E. steedmanii* C. Gardner	25
E. gomphocephala DC.	1	*E. tenera* L. Johnson & K. Hill	14
E. gongylocarpa Blakely	49	*E. tenuis* Brooker & Hopper	33
E. longissima Nicolle	43	*E. tephroclada* L. Johnson & K. Hill	20
E. guilfoylei Maiden	7	*E. transcontinentalis* Maiden	37
E. gypsophila Nicolle	48	*E. trivalvis* Blakely	50
E. histophylla Brooker & Hopper	38	*E. urna* Nicolle	18
E. incerata Brooker & Hopper	36	*E. victrix* L. Johnson & K. Hill	55
E. intertexta R. Baker	56	*E. wandoo* Blakely *wandoo*	5
E. jacksonii Maiden	8	*E. yilgarnensis* (Maiden) Brooker	34
E. jutsonii Maiden	57	*E. youngiana* F. Muell.	44
E. kingsmillii (Maiden) Maiden & Blakely	62		
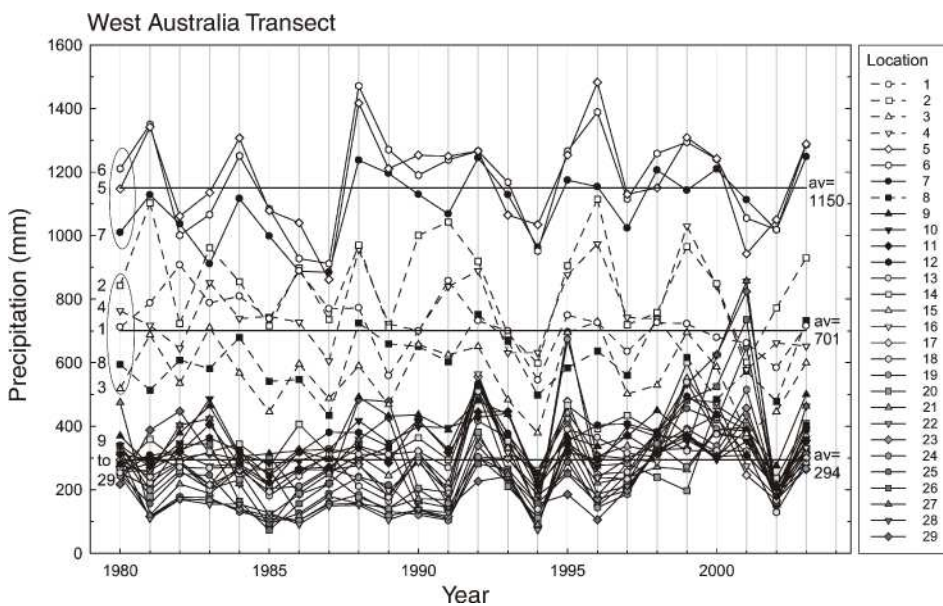
Figure 2. Rainfall patterns from 1980 to 2003 for the locations identified in Figure 1. Three rainfall ranges are distinguished: high rainfall near the southwest coast (mean annual rainfall 1150 mm), medium rainfall in the settled agriculture region (mean 700 mm) and the low rainfall rangelands (mean 294 mm).

ing. Based on previous experience (Schulze et al. 1998) there is no significant change in leaf area in *Eucalyptus* with drying. Care was taken to collect leaves that had grown in 2002. In the laboratory, the number of leaves was recorded, leaf area was measured with a leaf area meter (Model 3100, LI-COR, Lincoln, NE), leaves were dried to constant mass in a forced-draught oven at 70 °C and SLA was calculated. The leaf samples were then analyzed for $\delta^{13}C$ by isotope ratio mass spectrometry (Finnigan Delta+XL, Bremen, Germany). In addition, a wood core was taken from each of the trees and stem disks of the short multi-stemmed species for growth ring and $\delta^{13}C$ analysis. Isotopic analysis of the wood was carried out by a laser ablation technique (Schulze et al. 2004) in which a laser (Merchantek-New Wave UV Laser, Fremont, CA) disintegrates the wood to a fine dust and the carbon in the dust is oxidized in a catalytic oven and measured in a Finnigan isotope ratio mass spectrometer. The isotopic ratios are expressed in the ‰ notation and not as carbon isotope discrimination (Farquhar and Richards 1984) because the isotopic composition of carbon dioxide at the sites was not measured.

Growth ring widths were measured with the aid of a dissecting microscope. The identification of tree rings in *Eucalyptus* proved difficult, because some species showed continuous growth, whereas others had numerous growth rings. We only selected wood samples in which early wood growth could clearly be identified by a clear ring of larger pores. Despite this, the phenology of *Eucalyptus* wood growth remains a major uncertainty.

Statistical analysis

Leaves that had grown in 2002 and wood formed in 2002 were compared with 2002 rainfall. In addition, comparisons were made with the long-term mean annual rainfall between 1980 and 2003. Means and standard deviation were calculated: (1) for repeated sampling of individuals of the same species at a site; (2) for all species that were collected at any site; and (3) for all sites that were combined to form a location for which rainfall data were available. The dependence of $\delta^{13}C$ values on SLA, soil type, species identity and rainfall was described as a linear mixed model. Specific leaf area, soil type and rainfall were considered as fixed variables, whereas differences caused by species identity were modeled as a random variable. This takes into account the variable number of trees of the different species at any site and allows estimation of the contribution of species identity to the differences in $\delta^{13}C$ values. To quantify the relative contributions of direct and indirect effects of rainfall on the $\delta^{13}C$ values, a path analysis (Grace and Pugesek 1998) was performed using only the rainfall, SLA and $\delta^{13}C$ variables. To obtain standardized path coefficients, all variables were standardized before path analysis.

Results

Leaf $\delta^{13}C$ and rainfall

Along an annual rainfall gradient from 250 to 1350 mm in 2002, mean $\delta^{13}C$ values of the 2002 leaves of the various *Euca-*

lyptus species at the 29 locations decreased significantly from –27.0‰ at 300 mm to –28.2‰ at 1000 mm, a change of 1.63‰ per 1000 mm decrease in rainfall (Figure 3A). The data collected on the north–south axis along the west coast of Western Australia south of Perth, and the transect from the southwest to central Australia had the same regression line. The $\delta^{13}C$ response is about half that observed in Queensland, Australia (Stewart et al. (1995): an increase of 3.16‰ per 1000 mm decrease in rainfall), but larger than that observed for *Eucalyptus* species in the Northern Territory, Australia (Schulze et al. (1998): an increase of 0.6‰ per 1000 mm increase in rainfall). At locations where the rainfall in 2002 was below 300 mm, $\delta^{13}C$ in leaves produced in 2002 showed large variation (between –28.7 and –24.3‰) that was unrelated to rainfall. The range of $\delta^{13}C$ in the low rainfall locations extended over the full range observed at the high rainfall locations (Figure 3A). A further increase in $\delta^{13}C$ of leaves where annual rainfall was below 250 mm was also observed in *Eucalyptus* species in the Northern Territory (Schulze et al. 1998, Miller et al. 2001), but the range of values and in particular the decrease in $\delta^{13}C$ of leaves at some of the dry sites, has not been previously observed. The mean standard deviation of $\delta^{13}C$ of leaves at the different collection sites within a location was 0.49‰. The standard deviation of $\delta^{13}C$ of leaves of different species at any one site was similar (0.47‰), whereas the variation between individuals of the same species at any one site was slightly larger (0.59‰).

Only a few *Eucalyptus* species were found growing over a broad rainfall gradient. These were *E. marginata, E. wandoo wandoo* and *Corymbia calophylla*. The $\delta^{13}C$ response of these species (Figure 3B) to decreasing rainfall was not significantly different from the mean $\delta^{13}C$ response of all species at that location (Figure 3A). In the low rainfall range (< 300 mm), species that were collected at different sites showed increasing, decreasing or constant $\delta^{13}C$ values with decreasing rainfall (Figure 3B). Clearly, not only rainfall but also site conditions and species-specific traits determined the response of individual species. Species growing on clay had higher $\delta^{13}C$ values than species growing on sand (Figure 3B). A further decrease in $\delta^{13}C$ with decreasing rainfall was observed only on the sandy, clay-sand and floodplain soils. Leaf samples were taken in pairs on freshly burnt and unburnt sites, but the effect of burning was not significant in the context of the overall variation.

The SLA of the species at all sites varied between 2 and 6 $m^2\ kg^{-1}$ and there was a weak tendency for SLA to increase with rainfall in some species. Specific leaf area varied mainly with soil type and species (Figure 4A). Thus, a large part of the variation in the $\delta^{13}C$ response to rainfall can be explained by variation in SLA if soil type is considered (Figure 4B). On average, $\delta^{13}C$ increased by 0.67‰ for each 1 $m^{-2}\ kg^{-1}$ decrease in SLA. In Figure 4, each value represents a particular species at its location of sampling with some species being sampled at several sites (Figure 3). When classifying the sites according to soil type, parallel response lines emerged describing the change in $\delta^{13}C$ with SLA. For the same range of SLA values,

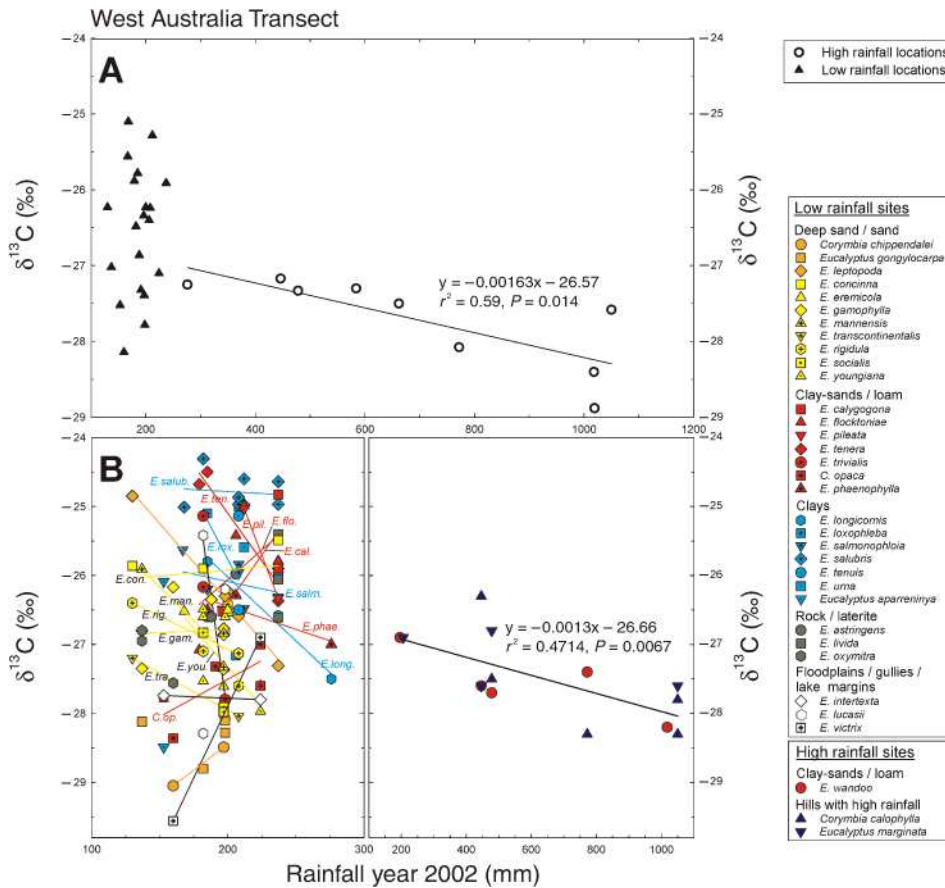Figure 3. (A) Relationship between mean isotopic ratio of ^{13}C to ^{12}C ($\delta^{13}C$) values of leaves grown in 2002 and mean rainfall in 2002 for the 29 locations. (B) Relationship between $\delta^{13}C$ values of leaves of individual species grown in 2002 and mean rainfall in 2002. Panel B is divided into data from sites with annual rainfall > 250 mm and annual rainfall < 250 mm. The solid circle represents the mean value of all data at sites with rainfall < 250 mm.

plants growing on clay soils had a significantly higher $\delta^{13}C$ than plants growing on sandy or on floodplain soils. Between an SLA of 3 and 4 m^2 kg^{-1}, $\delta^{13}C$ ranged between –29.5 and –24.3‰ because of changes in soil type. On any one type of soil, SLA changed not only with rainfall, but also with species.

The increase in SLA with rainfall was associated with a significant decrease in N content per unit leaf area (Figure 5), confirming model predictions made by Farquhar et al. (2002) that N content per area should decrease with rainfall. The highest N contents occurred in *Eucalyptus* species growing on sandy soils where annual rainfall was low. At the low rainfall sites, the variation in N content was largely a species effect. The species occurring at dry sites compensated for the short wet season by an increasing investment in leaf N content, which increased their photosynthetic efficiency. The small increase in $\delta^{13}C$ in combination with the high N content suggests that mesophyll resistance (Warren and Adams 2005) is not a major limitation under dry conditions. This is supported by the recent observation that specificity of Rubisco for CO_2 is higher in plant species from drier habitats and in species with persistent leaves (Galmés et al. 2005). Leaf N contents were higher in our study than those reported from the Northern Territory of Australia (Farquhar et al. 2002) mainly because of differences in SLA. This may reflect the winter-dominant rainfall in southern Australia compared with the summer-dominated rainfall in northern Australia.

A statistical analysis of the effects of SLA, soil type, species identity and rainfall on $\delta^{13}C$ showed that almost 27% of the variation of the whole data set (see Figure 4) was associated with SLA (Table 3). Soil type was associated with an additional 16% of the variation, whereas rainfall added only 1%. For the best model, including SLA and soil type as fixed effects, the estimated additional contribution of species identity amounted to 21%. In a subset of sites with > 300 mm rainfall, where we obtained evidence for a linear relationship between $\delta^{13}C$ and rainfall (Figure 3), 43% of the variation was explained by SLA, 13% by soil type and only 3% by rainfall. The species effect decreased to 9% because there were fewer species in this subset. Thus, even though rainfall is the overarching driving variable, its effect on $\delta^{13}C$ appears to be indirect, exerted through the water-holding capacity of the soil and through species-specific traits such as SLA and N content. The observation of a minor effect of rainfall is further supported by a path analysis (Figure 6) that considered only the parameters of rainfall, SLA and $\delta^{13}C$. The standardized path coefficient was high (0.38, $P < 0.0001$) for the effect of rainfall on SLA and for the effect of SLA on $\delta^{13}C$ (–0.50, $P < 0.001$), but low for the direct effect of rainfall on $\delta^{13}C$ (–0.05, $P = 0.456$). Thus, the direct effect of precipitation on $\delta^{13}C$ of leaves was low, with rainfall largely acting indirectly through soil type (water-holding capacity), species and the associated changes in SLA and N.
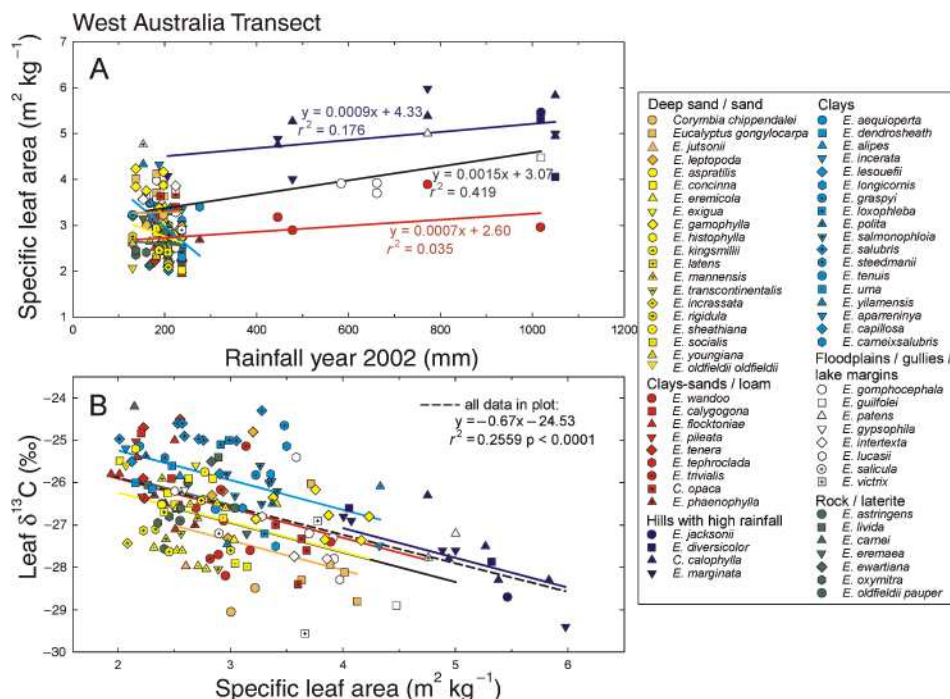
Figure 4. (A) Relationship between specific leaf area and rainfall. The lines depict the trends of individual species. (B) Relationship between leaf isotopic ratio of ^{13}C to ^{12}C ($\delta^{13}C$) values and specific leaf area. Each value represents a particular species growing at a particular site and on a particular soil type. Some species were repeatedly measured (see Figure 3B). Species were grouped according to soil type in the low rainfall region (< 250 mm). Species in the high rainfall region are shown as a separate group.

Tree growth and rainfall

Variation within and between species resulted in a weak relationship between tree stem growth and $\delta^{13}C$ of leaves that accounted for only 9% of the variation (Figure 7). The $\delta^{13}C$ in wood varied markedly between light and dark wood, which is the early and late wood after rain. In Figures 8–10, the $\delta^{13}C$ of wood samples of three species of *Eucalyptus* measured at the cellular level are shown for several years and several wetting and drying events. The association between $\delta^{13}C$ in the wood and rainfall and temperature was based on the assumption that light wood with many new vessels was triggered by rainfall events and may continue into the dry period, depending on soil type, rooting depth or access to groundwater. We are aware that this assumption needs further investigation.

In tall trees in southwestern Australia, for example, in *E. diversicolor* (Figure 8A), seasonal growth started with a distinct ring of vessels of light-colored wood that was followed by dense dark wood with scattered vessels. The distinct layer of early vessels gives the wood an appearance reminiscent of a ring-porous species. The widths of the bands of light-colored wood and dark-colored wood were variable. At the onset of early wood formation we observed a distinct decrease in $\delta^{13}C$ (Figure 8B). In many years, the first row of vessels showed the lowest $\delta^{13}C$ value (about –27‰ in 2002) for that year. Within the early wood, this decrease in $\delta^{13}C$ was followed by a sharp increase of about 1.5‰, so that the highest values of $\delta^{13}C$ were recorded (–25.67‰ in 2002) at the end of earlywood formation. The $\delta^{13}C$ then decreased continuously

Table 3. Linear mixed model analysis of the response of isotopic ratio of ^{13}C to ^{12}C ($\delta^{13}C$) to changes in specific leaf area (SLA), soil type and rainfall (fixed effects). The contribution of species identity is modeled as a random effect. The species effect is then taken from the best model (given in bold letters). Using all data, the rain effect was not significant, thus, the best model is represented by Model 2. For sites with > 300 mm rainfall, the rain effect was significant, thus, the best model is represented by Model 3.

| Model no.	Model	Degrees of freedom (df)	Likelihood ratio	*P* value	Variance explained		Total
					Fixed effects	Species effect	
a. Statistical model based on all data							
1	SLA	4	36.88	< 0.0001	26.8%	39.8%	66.6%
2	**SLA + soil type**	**10**	**24.42**	**0.0004**	**43.3%**	**21.1%**	**64.4%**
3	SLA + soil type + rain	11	2.66	0.1031	44.3%	20.6%	64.9%
b. Statistical model based on sites with > 300 mm rainfall							
1	SLA	4	41.82	< 0.0001	43.4%	29.1%	72.5%
2	SLA + soil type	10	13.71	0.0331	56.6%	11.5%	68.1%
3	**SLA + soil type + rain**	**11**	**6.07**	**0.0137**	**59.7%**	**9.4%**	**69.1%**
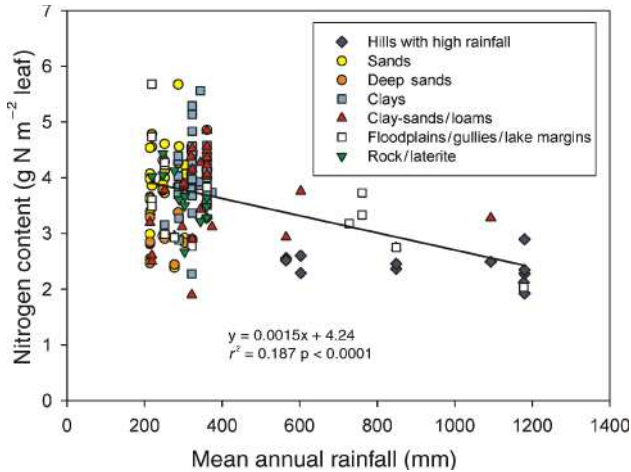
Figure 5. Leaf-area-based nitrogen content ($g\ N\ m^{-2}$) of leaves as related to rainfall. Species are grouped according to soil type. The colors of each soil type correspond to the groupings in Figures 3 and 4.

Figure 6. Path analysis of the relationships between rainfall, specific leaf area (SLA) and isotopic ratio of ^{13}C to ^{12}C ($\delta^{13}C$) of leaves. The numbers indicate the standardized path coefficients. Solid arrows indicate significant relationships and the dashed arrow indicates a non-significant relationship.

during the growing season (which is probably consistent with stomatal opening) despite low rainfall (Figure 8B). Similar patterns have been observed in temperate-zone forest species (e.g., *Pinus*: Schulze et al. 2004; *Populus*: Helle and Schleser 2004; *Fagus*: Demasin and Lelarge 2003). The trend in $\delta^{13}C$ of wood was not simply related to rainfall or temperature in that season (Figure 8B). Growth started with the first winter rains and the likely mobilization of carbohydrate reserves. We suggest that these carbohydrates were initially isotopically depleted, but with increasing use of reserves the $\delta^{13}C$ values increased. Thus, early wood did not appear to be formed from current assimilates, but from reserves. If the $\delta^{13}C$ values in the wood were only the result of assimilation by the leaves, the conditions for growth in spring with increasing rainfall, higher solar radiation and increasing temperatures would have produced a trend of $\delta^{13}C$ opposite to that observed. After the initial growth of early wood, the $\delta^{13}C$ values of the wood continued to decrease until growth of late wood ceased with the onset of summer drought when the values of $\delta^{13}C$ reached
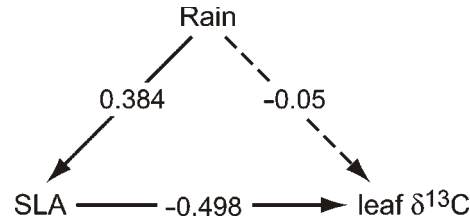
their lowest values. Thus, the isotopic signal in late wood does not reflect the effect of climate on leaf assimilation. The wood was isotopically most depleted at the time when growth stopped because of water shortage and high temperatures, indicating that wood growth in *E. diversicolor* is not always directly linked to carbon assimilation by the leaves and that additional fractionation steps occur between the leaf and formation of wood (Gleixner and Schmidt 1997, Demasin and Lelarge 2003).

The growth response of *E. concinna* (Figure 9A), a mallee species that sprouts from dormant buds of the lignotuber (a "sprouter"), showed similar patterns of tree ring growth and $\delta^{13}C$ as *E. diversicolor*. The wood showed a marked transition between early and late wood and a sharp border to the next tree ring. Nevertheless, the wood contained irregularities (false "tree rings"), for example, in 2003. The $\delta^{13}C$ analysis (Figure 9B) showed that there was considerable interannual variation in $\delta^{13}C$, but this variation was difficult to interpret because rainfall events occurred in both summer and winter. As in *E. diversicolor*, the highest $\delta^{13}C$ values were found in the early wood, suggesting that carbon reallocation from reserves was also important in this mallee species. This was followed by low $\delta^{13}C$ values even though drought was developing. Thus, assimilate transport again appeared to change the isotopic signal.

The $\delta^{13}C$ pattern was different in *E. phaenophylla* (Fig-

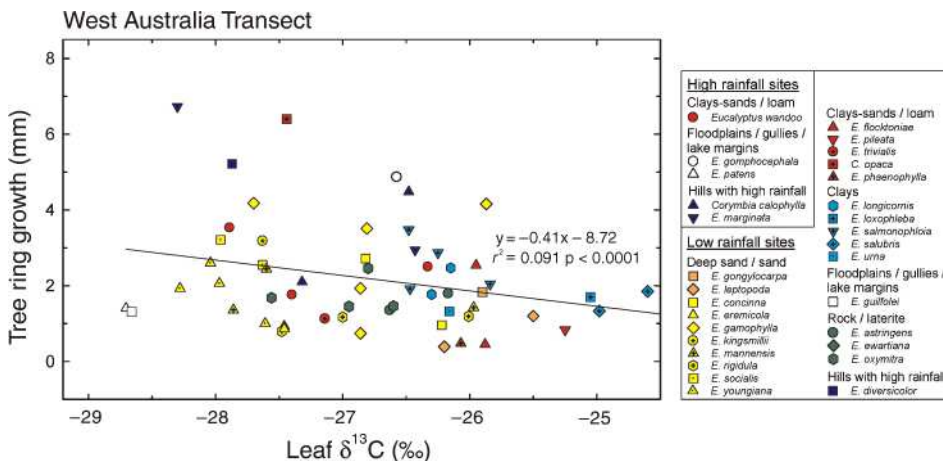Figure 7. Relationship between tree ring growth in 2002 and leaf isotopic ratio of ^{13}C to ^{12}C ($\delta^{13}C$) for 2002 for 30 species of *Eucalyptus* at high and low rainfall sites and growing on different soil types.
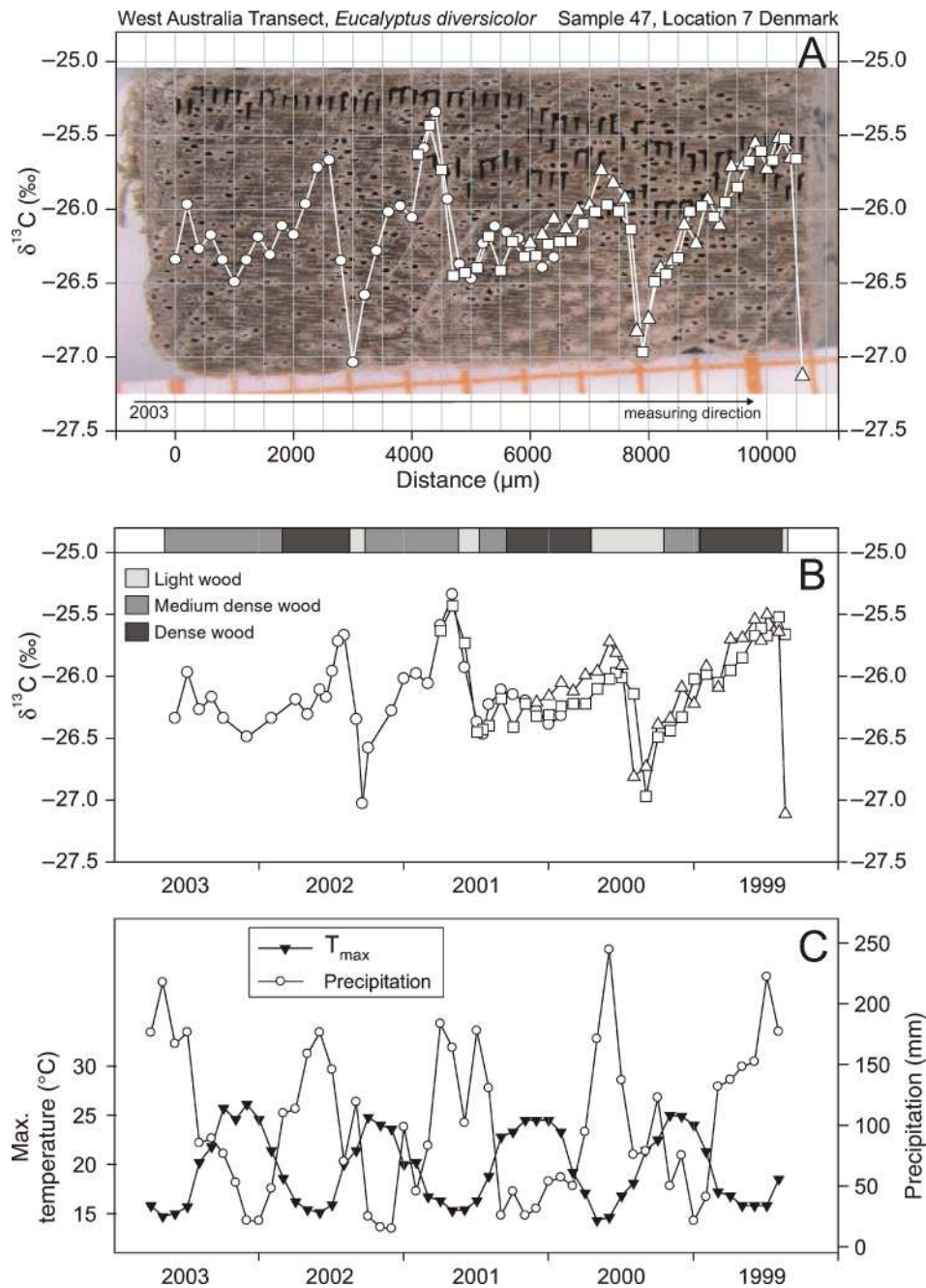
Figure 8. (A) Seasonal variation in wood anatomy and isotopic ratio of ^{13}C to ^{12}C ($\delta^{13}C$) and (B) $\delta^{13}C$ for *E. diversicolor* and mean monthly maximum temperature and rainfall at a site with 1017 mm of annual rainfall.

ure 10A), a species that regenerates vegetatively from a ligno-tuber. The wood showed a distinct border between light and dense wood, but a more gradual transition between dense and light-colored wood. At the site where the tree was sampled, rainfall occurred not only in winter, but also in late summer (Figure 10B). The $\delta^{13}C$ values of the wood reached a minimum at the onset of dense-wood formation and reached a maximum in the light-colored wood. Apparently, this species started to grow dense wood from carbohydrate reserves with the late summer rains and then produced light-colored wood with winter rainfall.

The patterns shown in Figures 8B to 10B suggest that there

are lag periods between assimilation and stem growth and possibly also isotopic discrimination during assimilate transport, storage and wood growth and that there may be additional metabolic steps determining the isotopic composition of early and late wood, or light and dense wood. For the trees investigated, the range of $\delta^{13}C$ in wood within an individual period of wood growth varied from about 2 to 4‰ (Figure 11) and on average, the mean $\delta^{13}C$ in wood was about 3‰ higher (less negative) than in the leaf (cf. Helle and Schleser 2004, Schulze et al. 2004, but these studies did not measure N). Nevertheless, in ^{13}C-depleted wood formed after rain the $\delta^{13}C$ values were similar to those in leaves (Figure 11). We suggest that this does not
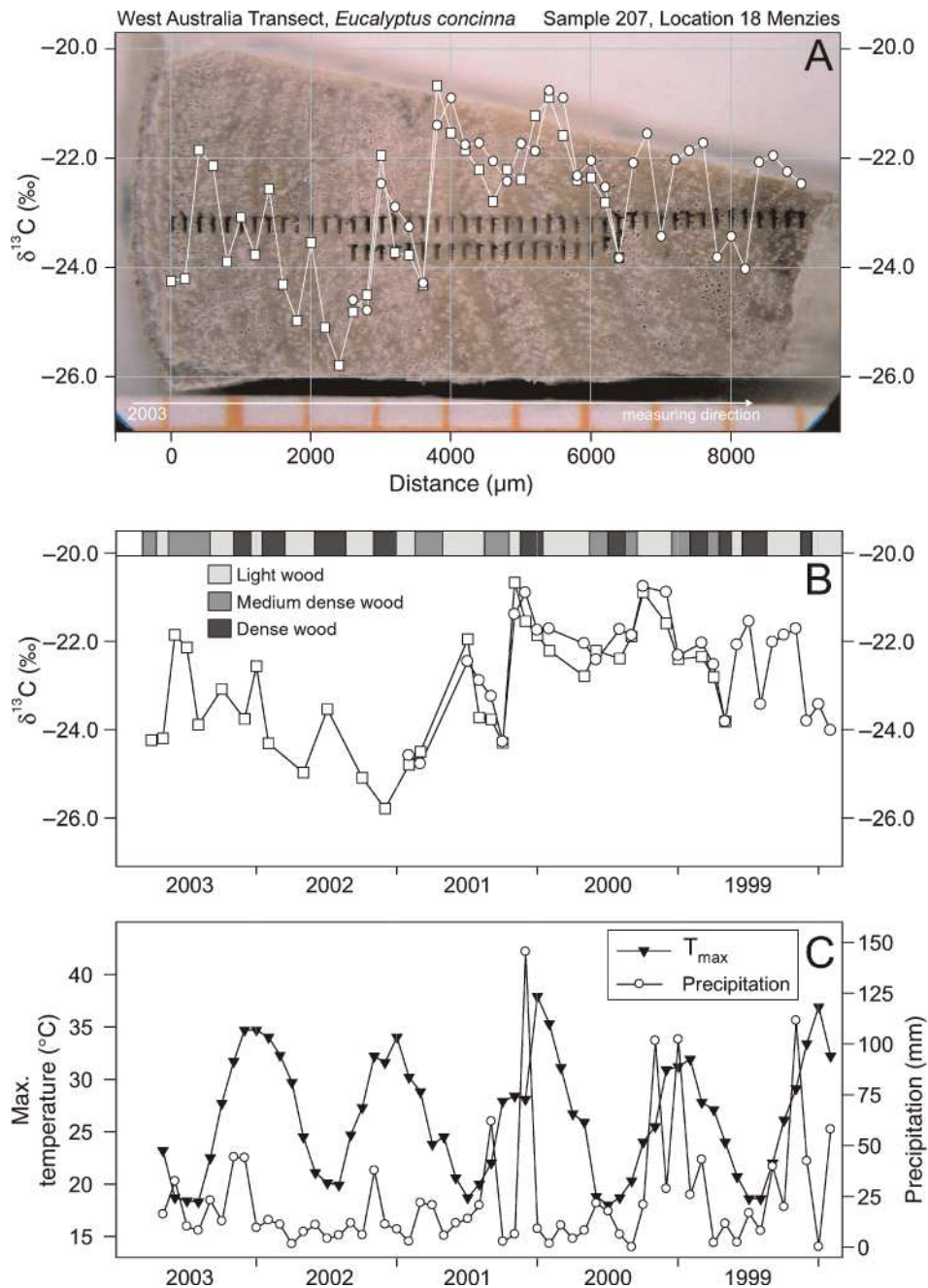
Figure 9. (A) Seasonal varia-
tion in wood anatomy and iso-
topic ratio of ^{13}C to ^{12}C ($\delta^{13}C$)
and (B) $\delta^{13}C$ of *E. concinna*
and mean monthly maximum
temperature and rainfall at a
site with 130 mm of annual
rainfall.

mean that the carbohydrates for the growth of early wood were
translocated directly from the leaves to the wood, but that
some reallocation occurred. The highest $\delta^{13}C$ values were ob-
served in the light wood when climatic conditions suggest that
the stomata should be open and the lowest $\delta^{13}C$ values were
observed in the dense wood when the climatic conditions were
such that stomata should be closed.

Discussion

Experimental studies based on a single species have shown
that water-use efficiency and $\delta^{13}C$ change with soil water

availability (Ngugi et al. 2003) or with changing seasonal rain-
fall (Searson et al. 2004). In a major review, Wright et al.
(2004) showed that, across a wide range of species in Austra-
lia, assimilation per mass was closely related to leaf N concen-
tration and N concentration was related to SLA. By using
SLA, we do not necessarily imply some measure of internal
diffusion that may change with SLA (Warren and Adams
2005), but we take SLA and the associated N content as neutral
measures that reflect photosynthetic capacity. Under light-
limited conditions, there is an adaptive advantage in producing
thin rather than thick leaves even though the life span of thin
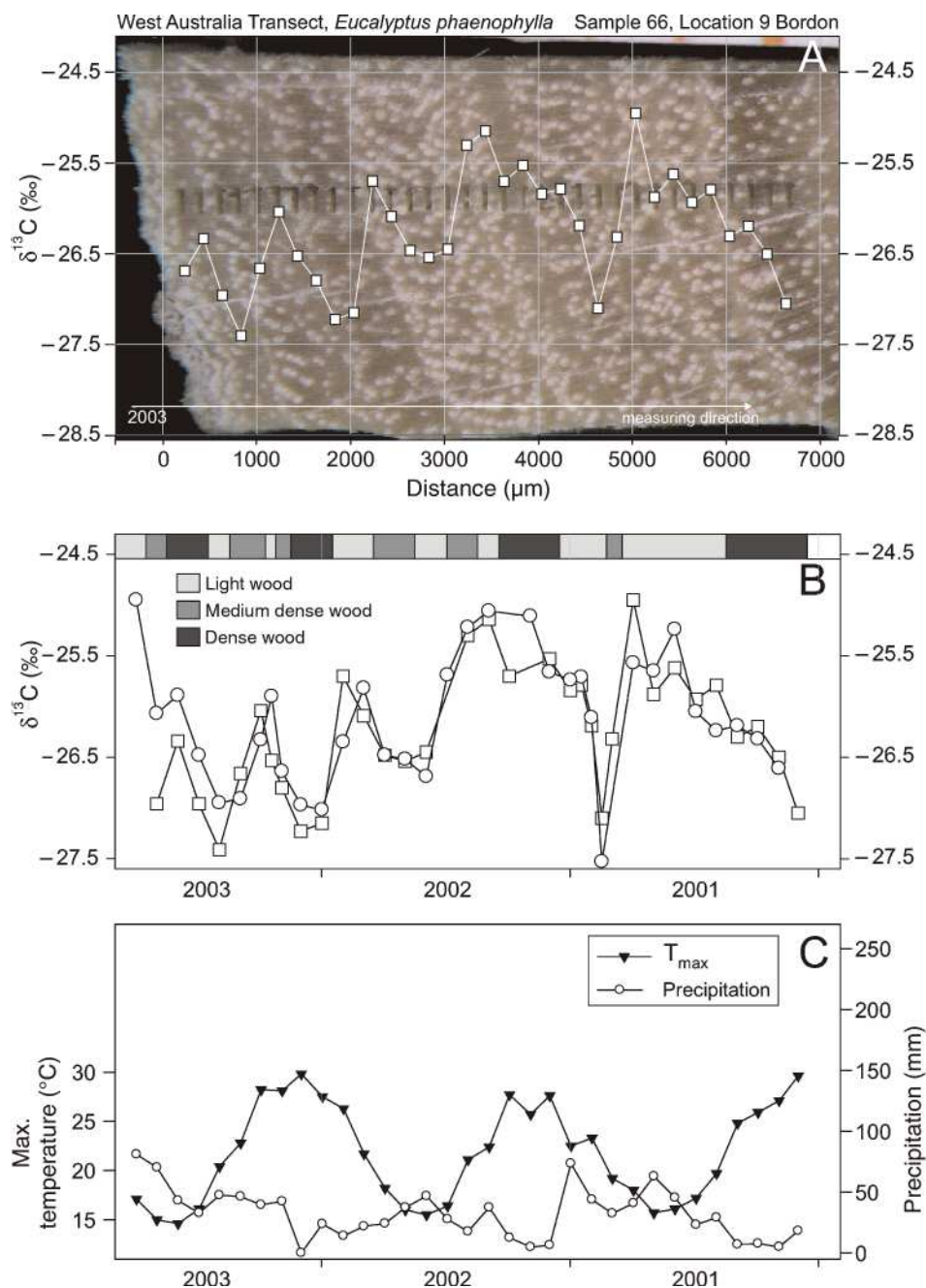leaves is generally shorter than that of thick leaves and thin

Figure 10. (A) Seasonal variation in wood anatomy and isotopic ratio of ^{13}C to ^{12}C ($\delta^{13}C$) and (B) $\delta^{13}C$ for *E. phaenophylla* and mean monthly maximum temperature and rainfall at a site with 275 mm of annual rainfall.

leaves are usually more sensitive to drought in most native species (Schulze and Hall 1982, Schulze et al. 1994). Farquhar et al. (2002) modeled these interactions and showed that as rainfall decreases plants adapt by decreasing the number of plants per unit area, reducing the number of leaves per plant, reducing the area per unit photosynthetic capacity and reducing stomatal conductance per unit area. The model showed how these responses "spread the responsibility" and how photosynthetic capacity in the form of N per unit area increased as rainfall decreased so that the changes in $\delta^{13}C$ were correlated with those of SLA even though stomatal conductance was also changing. Our data for *Eucalyptus* support this analysis. How-

ever, the range over which any one species can respond to its environment is limited, and it has been shown in common garden experiments (Anderson et al. 1996) and in plants from different origins (Read and Farquhar 1991) that genetic variation exists in the response of $\delta^{13}C$ to the environment. Thus, in addition to the response patterns partly by, species identity is important in the study of environmental gradients.

In the present study, only a few *Eucalyptus* species covered a large rainfall gradient, yet these species showed responses to rainfall not only in $\delta^{13}C$, as has been established in other dry climates (e.g. Kagawa et al. 2003, Midgley et al. 2004), but also in SLA and N content, indicating that they adapt to an arid
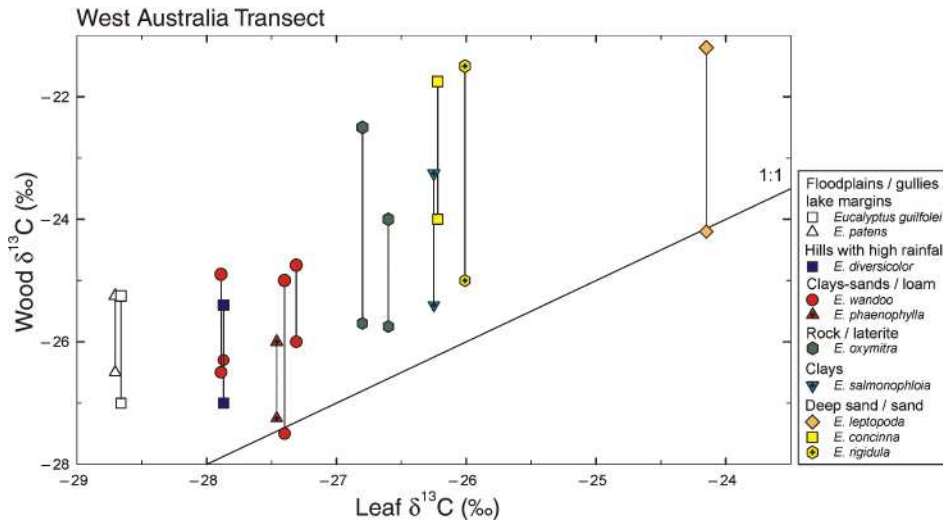
Figure 11. Relationship between the range of isotopic ratio of ^{13}C to ^{12}C ($\delta^{13}C$) values of wood and the $\delta^{13}C$ values of leaves for 11 individuals of different species of *Eucalyptus* in 2002. Because only one lumped sample of 5–10 leaves was taken per individual plant, there is no variation for leaf $\delta^{13}C$. In contrast, several growth periods of past rainy seasons were analyzed. Therefore there is variation in wood $\delta^{13}C$ between light and dense wood of the same tree.

climate partly by reducing SLA and partly by increasing N content and closing their stomata. However, the increase in $\delta^{13}C$ with decreasing rainfall was statistically significant only at high rainfall sites and in species covering a broad range of sites. It explains only a minor part of the variation at low rainfall where site-specific conditions and species-specific characteristics play an increasing role. The site-specific responses become most important in dry habitats, where not only the soil type (sand versus clay, Walter 1964), but also fire frequency (Mappin et al. 2003) and access to groundwater determine the response. This makes the ratio of changes in $\delta^{13}C$ to changes in rainfall quite variable below 300 mm.

Based on the few species that covered the widest rainfall range, there was a relatively strong response of $\delta^{13}C$ to drought. We investigated the community of *Eucalyptus* as a whole and the community-averaged response to drought was similar to that observed for a single species. However, as mean annual rainfall decreased, new species entered and each of these species tended to keep the community-averaged $\delta^{13}C$ at a low value. Thus, even in arid environments, the selection effect of biodiversity (Loreau and Hector 2001) contributed by maintaining the community-averaged intrinsic water-use efficiency constant. The effect of species identity on isotopic discrimination was also observed by Miller et al. (2001) and confirmed by common garden experiments (Anderson et al. 1996). We suggest that this compensatory response of species is reached by changes in SLA, and additionally by changing plant size and leaf area index from tall single trees to multiple-stemmed mallee types. Thus, the response to aridity must take into account plant biodiversity, otherwise predictions on changes in vegetation with global climate change based on generalized functional types will be unreliable (Berry and Roderick 2004).

Although there is an increasing mechanistic understanding about the relationship between leaf $\delta^{13}C$ and rainfall, the relationship between leaf $\delta^{13}C$ and wood growth remains complex for broad-leaved species (Fritts 1976, see also Koszlowski and Pallardy 1997, Vaganov et al. 2006). General relationships between wood growth and leaf $\delta^{13}C$ have been established based

on drought indices (e.g., Macfarlane and Adams 1998, Adams and Kolb 2004). A similar trend was observed in this study. Along the rainfall gradient, the annual range in $\delta^{13}C$ in wood was correlated with the annual range in $\delta^{13}C$ in leaves in the same year (Miller et al. 2001, Watmough et al. 2001). The offset was on average 3‰, as was expected from studies with cellulose and lignin (cf. Schulze et al. 2004). However, our more detailed investigation of $\delta^{13}C$ in wood indicates that several steps of discrimination appear to occur between assimilation by the leaf and the deposition of cellulose in the stem and that reallocation of carbohydrates from and into reserves seems to affect the isotopic signature of the carbon in the wood such that the general correlation between leaf and wood $\delta^{13}C$ may simply be fortuitous. As shown in *E. globulus* (Cernusak et al. 2003), there is increasing awareness that several discrimination steps exist between the assimilation of carbon by the leaves and the formation of wood in the stem. Demasin and Lelarge (2003) concluded that at least two fractionation steps occur, namely during sugar transfer from leaves to stems and during stem respiration. Gleixner and Schmidt (1997) pointed out the importance of the aldolase reaction converting triose-phosphate to fructose-1,6-diphosphate, as a metabolic step at which discrimination occurs. Our detailed analyses of $\delta^{13}C$ of wood using the laser ablation technique of Schulze et al. (2004) showed that, for the *Eucalyptus* species studied, the early wood was initially depleted of ^{13}C and then enriched in ^{13}C as a result of reallocation of carbohydrates from and to storage. This process of reallocation resulted in a $\delta^{13}C$ pattern opposite to that expected from the seasonal responses of the stomata to climatic conditions. High $\delta^{13}C$ values were found in wood when the stomata of leaves were likely to be open. This stage was followed by a period when $\delta^{13}C$ in wood decreased, even though rainfall decreased, and stomata of leaves presumably closed. This pattern was most obvious in a tall tree in the high rainfall region, but it was also observed in a "sprouter" mallee receiving low rainfall. The pattern was different from that observed by Pate et al. (1998) in *E. globulus* in which the $\delta^{13}C$ value in the phloem sap was close to the $\delta^{13}C$ in newly

formed xylem cells (Pate and Arthur 1998). We observed that the $\delta^{13}C$ of wood appeared to be generally more uncoupled from the processes in the leaf than was observed by Pate et al (1998) in *E. globulus*. Our observations of seasonal changes of $\delta^{13}C$ in wood that are apparently not synchronized with carbon assimilation lend credence to our finding that the relationship between width of wood growth and rainfall was not significant.

Conclusions

In *Eucalyptus,* variation in $\delta^{13}C$ with rainfall depends on species traits, particularly SLA, N content, general site conditions (burnt/unburnt, sand/clay) and on the degree of biodiversity in the region.

The $\delta^{13}C$ values in wood are associated with the $\delta^{13}C$ values in leaves, but there are additional discrimination processes and internal reallocation of carbon that result in a time-trend opposite to that expected from the change in stomatal conductance with rainfall and temperature.

Within a species, the relationship between radial wood growth and rainfall is weak based on observations in different years (interannual variability). We suggest that the reallocation of reserves buffers growth from the immediate effects of climate. Moreover, different species have different rates of annual growth at any one location, making it difficult to derive a generalized response of wood growth to climate.

In the arid region there was considerable variation in $\delta^{13}C$ among species, indicating that an understanding of biodiversity is important and that it is impossible to draw general conclusions from the study of a single species on the likely community response to, for example, climate change when the community contains multiple species.

Our study was confined to *Eucalyptus* species and the closely related genus *Corymbia*, and did not consider the wide range of other species present along the aridity gradient. The wide range of responses to aridity among the *Eucalyptus* species in this study would likely be multiplied in other species.

Acknowledgments

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