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Water availability and carbon isotope discrimination in conifers

Abstract The stable C isotope composition ($\delta^{13}C$) of leaf and wood tissue has been used as an index of water availability at both the species and landscape level. However, the generality of this relationship across species has received little attention. We compiled literature data for a range of conifers and examined relationships among landscape and environmental variables (altitude, precipitation, evaporation) and $\delta^{13}C$. A significant component of the variation in $\delta^{13}C$ was related to altitude (discrimination decreased with altitude in stemwood, 2.53‰ km^{-1} altitude, $r^2=0.49$, and in foliage, 1.91‰ km^{-1}, $r^2=0.42$), as has been noted previously. The decrease in discrimination with altitude was such that the gradient in CO_2 partial pressure into the leaf ($P_a–P_i$) and altitude were generally unrelated. The ratio of precipitation to evaporation (P/E) explained significant variation in $P_a–P_i$ of stemwood ($r^2=0.45$) and foliage ($r^2=0.27$), but only at low (<0.8) P/E. At greater P/E there was little or no relationship, and other influences on $\delta^{13}C$ probably dominated the effect of water availability. We also examined the relationship between plant drought stress (Ψ) and $\delta^{13}C$ within annual rings of stemwood from *Pinus radiata* and *Pinus pinaster* in south-western Australia. Differential thinning and fertiliser application produced large differences in the availability of water, nutrients and light to individual trees. At a density of 750 stems ha^{-1}, Ψ and $\delta^{13}C$ were less (more negative) than at 250 stems ha^{-1} indicating greater drought stress and less efficient water use, contrary to what was expected in light of the general relationship between discrimination and P/E. The greater $\delta^{13}C$ of trees from heavily thinned plots may well be related to an increased interception of radiation by individual trees and greater concentrations of nutrients in foliage – attributes that increase rates of photosynthesis, reduce P_i and increase $\delta^{13}C$. $\delta^{13}C$ was thus modified to a greater extent by interception of radiation and by nutrient concentrations than by water availability and the $\delta^{13}C$-Ψ relationship varied between thinning treatments. Within treatments, the relationship between $\delta^{13}C$ and Ψ was strong ($0.38<r^2<0.58$). We conclude that $\delta^{13}C$ may well be a useful indicator of water availability or drought stress, but only in seasonally dry climates ($P/E<1$) and where variation in other environmental factors can be accounted for.

Keywords Thinning · Fertiliser · *Pinus pinaster* · *Pinus radiata* · $\delta^{13}C$

Introduction

The heavier ^{13}C isotope of atmospheric CO_2 is discriminated against during photosynthesis. In C_3 species, the slower diffusion of the heavier isotope from the atmosphere to the site of carboxylation, and the intrinsically poorer reactivity of ^{13}C (Melander and Saunders 1979) with the primary carboxylating enzyme, ribulose-1,5-bisphosphate carboxylase (Park and Epstein 1960), are largely responsible for the observed depletion of plant material in ^{13}C relative to the atmosphere. Discrimination is linearly related to the ratio of intercellular to ambient CO_2 partial pressures (P_i/P_a), which reflects the balance between the rate of inward CO_2 diffusion, mediated by stomatal conductance (g_s), and the rate of CO_2 assimilation in photosynthesis (A). Water-use efficiency (WUE, the ratio of assimilation to transpiration) is also related to P_i/P_a, thus the stable C isotope composition ($\delta^{13}C$) and WUE are positively related (Farquhar et al. 1989).

The isotopic composition of plant tissue provides an estimate of environmental or physiological effects on A or g_s. Hence, $\delta^{13}C$ of tissues reflects the $\delta^{13}C$ of atmospheric CO_2 at the time it was fixed and the fractionation of CO_2 by plant processes between then and when it was

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deposited in the plant (Farquhar et al. 1982). A strong and consistent decrease in discrimination with altitude has been observed in many species (Körner et al. 1988, 1991; Marshall and Zhang 1994; Hultine and Marshall 2000). This decrease in discrimination reflects a combination of the decrease in CO_2 and O_2 partial pressures with altitude and variation in leaf anatomical, morphological and physiological traits with altitude (Hultine and Marshall 2000). However, altitude is an indirect environmental gradient and a host of inter-related climatic (e.g. atmospheric pressure, temperature, precipitation) and edaphic factors (e.g. soil age, depth, nutrient status, water-holding capacity) vary with altitude. Thus the decrease in discrimination with altitude cannot be generally ascribed to any single factor and is likely a response to many.

Of single environmental variables, discrimination has been related to temperature (Wilson and Grinsted 1977; Tans and Mook 1980; Freyer and Belacy 1983; Leavitt and Long 1982, 1991), irradiance (Leavitt and Long 1991; Pearcy and Pfitsch 1991) and humidity (Saurer and Siegenthaler 1989). In most cases, the strongest relationships between $\delta^{13}C$ and *single variables* have been between $\delta^{13}C$ and modelled soil water availability or transpiration (Dupouey et al. 1993; Livingston and Spittlehouse 1993; McNulty and Swank 1995; Walcroft et al. 1997). Walcroft et al. (1997) suggested that observed close relationships between soil water availability or transpiration, and $\delta^{13}C$, owe much to the integration of a number of environmental variables (e.g. precipitation, irradiance, temperature and air saturation deficit) by P_i, and to the relationship of transpiration and $\delta^{13}C$ through g_s. In contrast to strong relationships with water availability or transpiration, $\delta^{13}C$ is often only weakly related to precipitation and evaporation gradients (e.g. Leavitt and Long 1986; Niemelä et al. 1997), with the notable exception of the close relationship described for *Pinus radiata* D. Don by Korol et al. (1999). We incorporated a range of data from conifers in south-western West Australia with that published in a range of other studies, to investigate the general hypothesis (Stewart et al. 1995) that $\delta^{13}C$ is a useful integrator of water availability.

In conifers, few studies have tested the relationship between $\delta^{13}C$ and indices of drought stress such as pre-dawn water potential (Ψ). Such a relationship directly links $\delta^{13}C$ to the water status of the *plant* and ought produce a better relationship than that with the availability of *soil* water. We investigated the relationship between $\delta^{13}C$ and Ψ in thinning×fertiliser trials with *P. radiata* and *Pinus pinaster* Ait. in the expectation that thinning would increase the availability of soil water to individual trees (e.g. Butcher 1977; Aussenac and Granier 1988), thereby reducing drought stress [i.e. Ψ (Whitehead et al. 1984; Aussenac and Granier 1988; Bréda et al. 1995)], WUE (Lauteri et al. 1997) and $\delta^{13}C$. Thinning also frequently increases interception of radiation and foliar nutrient concentrations, and these might be expected to increase WUE (e.g. Leavitt and Long 1991; Guehl et al. 1995) and $\delta^{13}C$. We thus sought further to examine these possible influences on $\delta^{13}C$ in a rigorous field trial.

In areas of lesser rainfall in south-western West Australia (<750 mm year^{-1}) *P. pinaster* is favoured over *P. radiata* because of greater drought resistance (Hopkins 1971; Warren and Adams 2000a). Our overall hypothesis was that *P. pinaster* would use water more efficiently than *P. radiata* and that this would be manifest as a greater (less negative) $\delta^{13}C$ in wood.

Materials and methods

Review of literature $\delta^{13}C$ data for conifers

The ratio of precipitation (P) to evaporation (E) was used as an index of water availability and compared to $\delta^{13}C$ of stemwood and foliage in a range of conifers from across the globe (Tables 1, 2). Our literature review was hampered by the absence of adequate site descriptions in many studies, thus studies that did not provide

Table 1 Sources of stemwood stable C isotope composition ($\delta^{13}C$) data for conifers. Data are plotted in Figs. 2a, c and 3a, c

Species	Location	Study
Araucaria angustifloia	Brazil	Rebello and Wagener (1976)
Athrotaxis selaginoides	Tasmania	Francey (1981)
Fitzroya cupressoides	Chile	Leavitt and Lara (1994)
Larix decidua	Scotland	Farmer and Baxter (1974)
Phyllocladus aspleniifolius	Tasmania	Francey (1981)
Picea abies	Switzerland	Saurer et al. (1995)
Pinus banksiana	Central Canada	Brooks et al. (1998)
Pinus edulis	New Mexico	Leavitt and Long (1986)
Pinus pinaster	SW France	Nguyen-Queyrens et al. (1998)
Pinus pinaster	Western Australia	Present study
Pinus radiata	Western Australia	Present study
Pinus radiata	New Zealand	Walcroft et al. (1997)
Pinus radiata	Australia and New Zealand	Korol et al. (1999)
Pinus strobus	North Carolina	McNulty and Swank (1995)
Pinus sylvestris	Black Forest	Freyer (1979)
Pinus sylvestris	Switzerland	Saurer et al. (1995)
Pinus tabulaeformis	China	Yu et al. (1996)
Pseudotsuga menziesii	Oregon	Panek and Waring (1997)
Pseudotsuga menziesii	Vancouver Island	Livingston and Spittlehouse (1996)

Table 2 Sources of foliage δ13C data for conifers. Data are plotted in Figs. 2b, d and 3b, d

Species	Location	Study
Juniperus monosperma	New Mexico	Lajtha and Barnes (1991)
Juniperus monosperma	New Mexico	Lajtha and Getz (1993)
Juniperus osteosperma	Utah	Ehleringer and Cerling (1995)
Juniperus osteosperma	Nevada	DeLucia and Schlesinger (1991)
Juniperus osteosperma	Utah	Marshall et al. (1994)
Juniperus virginiana	Nebraska	Zhang and Cregg (1996)
Lagarostrobus franklinii	Tasmania	Francey et al. (1985)
Larix lariciana	Wisconsin, New Brunswick, Sasketchwan, Manitoba	Kloeppel et al. (1998)
Larix leptolepis	Nebraska	Zhang and Cregg (1996)
Larix lyallii	Montana	Kloeppel et al. (1998)
Larix occidentalis	Montana	Kloeppel et al. (1998)
Larix siberica	Russia	Kloeppel et al. (1998)
Larix siberica	Iceland	Kloeppel et al. (1998)
Picea abies	Bavaria	Gebauer and Schulze (1991)
Picea abies	Sweden	Högberg et al. (1993)
Picea mariana	Central Canada	Flanagan et al. (1997)
Picea mariana	Wisconsin, New Brunswick, Sasketchwan, Manitoba	Kloeppel et al. (1998)
Picea obovata	Russia	Kloeppel et al. (1998)
Picea pungens	Nebraska	Zhang and Cregg (1996)
Picea sitchensis	Iceland	Kloeppel et al. (1998)
Pinus albicaulis	Montana	Kloeppel et al. (1998)
Pinus banksiana	Central Canada	Flanagan et al. (1997)
Pinus contorta	Montana	Kloeppel et al. (1998)
Pinus edulis	New Mexico	Lajtha and Barnes (1991)
Pinus edulis	Utah	Flanagan et al. (1992)
Pinus edulis	New Mexico	Lajtha and Getz (1993)
Pinus jeffreyi	Nevada	DeLucia and Schelsinger (1991)
Pinus massoniana	Sub-tropical China	Ehleringer et al. (1986)
Pinus monophylla	Nevada	DeLucia and Schlesinger (1991)
Pinus nigra	Nebraska	Zhang and Cregg (1996)
Pinus pinaster	Western Australia	Warren and Adams (2000b)
Pinus ponderosa	Nebraska	Zhang and Cregg (1996)
Pinus ponderosa	Utah	Ehleringer and Cerling (1995)
Pinus ponderosa	Oregon	Hubbard et al. (1999)
Pinus ponderosa	Nevada	DeLucia and Schelsinger (1991)
Pinus radiata	New Zealand	Waring and Silvester (1994)
Pinus resinosa	Nebraska	Zhang and Cregg (1996)
Pinus resinosa	Ontario	Flanagan and Varney (1995)
Pinus strobiformis	Nebraska	Zhang and Cregg (1996)
Pinus strobus	Nebraska	Zhang and Cregg (1996)
Pinus sylvestris	Nebraska	Zhang and Cregg (1996)
Pinus taeda, P. echinata	Tennessee	Garten and Taylor (1992)
Pseudotsuga menziesii	Oregon	Panek and Waring (1997)
Pseudotsuga menziesii	Nebraska	Zhang and Cregg (1996)
Pseudotsuga menziesii	Montana	Kloeppel et al. (1998)

(or where we could not infer or interpolate) latitude, longitude and altitude were excluded since we could not estimate P and/or E. In addition, we include only those studies that give δ13C of individual species not means of multiple species from a community. Where data were not given in the original publication, estimates of precipitation and pan evaporation were interpolated from the closest weather stations, using records held by various meteorology agencies (Müller 1982). Where Penman potential evaporation (PET) was given, pan evaporation was calculated as: E=PET/0.7 (Linacre 1992). Pan evaporation is often greater than potential evaporation from vegetated surfaces (Linacre 1992) but the limited availability of climatic data for most published studies prevented estimation of PET. To avoid confounding analysis of climatic effects with differences in tissue composition, our comparison was based on isotope measurements on cellulose from stemwood or untreated foliage. These data are available for many of the studies listed in Tables 1, 2. For those studies of *Pinus* spp., data were converted to cellulose or untreated values using the offset (δ13C cellulose-δ13C untreated) of 0.9‰ found for *P. radiata* and *P. pinaster*. We acknowledge that applying a correction of 0.9‰ to all

Pinus spp. is a potential source of error given intra-generic variation, and differences in techniques of "cellulose" extraction. Nonetheless this was the only feasible approach given the few studies that have reported the difference in δ13C between whole tissue and cellulose.

The dataset spanned an altitudinal range of almost 2,500 m. We accounted for the altitudinal decrease in partial pressures of CO_2 and O_2 using the CO_2 partial pressure gradient into the leaf (P_a–P_i) calculated as described by Hultine and Marshall (2000). We assumed a constant mole fraction of CO_2 (360.9 μmol mol^{-1}), estimated mean air temperature from climate records, and P_i from measured δ13C (Farquhar et al. 1982).

Study site

The experimental study was conducted in south-western Australia, 20 km northwest of Harvey [latitude 32.97S and longitude 115.43E; 15–30 m above sea level (ASL)] and has been previously described (Warren and Adams 2000a). The region has a Mediter-

ranean climate with cool, wet winters and hot, dry summers. In July (mid-winter), the mean daily minimum temperature is 8.0°C and the maximum temperature is 16.7°C. In January (mid-summer), the mean daily minimum temperature is 15.5°C and the maximum temperature is 30.9°C. Mean annual rainfall at Harvey is 978 mm and open pan evaporation is about 1,788 mm, with 77% of the total annual rainfall between May and October. The site gently undulates between 15 and 30 m ASL with deep sandy soils overlying the water table at a depth of 15–20 m (Commander 1988).

Measurements were made on stands of *P. radiata* and *P. pinaster* about 5 km apart within the uniform landscape of the Swan Coastal Plain. Aspect, slope and soil type were the same in both stands as were soil depth, depth to groundwater and water storage capacity [based on long-term measurements to 8 m depth by neutron probe by the West Australian Department of Conservation and Land Management (CALM)].

Treatments

In 1966 *P. pinaster* was planted at a spacing of 3.0×3.0 m (1,111 stems ha^{-1}) and by winter 1993 was at a mean density of 992 stems ha^{-1}. *P. radiata* was planted in 1970 at 3.7 m×1.8 m (1,500 stems ha^{-1}), the plantation was thinned in 1983/1984 to remove malformed trees and by winter 1993 was at a mean density of 878 stems ha^{-1}. In winter 1993, CALM established an experiment with a randomised complete block design (six treatment plots assigned at random to each of three blocks) in each stand. At that time, mean overbark diameter at breast height (1.3 m; DBH) and plot basal area were similar for both species (*P. pinaster* 21.3 cm; *P. radiata* 21.2 cm), although *P. pinaster* was significantly shorter than *P. radiata* (15.5 m vs. 20.2 m). Treatment plots were around 0.2 ha and measurement plots 0.075 ha, with some variation owing to slight differences in row spacing between species. Treatments included three tree densities: 750, 500 or 250 stems ha^{-1}, and two amounts of fertiliser: none, or NH_4NO_3 (800 kg ha^{-1}) plus super-Cu-Zn (1,000 kg ha^{-1}) plus muriate of potash (500 kg ha^{-1}) – equivalent to (in kg ha^{-1}): 272 N, 91 P, 101 S, 185 Ca, 249 K, 6 Cu and 6 Zn. Fertiliser was applied once in winter 1993. Only the 750 and 250 stems ha^{-1} treatments are dealt with here.

Growth and leaf area measurements

DBH of five co-dominant trees was measured monthly, DBH of all other trees was measured annually. The leaf area index (LAI) of each plot was estimated at the beginning and end of the experiment based on allometric relationships between DBH and leaf area (Warren and Adams 2000a). All leaf areas are expressed on a projected basis and may be converted to total leaf areas with the factors derived by Grace (1987) (*P. pinaster*, 2.57; *P. radiata,* 3.14).

Plant water relations

Ψ was measured monthly during the dry season (September–May) with a pressure chamber. One fascicle, produced during the previous growing season, was cut from low branches (8–10 m above the ground) before dawn from each of five co-dominant trees in two replicate plots, giving a total of ten replicates per treatment. Each fascicle was placed inside a tube made of aluminium foil and stored within plastic bags at 0–4°C, measurements were made within 2 h of collection (Myers and Craig 1988).

C isotope analysis

In November 1997 wood cores were taken from five co-dominant trees of each treatment. Half-inch cores were taken at breast height from each of the four cardinal directions to account for circumferential variability in δ^{13}C (Leavitt and Long 1986) and dried to
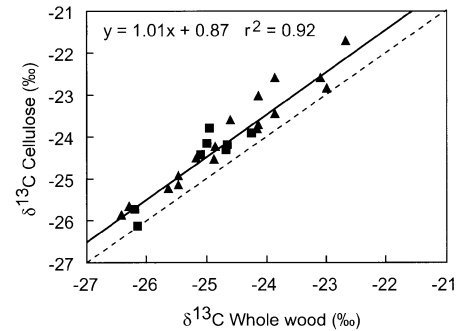

Fig. 1 The relationship between the stable C isotope composition (δ^{13}C; ‰) of whole wood and δ^{13}C of cellulose extracted with Diglyme-HCl (Macfarlane et al. 1999). Data are single measurements of wood samples pooled from five trees of *Pinus radiata* (■) and *P. pinaster* (▲)

constant mass at 60°C. For the 4 years of the experiment (August 1993–August 1997), cores were carefully separated into years on the basis of distinct changes in colour and hardness at the late-wood-earlywood transition. These transition zones are readily matched to the start of the spring growing season in August each year. Wood of each year was further subdivided into the four seasons based on measurements of DBH and wood properties. In the strongly Mediterranean climate of south-western Australia, seasons are well defined and associated with obvious changes in wood properties. In summary, seasons were characterised by different growth rates: (1) a period of rapid growth during spring (August–November) corresponding to abundant soil moisture and warm temperatures; (2) a period of slow growth over the hot, dry summer (November–March); (3) a period of intermediate growth during the cooler and wetter autumn (March–May); and (4) a second period of slow growth during the cool, wet winter (May–August). Wood from the four cores of each tree was pooled for analysis. In trees growing at 750 stems ha^{-1} there was little change in DBH over summer with wood development being offset by shrinkage of wood and bark. In some instances, increments of DBH were negative and we collected the smallest sample that would provide enough material for analysis (usually <0.5 mm of core). Changes in tissue composition affect the δ^{13}C of whole wood due to differences in ^{12}C:^{13}C fractionation among biosynthetic pathways (Park and Epstein 1961). A preliminary experiment determined the necessity of extracting cellulose for the determination of δ^{13}C rather than simply using whole wood. Cellulose was extracted from wood samples with the Diglyme-HCl method (Macfarlane et al. 1999). δ^{13}C$_{wood}$ and δ^{13}C$_{cellulose}$ were closely related (r^2=0.92) with whole wood samples being consistently 0.87‰ more negative than cellulose samples (Fig. 1). The difference between δ^{13}C$_{wood}$ and δ^{13}C$_{cellulose}$ was similar for all treatments and, consequently, all subsequent analyses were performed on whole wood samples. Finely ground samples (1.5–2 mg dry mass) were combusted to CO_2 in the presence of O_2 (Roboprep-CN; Europa Scientific, Crewe, UK) before passing into a mass spectrometer (Tracermass; Europa Scientific). δ^{13}C (‰) was calculated with respect to the PDB standard $\{=[^{13}C/^{12}C_{sample}]/(^{13}C/^{12}C_{standard})-1]\times1000\}$.

Statistics

All data were analysed using the routines contained within the commercial packages StatView or SuperAnova. The global data were analysed using simple linear or exponential regression models. The experimental data were analysed using several techniques. A nested ANOVA (age of wood nested within sample trees) was used to test the significance of treatment effects. The distribution of δ^{13}C data was close to normal and the data were homoscedastic. Analysis of co-variance (ANCOVA) with Ψ as a

co-variate was used to test for differences in the relationship between $\delta^{13}C$ and Ψ.

Results

General relationship between water availability and $\delta^{13}C$ in conifers

Data on the $\delta^{13}C$ signature of wood were compiled from 14 conifer species from across the globe (Tables 1, 2), these data were related to altitude and water availability (Fig. 2). The $\delta^{13}C$ signature of wood and altitude of collection were positively related ($r^2=0.49$) with an increase in $\delta^{13}C$ of 2.53‰ per kilometre of altitude (Fig. 2a). There was a weak positive relationship between the gradient in CO_2 partial pressure into the leaf (P_a-P_i) and altitude ($r^2=0.14$, $P<0.0001$, Fig. 2b). However, this relationship was solely due to sites where the ratio of precipitation to evaporation (P/E) was <1 ($r^2=0.27$, $P<0.0001$), and thus for sites where $P/E>1$ there was no relationship ($r^2=0.00$, $P>0.50$). P_a-P_i were closely related when $P/E<1$ ($P_a-P_i=8.51\times(P/E)^{-0.211}$, $r^2=0.45$, $P<0.001$) (Fig. 3c, d). This corresponds to a decrease in $\delta^{13}C$ of approximately 3.2‰ per unit P/E when normalised to sea level. At greater P/E, P_a-P_i were unrelated (Fig. 3a, b).

The relationship between foliage $\delta^{13}C$ and altitude was similar to that of wood, however, the rate of increase in $\delta^{13}C$ was less at 1.91‰ per kilometre of altitude (Fig. 2b). As with wood $\delta^{13}C$, there was a weak positive relationship between foliage P_a-P_i and altitude ($r^2=0.10$, $P<0.01$) due to sites with $P/E<1$ ($r^2=0.06$, $P<0.05$), there being no relationship for sites with $P/E>1$ ($r^2=0.02$, $P>0.50$) (Fig. 2d). The relationship between P_a-P_i and P/E was best described by a power relationship ($P_a-P_i=8.050\times(P/E)^{-0.105}$, $r^2=0.21$, $P<0.001$ Fig. 3d). However, at low P/E this relationship gave a poor fit in comparison with a linear relationship ($P_a-P_i=-3.81\times P/E+10.68$, $r^2=0.27$, $P<0.001$). This corresponds to a decrease in $\delta^{13}C$ of approximately 2.4‰ per unit P/E when normalised to sea level.

Experimental study

Growth

By 1995, the annual increase in basal area of individual trees in plots thinned to 250 stems ha^{-1} was 2–3 times greater than that of trees growing at 750 stems ha^{-1} (Table 3). Hence, on a land area basis, increases in basal area were only slightly reduced at the lesser stand density.
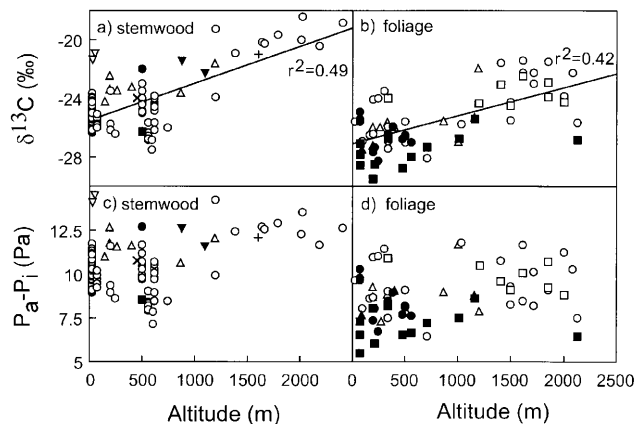

Fig. 2 Relationships between **a** $\delta^{13}C$ of stemwood cellulose and the altitude of collection, **b** $\delta^{13}C$ of foliage and the altitude of collection, **c** CO_2 partial pressure gradient into the leaf [ambient CO_2 partial pressure-internal CO_2 partial pressure (P_a-P_i)] and altitude of stemwood, and **d** CO_2 partial pressure gradient into the leaf (P_a-P_i) and altitude of foliage. Sources of data are listed in Tables 1 and 2. Symbols are: *Araucaria angustifolia* (**+**), *Fitzroya cupressoides* (**✗**), *Larix* spp. (**■**), *Picea* spp. (**●**), *Pinus* spp. (○), *Pseudotsuga menziesii* (△), *Phyllocladus asplenifolius* (▽), *Athrotaxis selaginoides* (▼), *Juniperus* spp. (□), *Lagarostrobus franklinii* (▲)
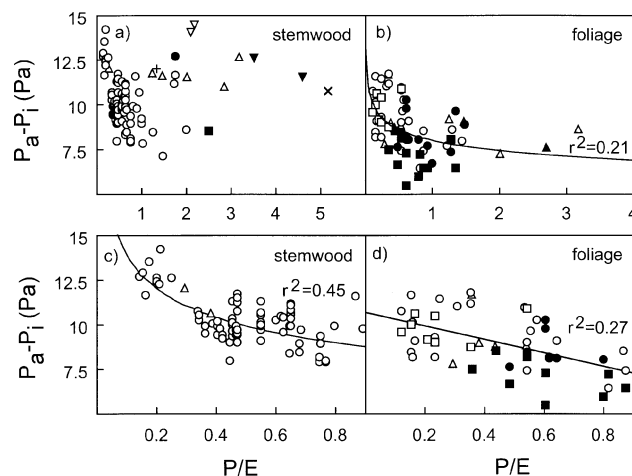

Fig. 3 Relationships between CO_2 partial pressure gradient into the leaf (P_a-P_i) and the ratio of precipitation to evaporation (P/E) in stemwood cellulose (**a, c**), and foliage (**b, d**). Sources of data are listed in Tables 1 and 2. Symbols are as in Fig. 2

Table 3 Annual increments of basal area (*BAI*; cm^2 tree^{-1} year^{-1}). Data shown are for the 1995/1996 season, trends between treatments were similar for the other years. Data were obtained from monthly measurements of DBH of five trees per plot (±SE)

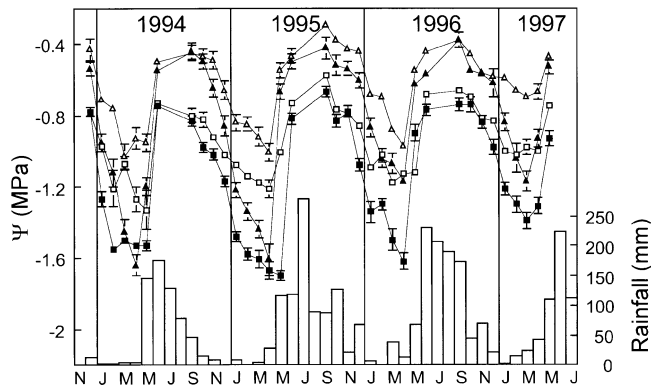| BAI	*Pinus pinaster*		*Pinus radiata*	
	250 Stems ha^{-1}	750 Stems ha^{-1}	250 Stems ha^{-1}	750 Stems ha^{-1}
Plus fertiliser	103 (±13)	32 (±3)	101 (±9)	33 (±10)
Minus fertiliser	45 (±7)	21 (±2)	42 (±5)	21 (±4)

Fig. 4 Seasonal trends of rainfall and pre-dawn water potential (Ψ; MPa) in *P. radiata* (■, □) and *P. pinaster* (▲, △) thinned to 750 stems ha⁻¹ (■, ▲) or 250 stems ha⁻¹ (□, △) in the spring of 1993. *Every second month* of the year is indicated (*J* January, *M* March, *M* May, etc.). Rainfall was measured with a tipping bucket gauge in a clearing adjacent to the *P. radiata* plantation. Water potential data are means±SE; *n*=10. (after J. F. McGrath, West Australian Department of Conservation and Land Management, unpublished data)

Table 4 Leaf area index (one-sided) of plots in October 1993 and October 1997. Data were obtained from allometric relationships between DBH and leaf area

Treatment	Pinus pinaster		Pinus radiata	
	1993	1997	1993	1997
250 Stems ha⁻¹ plus fertiliser	1.29	2.53	1.35	3.38
250 Stems ha⁻¹ minus fertiliser	1.29	1.89	1.33	2.54
750 Stems ha⁻¹ plus fertiliser	3.33	4.65	3.31	4.73
750 Stems ha⁻¹ minus fertiliser	3.41	3.93	3.21	4.29

Fig. 5 The seasonal course of stemwood δ¹³C (‰) in **a** *P. radiata* 750 stems ha⁻¹ plus fertiliser, **b** *P. pinaster* 750 stems ha⁻¹ plus fertiliser, **c** *P. pinaster* 250 stems ha⁻¹ plus fertiliser, and **d** *P. pinaster* 250 stems ha⁻¹ minus fertiliser. Results are means; *n*=5; 1 SE was <0.7‰ in all cases. *Sp* Spring, *Su* summer, *Au* autumn, *Wi* winter

Fertiliser application increased growth (relative to unfertilised trees) by 150% at 250 stems ha⁻¹ and approximately 50% at 750 stems ha⁻¹.

Plant water status

Ψ exhibited distinct intra-seasonal trends (Fig. 4). Water potential was greatest (least negative) in spring, at the end of the winter rains, decreasing to a minimum in late summer/autumn, before increasing with the arrival of rain in late autumn/winter. Minimum annual Ψ was less in the drier years 1993/1994 and 1994/1995 than in the years 1995/1996 and 1996/1997. Plots thinned to 250 stems ha⁻¹ had generally greater Ψ than those with 750 stems ha⁻¹ and the difference in Ψ was greatest in summer and autumn. In plots thinned to 250 stems ha⁻¹, Ψ never fell below –1.1 MPa (*P. pinaster*) or –1.3 MPa (*P. radiata*), whereas in plots thinned to 750 stems ha⁻¹, Ψ reached a minimum of –1.7 MPa (*P. pinaster*) or –1.8 MPa (*P. radiata*). Ψ was greater in *P. pinaster* than *P. radiata* by between 0.1 and 0.4 MPa throughout the measurement 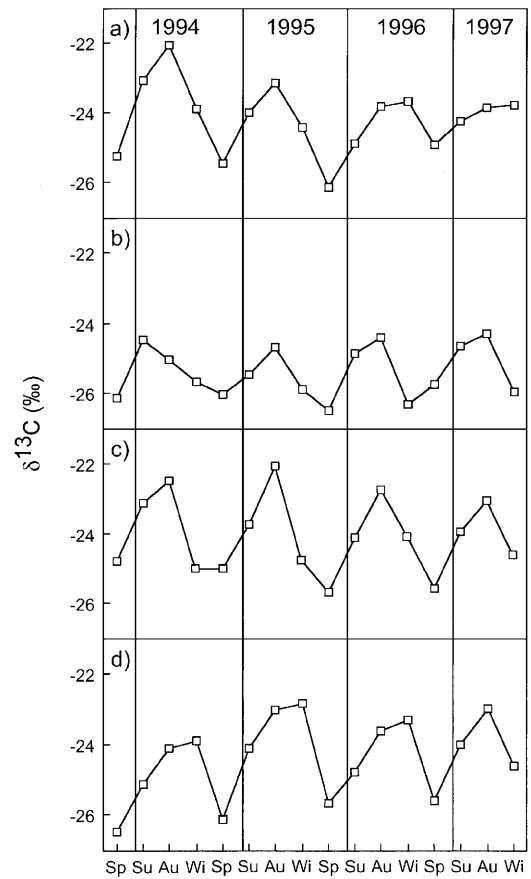period. Addition of fertiliser had a small and inconsistent effect on Ψ. Nonetheless, the maximum (spring and winter) Ψ was greater, and the minimum (summer/autumn) Ψ was less (by approximately 0.1 MPa) in fertilised plots than in control plots (data not shown).

Leaf area index

In 1993 the LAI of the two species was similar. Plots thinned to 250 stems ha⁻¹ had between 2 and 2.1 m² m⁻² less leaf area than plots thinned to 750 stems ha⁻¹ (Table 4). LAI of all plots increased during the study period; the increase being greatest in heavily thinned plots and those to which fertiliser was added. By 1997, the LAI of plots thinned to 750 stems ha⁻¹ was between 1.4 and 2.1 m² m⁻² greater than that of plots thinned to 250 stems ha⁻¹, and the LAI of plots which received fertiliser was between 0.4 and 0.8 m² m⁻² greater than that of unfertilised plots.
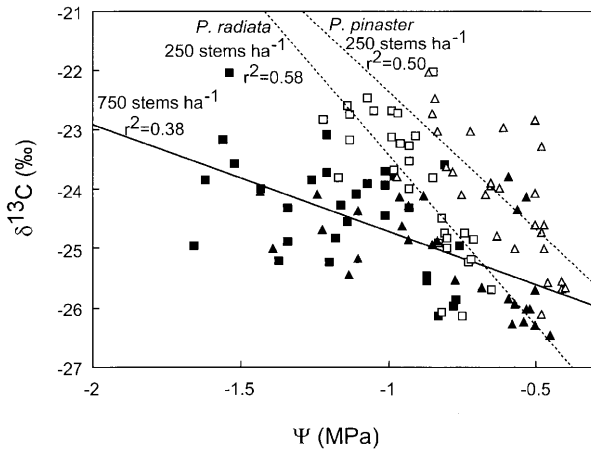
Fig. 6 The relationship between Ψ (MPa) and stemwood $\delta^{13}C$ (‰) in *P. radiata* (■, □) and *P. pinaster* (▲, △) thinned to 750 stems ha^{-1} (■, ▲) or 250 stems ha^{-1} (□, △). Data are derived from monthly Ψ measurements (n=10 per month) and seasonal $\delta^{13}C$ measurements (n=5). *Each point* is an average of several monthly Ψ measurements which correspond to the time when the wood for the $\delta^{13}C$ measurement was laid down

Stemwood $\delta^{13}C$

Clear seasonal patterns in stemwood $\delta^{13}C$ were evident in all tree rings. Wood developed in spring was depleted in ^{13}C ($\delta^{13}C$ was less) relative to wood developed in summer, autumn or winter (Fig. 5). The seasonal $\delta^{13}C$ pattern and mean ^{13}C depletion differed between species (ANOVA, $P<0.0001$). In *P. pinaster*, the $\delta^{13}C$ of wood was less than that of *P. radiata* by an average of 0.5‰; this difference was greater in summer (mean difference 1.1‰) and spring (0.9‰) than in winter (0.3‰) (compare Fig. 5a, b).

The addition of fertiliser significantly affected the $\delta^{13}C$ of annual rings ($P<0.04$, compare Fig. 5c, d). The effect of fertiliser varied between years: in the 1993/1994 season, the $\delta^{13}C$ of wood from trees to which fertiliser had been added was, on average, 0.6‰ less than that from unfertilised trees; in the three subsequent seasons, fertiliser application had no effect on $\delta^{13}C$. Fertiliser also affected the seasonal pattern of $\delta^{13}C$, such that $\delta^{13}C$ reached a maximum at a later date each year. This effect also diminished with time and was not evident by the 1996/1997 season.

In plots thinned to 250 stems ha^{-1}, the $\delta^{13}C$ of tree rings were generally greater than those of plots thinned to 750 stems ha^{-1}; the average difference was 0.75‰ ($P<0.0001$, compare Fig. 5b, c). Differences were greater in summer (mean difference 1.5‰) and autumn (1‰) than in spring (0.6‰) and winter (0.1‰).

$\delta^{13}C$ was negatively related to Ψ (Fig. 6). The relationship was poor when analysed across all treatments (r^2=0.14), but was considerably stronger within treatments or when the two thinning regimes were treated separately (r^2=0.38, 0.50, 0.58). Slopes and intercepts of regressions of $\delta^{13}C$ on Ψ were not significantly different for trees of either species growing at 750 stems ha^{-1}

(ANCOVA, $P>0.05$) and these data were combined into a single data set. At 250 stems ha^{-1}, the $\delta^{13}C$ of *P. pinaster* was significantly greater than that of *P. radiata* at any given Ψ ($P<0.04$). Moreover, the change in $\delta^{13}C$ per unit Ψ was approximately twice as great at 250 stems ha^{-1} compared to 750 stems ha^{-1} (–3.1‰ MPa^{-1} vs. –1.8‰ MPa^{-1}; $P<0.0001$).

Discussion

Predictive capabilities of $\delta^{13}C$

C isotope discrimination consistently decreased ($\delta^{13}C$ increased) with altitude for a wide range of conifers (Fig. 2a, b). Similar results have been reported for conifers (e.g. Marshall and Zhang 1994; Hultine and Marshall 2000) and other species (Körner et al. 1988; Vitousek et al. 1990; Körner et al. 1991). For plants unlikely to experience drought stress (i.e. $P/E>1$), the gradient in CO_2 between the atmosphere and the leaf (P_a–P_i) was unrelated to altitude (Fig. 2c,d). The positive relationship between these variables for drier sites ($P/E<1$) probably owes more to an autocorrelation between altitude and drought stress than to any effect of altitude per se. As with other studies of this type, considerable variation in P_a–P_i (calculated using $\delta^{13}C$) could not be explained by altitude. The variation may have at least three sources: (1) species differences in altitude-$\delta^{13}C$ relationships (e.g. Hultine and Marshall 2000); (2) inherent species differences in discrimination (e.g. Zhang and Cregg 1996); (3) or variation between sites in the multitude of other factors that affect discrimination (e.g. water availability). Our data indicate that water availability (P/E) explains some of the residual scatter in P_a–P_i (sensu Stewart et al. 1995) but only at low P/E (Fig. 3). Even here, there is considerable spread about the line-of-best-fit and we acknowledge that a multitude of factors including water availability modify P_i under *all* climatic conditions. Inherent species differences in discrimination, for example that between the deciduous *Larix* and evergreen species (Kloeppel et al. 1998), also accounts for much variation in discrimination. The absence of a consistent relationship at high P/E (and Ψ) may well indicate that when water is not a primary limitation to g_s, other factors such as light intensity and air saturation deficit have a greater effect on $\delta^{13}C$. In addition, when P/E is very large (e.g. >2), flooding or water-logging are likely on some sites, at least on a seasonal basis, and may also induce stomatal closure (Drew 1983). Some of the greater $\delta^{13}C$ signatures at large P/E are probably the result of excessive water.

Species differences in $\delta^{13}C$ signature

The results did not support our hypothesis, that *P. pinaster*, the more drought-tolerant species (Hopkins 1971), is more efficient in the use of water than *P. radiata*. Wood

Table 5 Relationship between environmental factors and $\delta^{13}C$ [or ambient CO_2 partial pressure-internal CO_2 partial pressure (P_a-P_i)]. Data were obtained from various sources and this study. Data from non-conifers is included due to a paucity of data for some environmental factors

Environmental factor	Species	Author(s)	Relationship
Water availability			
$\delta^{13}C$ (leaves) vs. Ψ	*Quercus ilex*, *Q. pubescens*	Damesin et al. (1998)	–0.18‰ per MPa
$\delta^{13}C$ (wood) vs. Ψ	*P. pinaster*, *P. radiata*	Present study	–1.8 to –3.1‰ per MPa
$\delta^{13}C^a$ (leaves) vs. *P/E*	Various conifers	Present study	–2.4‰ per mm mm^{-1}
$\delta^{13}C^a$ (wood) vs. *P/E*	Various conifers	Present study	–3.2‰ per mm mm^{-1}
Altitude			
$\delta^{13}C$ (leaves) vs. altitude	Various conifers	Present study	1.91‰ per km
$\delta^{13}C$ (wood) vs. altitude	Various conifers	Present study	2.53‰ per km
Light			
$\delta^{13}C$ vs. daily PAR received	Various species	Ehleringer et al. (1986)	0.125‰ per mol m^{-2} day^{-1}
$\delta^{13}C$ vs. daily PAR received	*Adenocaulon bicolor*	Pearcy and Pfitsch (1991)	0.41‰ per mol m^{-2} day^{-1}
Nutrients			
$\delta^{13}C$ (leaves) vs. mass-based N	*Juniperus osteosperma*	Marshall et al. (1994)	1.16‰ per mmol g^{-1}
$\delta^{13}C$ (leaves) vs. area-based N	*Citrus* spp.	Syvertsen et al. (1997)	6–18‰ per mol m^{-2}

[a] $\delta^{13}C$ normalised to sea level (360.9 µmol mol^{-1} CO_2; 101.3 kPa barometric pressure)

of *P. pinaster* was, on average, 0.5‰ depleted in ^{13}C relative to that of *P. radiata* (Fig. 5), although if compared at common Ψ, there was no difference in $\delta^{13}C$ between species (Fig. 6). Rates of aboveground biomass production and leaf area were similar for both species at this site (Tables 3 and 4, see also Warren and Adams 2000a). The conflicting evidence of less negative Ψ yet greater $\delta^{13}C$ (and supposedly poorer WUE) is consistent with *P. pinaster* having better access to soil water than *P. radiata*. In this instance, drought tolerance of *P. pinaster* can be equated with avoidance of water stress, probably via a greater root/shoot ratio and development of a deep taproot. Our results for these two *Pinus* spp. along with those of others (e.g. Lauteri et al. 1997; Zhang et al. 1997), challenge the view that a greater WUE is advantageous in water-limited habitats.

Thinning and $\delta^{13}C$

Our data do not support the hypothesis that heavy thinning, by increasing water availability (Fig. 4), leads to a decrease in WUE (and $\delta^{13}C$) (Fig. 5). These data do not disprove the general relationship between $\delta^{13}C$ and water availability (e.g. Fig. 3), rather they indicate that thinning also modifies the availability of nutrients and interception of radiation and that these may mask the effect of water availability on discrimination. Increased interception of radiation by trees in the heavily thinned plots should be associated with increased rates of photosynthesis, a reduction in P_i and less ^{13}C discrimination (greater $\delta^{13}C$, Francey et al. 1985; Ehleringer et al. 1986; Farquhar et al. 1989; Leavitt and Long 1991; Waring and Silvester 1994). Severe thinning is also likely to reduce competition for soil nutrients and may increase N concentrations in foliage. We observed an increase of ap-

proximately 0.2% in thinned *P. radiata* and 0.3% in thinned *P. pinaster* (C. R. Warren and M. A. Adams, unpublished data) and such increases will contribute to faster rates of photosynthesis and greater $\delta^{13}C$ (Table 5; Lajtha and Getz 1993; Marshall et al. 1994; Syvertsen et al. 1997).

The density-dependent sensitivity of $\delta^{13}C$ to Ψ (Fig. 6) suggests that soil water deficits or some correlated variable (e.g. air saturation deficit) are modified by density. Greater nutrient concentrations in trees from heavily thinned plots may be at least partially responsible (Thompson and Wheeler 1992) for the greater stomatal sensitivity to water deficits in severely thinned stands. Alternatively, the development of more negative water potentials during the day in trees from heavily thinned plots is probably due to their greater leaf area and interception of radiation (Jarvis 1975). g_s is regulated in conifers such that xylem embolisms are avoided by the maintenance of water potential above a critical threshold for xylem dysfunction (Tyree and Sperry 1988; Lu et al. 1996). Increased tension in the xylem (more negative water potential in foliage) during daytime can trigger stomatal closure, and increases in resistance of the pathway between soil and atmosphere or increased transpirational flows will both lead to greater diurnal variation in Ψ. This interpretation is supported in our case since, although the stand LAI was reduced (by ~2 m^2 m^{-2}) by thinning, aboveground production of individual trees was about twice that of unthinned trees (Table 3).

Drought stress and $\delta^{13}C$

Ψ is a useful indicator of the availability of soil water (Slatyer 1967), while $\delta^{13}C$ indicates the ratio P_i/P_a (Farquhar et al. 1982). Ψ and $\delta^{13}C$ integrate many com-

mon variables, and were indeed negatively correlated. Nonetheless, the relationship between $\delta^{13}C$ and Ψ varied between thinning treatments, largely as a function of the aforementioned variation in radiation interception and nutrient status between thinning treatments. Furthermore, comparing species and treatments at common Ψs may be confounded by variation in other factors that modify $\delta^{13}C$ (i.e. leading to common Ψs at different times of the year in different treatments). Hence, for any given Ψ, other micrometeorological influences on $\delta^{13}C$ (e.g. air saturation deficit and irradiance) may differ greatly. These variations contribute to the "weakness" of any relationship between $\delta^{13}C$ and Ψ. In light of the global trends in $\delta^{13}C$ and water availability (see above and Fig. 3), it is also worth noting that the relationship between $\delta^{13}C$ and Ψ was weakest as Ψ approached 0 MPa during winter and spring.

Fertiliser addition and $\delta^{13}C$

The positive response of WUE (and $\delta^{13}C$) to the addition of fertiliser was expected in light of other studies of water use and nutrient status (Fig. 5; Field et al. 1983; Sheriff et al. 1986; Lajtha and Getz 1993; Guehl et al. 1995). In our case the large and prolonged increases in LAI (Table 4) and transpiration have increased growth (Table 3) and water use (CALM, unpublished data) and WUE (Fig. 6). Consistent with these observations is that during dry periods, tree Ψ was less in plots to which fertiliser had been added (data not shown), implying a reduction in soil water content with fertiliser additions. Nonetheless, the nutrient-promoted increase in WUE was transient (lasting for the first season after fertiliser application) and probably related to the transformation of nutrients within foliage (e.g. withdrawal of nutrients during leaf senescence). WUE and foliar concentrations of N and/or P have been positively correlated (Sheriff et al. 1986; Lajtha and Getz 1993) and thus the decline in WUE after 1 year matches a likely fall in nutrient concentrations, especially since increased growth of foliage and other tissues was not matched by continuing fertiliser inputs (e.g. Carlyle 1995).

In winter and spring, a proportion of new wood is formed via remobilisation of C fixed during autumn (Egger et al. 1996). Hence the $\delta^{13}C$ signal of winter and spring wood represents a variable amount of retranslocated C as well as some from current photosynthesis. Fertiliser-induced differences in the seasonal pattern of $\delta^{13}C$ (Fig. 6) probably reflect these patterns of retranslocation. In trees to which fertiliser had been added, the $\delta^{13}C$ signature of winter wood was considerably less than that of autumn wood and more in line with our expectations of the $\delta^{13}C$ signature of products of current photosynthesis. In non-fertilised trees, the $\delta^{13}C$ signature was greater than expected on the basis of current photosynthesis and more consistent with a retranslocation interpretation.

Conclusions

The $\delta^{13}C$ signature of plant tissue is an information-rich signal that can provide useful insights into plant function. In a global survey of C isotope discrimination in conifers, P_a–P_i and water availability were significantly related. However, the relationship was fairly scattered due to inherent species differences in discrimination and variation between sites in other factors that affect discrimination. Variation in irradiance and N nutrition can have as large an effect on discrimination as water availability and altitude (Table 5). In one study, for example, an increase in daily PAR received from 20 to 50 mol m^{-2} day^{-1} increased $\delta^{13}C$ by over 3.5‰ (Ehleringer et al. 1986). Our examination of the water stress-$\delta^{13}C$ hypothesis was confounded by variation in radiation interception and plant nutrition. $\delta^{13}C$ was greater at reduced stand density where water availability was greatest, contrary to what might have been expected on the basis of the generally close relationship between $\delta^{13}C$ and water availability. Rather than disproving the general relationship between discrimination and water availability, such data highlights that radiation interception and N nutrition also affect $\delta^{13}C$. Thus $\delta^{13}C$ may well be a useful indicator of drought stress, but only in seasonally dry climates ($P/E < 1$) and where variation in other environmental factors is minimal.

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