

Tamir Klein · Deborah Hemming · Tongbao Lin
José M. Grünzweig · Kadmiel Maseyk · Eyal Rotenberg
Dan Yakir

Association between tree-ring and needle $\delta^{13}\text{C}$ and leaf gas exchange in *Pinus halepensis* under semi-arid conditions

Received: 20 June 2004 / Accepted: 29 December 2004 / Published online: 11 May 2005
© Springer-Verlag 2005

Abstract Associations between $\delta^{13}\text{C}$ values and leaf gas exchanges and tree-ring or needle growth, used in eco-physiological compositions, can be complex depending on the relative timing of CO_2 uptake and subsequent redistribution and allocation of carbon to needle and stem components. For palaeoecological studies it is often interpreted in terms of a simple model of $\delta^{13}\text{C}$ fractionation in C_3 plants. However, in spite of potential complicating factors, few studies have actually examined these relationships in mature trees over inter- and intra-annual time-scales. Here, we present results from a 4 years study that investigated the links between variations in leaf gas-exchange properties, growth, and dated $\delta^{13}\text{C}$ values along the needles and across tree rings of Aleppo pine trees growing in a semi-arid region under natural conditions or with supplemental summer irrigation. Sub-sections of tissue across annual rings and along needles, for which time of formation was resolved from growth rate analyses, showed rapid growth and $\delta^{13}\text{C}$ responses to changing environmental conditions. Seasonal cycles of growth and $\delta^{13}\text{C}$ (up to $\sim 4\text{‰}$) significantly correlated ($P < 0.01$) with photosynthetically active radiation, vapour pressure deficit, air temperature, and soil water content. The irrigation significantly increased leaf net assimilation, stomatal conductance and needle and tree-ring growth rate, and markedly decreased needle and tree-ring $\delta^{13}\text{C}$ values and its sensitivity to environmental parameters. The $\delta^{13}\text{C}$ estimates derived from gas-exchange parameters, and weighted by assimilation, com-

pared closely with seasonal and inter-annual $\delta^{13}\text{C}$ values of needle- and tree-ring tissue. Higher stomatal conductances of the irrigated trees (0.22 vs. $0.08 \text{ mol m}^{-2} \text{ s}^{-1}$ on average) corresponded with $\sim 2.0\text{‰}$ lower average $\delta^{13}\text{C}$ values, both measured and derived. Derived and measured $\delta^{13}\text{C}$ values also indicated that needle growth, which occurs throughout the stressful summer was supported by carbon from concurrent, low rate assimilation. For Aleppo pine under semi-arid and irrigated conditions, the $\delta^{13}\text{C}$ of tree-ring and needle material proved, in general, to be a reasonable indicator of integrated leaf gas-exchange properties.

Keywords ^{13}C discrimination · Soil moisture · Dendroecology · Stomatal conductance · Carbon allocation

Introduction

Tree-rings and needles provide a natural archive of environmental information that can be dated absolutely and used for palaeoecological reconstructions (Fritts 1976; Hughes et al. 1982; Van der Water et al. 1994; Schweingruber 1996). One major limitation of interpreting structural features, such as ring width or density, is that no universal theoretical model exists that links leaf gas exchanges with individual structural characteristics. In contrast, a simple theoretical model exists that associates the stable carbon isotope composition, $\delta^{13}\text{C}$, of photosynthates with leaf CO_2 exchange (Farquhar et al. 1982). This provides a powerful tool for environmental reconstruction using $\delta^{13}\text{C}$ measurements on tree-rings, $\delta^{13}\text{C}_t$, and needles, $\delta^{13}\text{C}_n$ (Leavitt and Long 1989; Saurer and Siegenthaler 1989; Schleser 1985; Robertson et al. 1997; Switsur and Waterhouse 1989). However, care must be taken when interpreting such data because other factors, including variations in the allocation and redistribution of carbohydrates as well as wood chemical composition, can influence the $\delta^{13}\text{C}$ of

Communicated by Christian Koerner

T. Klein · D. Hemming · T. Lin · J. M. Grünzweig
K. Maseyk · E. Rotenberg · D. Yakir (✉)
Department of Environmental Science and Energy Research,
Weizmann Institute of Science, Rehovot, 76100 Israel
E-mail: dan.yakir@weizmann.ac.il
Tel.: +972-8-9342549

Present address: D. Hemming
Hadley Centre for Climate Prediction and Research, Met Office,
Fitzroy Road, Exeter, EX1 3PB, UK

photosynthates and their translation into tree-ring and needle tissue (Gleixner 1993; Ogle and McCormac 1994; Loader et al. 1995; Terwilliger 1996; Schleser et al. 1999; Hemming et al. 2001; Tcherkez et al. 2003). Although the reliability of a simple $\delta^{13}\text{C}$ fractionation model has been verified using on-line techniques (Evans et al. 1986), few such studies exist for mature tree species over multi-seasonal time periods (Dawson et al. 2004). These studies are particularly important because the time lags between CO_2 uptake and carbon fixation into structural components may be the longest for the tree systems, and this may cause significant differences in the $\delta^{13}\text{C}$ of newly assimilated carbohydrates and that fixed as needle or tree-ring tissue (Marshall and Monserud 1996). Furthermore, trees in the semi-arid sites are relatively less studied but can provide information on ecophysiological and climatic aspects of an important climatic zone in the current terrestrial biosphere as well as under different climate scenarios (Fang and Epstein 1995).

Here, we examine the seasonal growth and $\delta^{13}\text{C}$ responses of tree-rings and needles of Aleppo pine trees growing in Yatir forest, Israel, to natural (semi-arid) and irrigated environmental conditions. Specifically, we test the assumption that $\delta^{13}\text{C}_t$ and $\delta^{13}\text{C}_n$ values of the whole-tissue represent an integrated record of prevailing leaf gas-exchange processes, measured regularly throughout the two growth seasons.

Materials and methods

Site description

Yatir forest is an Aleppo pine (*Pinus halepensis* Miller) dominated afforestation located at the northern edge of the Negev desert (31°20'N, 35°00'E, ~650 m a.s.l) and covering an area of 2,800 ha. The forest was initially planted in the mid-1960s, largely for landscaping and recreation purposes. Stand densities are ~360 trees ha^{-1} on predominantly light brown Rendzina, 25–100 cm deep. The general climatic regime is Mediterranean; a prolonged summer drought period from May to October (average temperature ~28°C) is followed by a winter period of moderate precipitation (annual mean is ~270 mm) and temperature (~15°C).

Irrigation experiment

Between 15 May 2001 and 15 November 2002 gravitational drip irrigation, 3.5–4.0 mm of H_2O day^{-1} was supplied to 15 trees. This rate of irrigation was chosen to best simulate average winter precipitation amounts. During the rainy and winter months, soil moisture levels were maintained relatively constant by supplying water only when the monthly precipitation was below 100 mm. Trees growing under natural moisture levels surrounding (within 30 m) the irrigation experiment were used for control measurements. The term

'irrigated' will be used to refer to the trees selected for irrigation (regardless of whether they were experiencing irrigation or not), and 'control' to the trees growing outside the irrigation plot.

Meteorological and physiological measurements

Since May 2000 an eddy covariance flux tower, located within Yatir forest at the 'tower plot' (1.2 km northeast of the irrigated plot), has provided continuous (averaged every 30 min) in situ measurements of CO_2 , water vapour and energy fluxes, together with an array of other hydrological and meteorological observations; including air temperature, relative humidity, precipitation and soil temperature and moisture (Rotenberg et al., in preparation). Fortnightly measurements of trunk growth and needle extension were made on ~10 trees per plot and 5 needles per tree at the tower, control and irrigated plots. Band dendrometers and calipers were used to record trunk and needle growth with a precision of 0.1 mm.

Leaf gas-exchange measures

During the irrigation experiment (15 May 2001 to 15 November 2002) leaf gas-exchange properties, such as net assimilation (A), stomatal conductance (g_s) and leaf internal CO_2 concentration (C_i), were measured on ten control and ten irrigated plot trees (Grünzweig et al. 2003; Lin et al., in preparation). Measurements were made every 1–2 weeks on sunlit needles on the south side of trees using a LI-6400 Photosynthesis System (Licor Inc., Lincoln, NE, USA). Up to five cycles of measurements were made throughout a day and the averages of all diurnal measurements were used in this analysis. To compare directly the leaf gas-exchange measurements with the observed $\delta^{13}\text{C}_t$ and $\delta^{13}\text{C}_n$ values, $\delta^{13}\text{C}$ was estimated from the following basic equation of $\delta^{13}\text{C}$ fractionation in C_3 plants (Farquhar et al. 1982) using the gas exchange derived values of C_i :

$$\delta^{13}\text{C}_{\text{gx}} = \delta^{13}\text{C}_a + a + (b - a) \times \frac{C_i}{C_a} \quad (1)$$

where, $\delta^{13}\text{C}_{\text{gx}}$ is the gas-exchange derived $\delta^{13}\text{C}$, $\delta^{13}\text{C}_a$ is the $\delta^{13}\text{C}$ of atmospheric CO_2 , a is the maximum ^{13}C fractionation from diffusion of CO_2 into the leaf (−4.4‰), b is the maximum ^{13}C fractionation from carboxylation reactions (−27‰), and C_i and C_a are CO_2 concentrations in the leaf intercellular cavity and atmosphere respectively.

This simplified version of the discrimination model, assumes that the effects of respiration on $\delta^{13}\text{C}_{\text{gx}}$ could be neglected in context of this study. C_a for the relevant dates were obtained from CO_2 concentration measured at the Yatir flux tower. However, due to the lack of continuous CO_2 measurements at Yatir, $\delta^{13}\text{C}_a$ data was obtained from bi-weekly NOAA-CMDL flask sampling measurements made on air collected at Sede Boker, a

sparsely vegetated, desert site ~ 50 km south of Yatir forest (<http://www.cmdl.noaa.gov/index.html>). Since CO_2 concentrations measured in Sede Boker showed excellent agreement with those measured at the Yatir sites, they were confidently used. As single $\delta^{13}\text{C}_{\text{gx}}$ values do not necessarily reflect a period of tree-ring or needle formation it may be misleading to compare $\delta^{13}\text{C}_{\text{gx}}$ with $\delta^{13}\text{C}_t$ and $\delta^{13}\text{C}_n$ values directly. Therefore, where annual averages are compared, the $\delta^{13}\text{C}_{\text{gx}}$ results are actually weighted averages. Each $\delta^{13}\text{C}_{\text{gx}}$ measurement was multiplied by the proportion of A on the specific day of $\delta^{13}\text{C}_{\text{gx}}$ determination relative to the sum of A over the period of tree-ring or needle formation. In this way, times of high assimilation by the tree, which contribute significantly to tissue formation, (and hence bias annual averages of $\delta^{13}\text{C}_t$ or $\delta^{13}\text{C}_n$) are reflected in higher weightings given to the corresponding gas-exchange measurements.

Tree-core and needle samples

Tree-cores and needles were sampled from ten irrigated and ten control trees. From each tree, two trunk cores (6 mm diameter) were collected, at a height of 1.3 m and at 0°N and 180°S . Three needles were collected from each of the last three annual age classes from the same trees and directions (usually Aleppo pine in Yatir forest retains needles for 2–4 years). All samples were oven-dried at 80°C prior to analyses.

Basal area increment calculation

Tree-cores were sanded to reveal the tree-ring cellular structure before the widths of the last four rings were measured (with precision of 0.1 mm) using calipers and a binocular microscope. The four ring widths measured from each core were cross matched graphically, comparing intra- and inter-tree-cores from the same plot. Tree-ring width chronologies were constructed by averaging the width sequences from each tree. To provide a

measure of the changes in surface area of each increment around the entire trunk, these chronologies were converted to tree basal area increment, BAI:

$$\text{BAI}_n = \pi r_n^2 - \pi r_{n+1}^2 \quad (2)$$

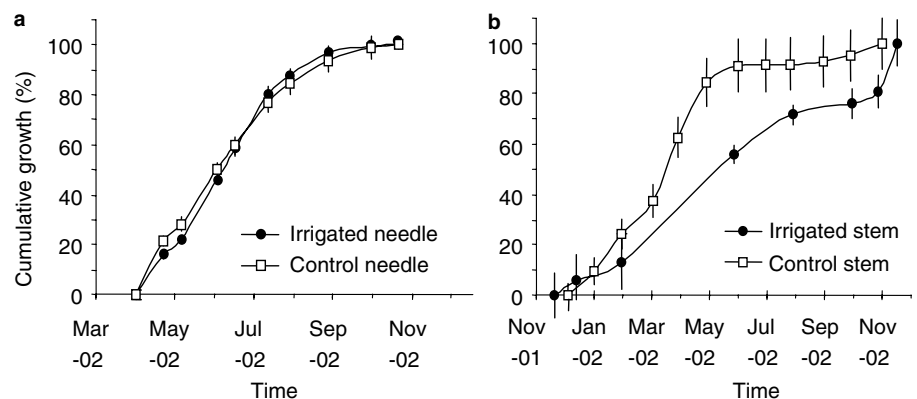
where, n is the number of tree rings (increasing towards the tree centre) from the outermost whole ring (where $n=1$), r_n is the radius at breast height (1.3 m) minus the radial increment contributed by bark and the current year partial ring formation at $n=1$, and r_{n+1} is the radius at increment $n+1$, calculated by subtracting the width of the n increment from r_n . The average BAI for the irrigated and control plots were determined separately by averaging all tree BAI chronologies per plot.

Tree-ring and needle $\delta^{13}\text{C}$

The whole-wood sample materials of 0.1–0.4 mg, taken from ten equal inter-annual sub-sections from the same tree-ring or needle, were weighed into tin foil capsules (Elemental Microanalysis Ltd. 3×5 mm #D1002). For particularly narrow tree-rings it was only possible to slice seven equal sub-sections. As formation of the tree tissue is not equally distributed along the year, these sub-sections were further subjected to careful dating, according to the specific growth curve (see below). Sample $\delta^{13}\text{C}$ values were determined with an elemental analyzer (Carlo Erba 1108) linked to a continuous flow isotope ratio mass spectrometer (Optima, Micromass, UK).

To minimize the number of analyses, but retain representative $\delta^{13}\text{C}$ variations among trees, a test was conducted on intra-tree needle $\delta^{13}\text{C}$ values using 2001's needles from north- and south-sides of a control and an irrigated tree. From these data, it was evident that intra-tree $\delta^{13}\text{C}_n$ variations were negligible relative to seasonal fluctuations ($P=0.87$ for control tree-rings; $P=0.68$ for irrigated tree-rings; data not shown). Therefore, further $\delta^{13}\text{C}$ analyses were made on a single needle and tree-core from the south-side of six trees: three irrigated and three controls. The $\delta^{13}\text{C}$ results are expressed in delta notation

Fig. 1 **a** Cumulative growth percent of irrigated and control ($n=8$) needles. Error bars indicate the standard error of the mean. **b** Cumulative growth percent of irrigated ($n=4$) and control ($n=8$) stems, where 100% is the complete annual growth of irrigated or control stems, respectively ($\sim 1,000 \text{ mm}^2$ or $\sim 4,000 \text{ mm}^2$, see Figure, year 2002); Slopes represent growth rate by percent. Error bars indicate the standard error of the mean



as parts per thousand (‰) deviations from the VPDB international carbon isotope standard (Coplen 1994).

Growth rates and dating of tree-ring and needle sub-sections

Tree-trunk and needle growth measurements were made on trees in the irrigated and control plots (ten trees in each). Dendrobands were installed on all study trees, and both stem and needle (measured on marked branches) increments were measured fortnightly. The control trees were a part of a larger-scale, long-term monitoring program of growth pattern analysis (Lin et al., unpublished results). To compare $\delta^{13}\text{C}_t$ and $\delta^{13}\text{C}_n$ results with gas-exchange and meteorological data of particular dates, the period of formation of each sub-section was estimated. For this purpose, empirical needle and tree-ring growth curves were constructed by plotting the cumulative ring width or needle length taken from the individual dendroband and needle-length observations (in % of whole ring width or needle length) as a function of the date of the individual observations. Points along these curves (y -axis) were then applied to the observed growth curves reported in Fig. 1 to extract the estimated date of formation (x -axis). Date estimates were verified by comparison with dendroband and needle length measurements made concurrently on trees growing at the flux tower plot, and with reference to total tree-ring widths. Each date represents the central date of each sub-section formation period.

Results

Tree-ring and needle growth

The well-calibrated growth rate analyses of the Aleppo pine growth at this site highlighted an unusual feature; the relative timings of BAI and needle extension were offset into two distinct periods. Trunk-growth rate maxima occurred in winter, November–April, whereas, needles emerged in April and extended throughout spring and summer (Fig. 1). The period of needle growth was highly conserved regardless of environmental conditions (e.g. irrigation), and total needle extension (e.g.

short and long needles grew within the same seasonal window; Fig. 1a). In contrast, stem growth for irrigated trees occurred between October and August, which was ~ 4 months longer than that of non-irrigated trees (Fig. 1b).

Annual BAI was not significantly different between trees in the control and irrigated plots during non-irrigation years. However, with supplemental summer irrigation, the average BAI and needle extension were, on average, significantly larger than those of the control trees, 325% and 13.5% respectively, and the variance between trees was higher (Fig. 2, Table 1). In 2001, when irrigation commenced in the middle of the stem growth season, average BAI was 250% higher in irrigated trees, and this increased to 400% in 2002, under full-season irrigation. Needles of irrigated trees were also significantly longer, by 27%, during the first year of irrigation, but not significantly different in 2002. However, in 2003, when no irrigation was applied to the irrigated plot trees, their needle length was significantly lower than those of control plot trees.

Tree-ring and needle $\delta^{13}\text{C}$

The values of $\delta^{13}\text{C}_n$ were typically lower, by $\sim 2\text{‰}$, than $\delta^{13}\text{C}_t$ values during the same growth period, regardless of the irrigation conditions (Fig. 3, Table 2, compare upper row to third below, and fifth row to seventh). Like the BAI results, annual average $\delta^{13}\text{C}_t$ and $\delta^{13}\text{C}_n$ were not significantly different between the irrigated and control plot trees during the non-irrigation years (average difference = 0.5‰ , $P > 0.05$). However, under irrigated conditions in 2001 and 2002, $\delta^{13}\text{C}_t$ and $\delta^{13}\text{C}_n$ values were significantly lower, by an average of 1.5‰ and 2.5‰ respectively, than those of control plot trees (Table 2, under ‘2001’ and ‘2002’ columns, compare upper row to fifth below, and third row to seventh).

Clear seasonal cycles existed in tree-ring sub-section $\delta^{13}\text{C}_t$ values of the irrigated and control trees (Fig. 3a). The lowest $\delta^{13}\text{C}_t$ values occurred during the relatively humid and cool winters (November–April), and the highest during the dry, hot summers (May–October). In accordance with these trends, $\delta^{13}\text{C}_n$ also increased progressively throughout the needle extension period, predominantly during spring-time (Fig. 3b).

Fig. 2 **a** Mean BAI and needle length of 2000–2003. Each point represents the mean value of ten trees, except for needles of 2000, where $n = 3$. Error bars indicate the standard error of the means. **b** A binocular microscope image of two cores taken at 180° from control tree (*lower*) and a tree from irrigation experiment (*upper*) ($\times 10$)

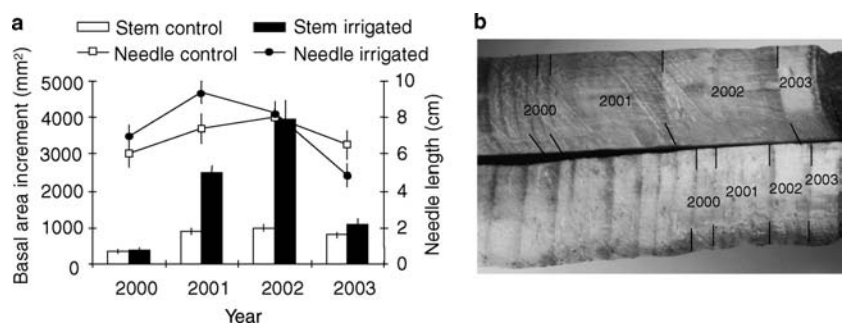


Table 1 Probability (P values) of significant differences between irrigated and control trees, calculated by a two-tailed Student's t test

	2000	2001	2002	2003
BAI	0.88	< 0.001	< 0.001	0.16
Needle	0.42	< 0.001	0.72	0.018
Stem $\delta^{13}\text{C}$	0.23	0.096	< 0.001	0.14
Needle $\delta^{13}\text{C}$		< 0.001	< 0.001	0.34

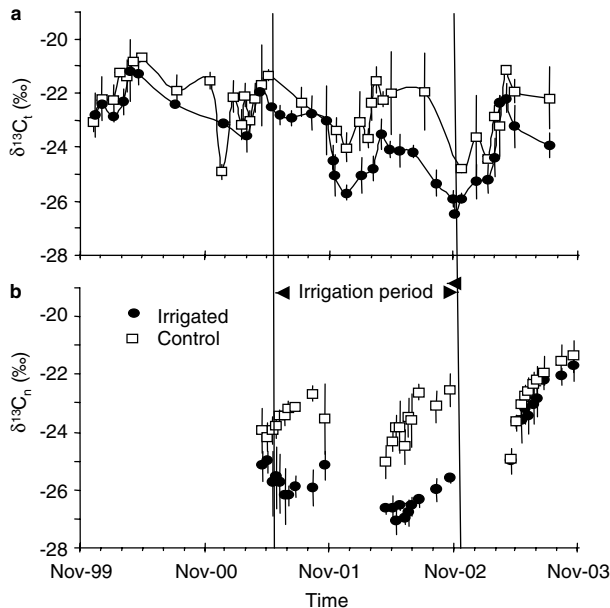


Fig. 3 **a** Mean $\delta^{13}\text{C}$ values of stems for 2000–2003. Irrigation period is pointed out as space between two vertical lines. Error bars indicate the standard error of the mean ($n=1-3$). **b** Mean $\delta^{13}\text{C}$ values of needles for 2001–2003. Irrigation period is pointed out as space between two vertical lines. Error bars indicate the standard error of the mean ($n=1-3$)

The $\delta^{13}\text{C}_t$ values annually decreased from 2000 to 2003, although, notably high values were again observed during the spring of 2003 (Fig. 3a). This general trend

was also apparent in $\delta^{13}\text{C}_n$ values (Fig. 3b), and it correlates with the increase in growth of the control trees from 2000 to 2003 (Fig. 2). Such high inter-annual variance is common in trees growing in semi-arid conditions, and it matches with general increase in precipitation; annual rainfall at Yatir forest was 156, 279, and 313 mm in 2000, 2001, and 2002, respectively. Nevertheless, during the rainiest year (2003; 365 mm), $\delta^{13}\text{C}$ values of control trees were higher, and their cumulative growth was lower than the previous 2 years. This apparent exception is explained by the special precipitation regime in the winter of 2003 when the rains were scarce during the peak cambial activity period (as reflected by the soil water content in Fig. 4b), and hence growth slowed down and $\delta^{13}\text{C}$ increased.

The values of both $\delta^{13}\text{C}_t$ and $\delta^{13}\text{C}_n$ decreased by $\sim 0.8\text{‰}$, from -22.0‰ to -22.8‰ and -25.0‰ to -25.8‰ respectively, in the first sub-section following the start of irrigation (Fig. 3, compare “irrigated” data points before and after left vertical line). After cessation of irrigation, in 2003, irrigated plot tree-rings maintained generally (but not significantly) lower $\delta^{13}\text{C}$ values, $\sim 1\text{‰}$ lower than control tree-rings. A similar non-significant offset was evident in $\delta^{13}\text{C}_n$ values for 2003, which were otherwise closely comparable between the plots.

Relationships between environmental variables and tree-ring and needle $\delta^{13}\text{C}$

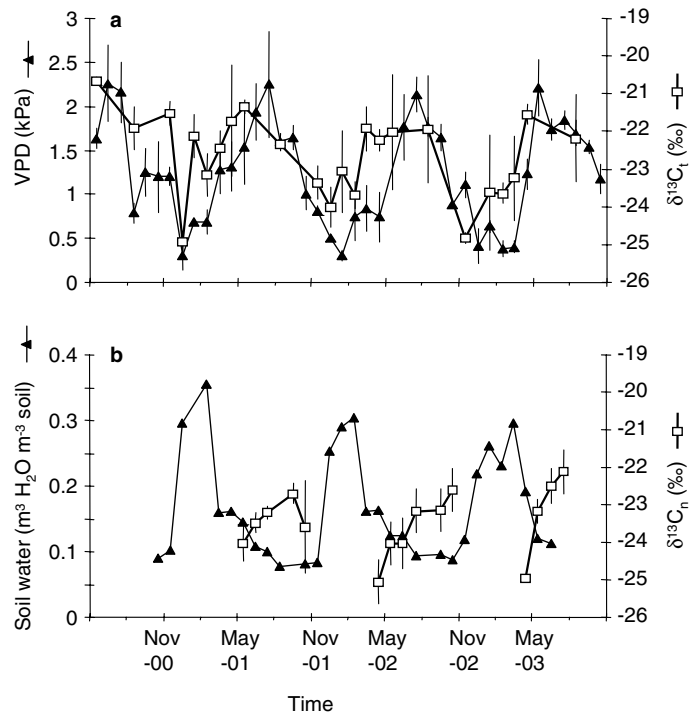
The $\delta^{13}\text{C}_n$ and $\delta^{13}\text{C}_t$ time series were correlated with various environmental parameters measured continuously at the nearby flux tower site: including precipitation (P), air temperature (air T), relative humidity (RH), vapor pressure deficit (VPD), photosynthetically active radiation (PAR), soil temperature (soil T) and tower plot soil water content (SWC). Parameters were averaged for 10 days or 1 month prior to the ‘central date’ of a tree-ring or needle sub-section in order to capture the dominant period influencing the sub-section formation. Pearson correlation coefficients (r) and their significance values (P) were calculated for control trees from May 2000 to October 2003, and for irrigated trees during the

Table 2 Mean $\delta^{13}\text{C}$ of tree-ring and needle sub-sections, $\delta^{13}\text{C}$ ($n=7-10$), and weighted average $\delta^{13}\text{C}$ derived from gas exchange measurements, $\delta^{13}\text{C}_{\text{gx}}$ ($n=14-17$) for each growth year

			2000	2001	2002	2003
Control	Stem	$\delta^{13}\text{C}_t$	-21.7	-22.4	-22.7	-23.0
		$\delta^{13}\text{C}_{\text{gx}}$		-22.5	-24.7	
	Needle	$\delta^{13}\text{C}_n$		-23.6	-23.7	-22.7
		$\delta^{13}\text{C}_{\text{gx}}$		-21.8	-23.7	
Irrigated	Stem	$\delta^{13}\text{C}_t$	-22.2	-23.2	-24.9	-24.0
		$\delta^{13}\text{C}_{\text{gx}}$		-25.4	-26.9	
	Needle	$\delta^{13}\text{C}_n$		-25.7	-26.5	-23.1
		$\delta^{13}\text{C}_{\text{gx}}$		-24.7	-26.6	

Individual $\delta^{13}\text{C}_{\text{gx}}$ values were weighted by the net photosynthesis during each gas exchange measurement according to the total net photosynthesis during the period of stem or needle growth

Fig. 4 a Monthly mean $\delta^{13}\text{C}$ values of control stems and monthly mean VPD values. *Error bars* indicate the standard error of the means ($n=3-6$ for $\delta^{13}\text{C}$ and $n=3$ for VPD). **b** Monthly mean $\delta^{13}\text{C}$ values of control needles and monthly measured soil water content values. *Error bars* indicate the standard error of the mean ($n=3-9$ for $\delta^{13}\text{C}$)



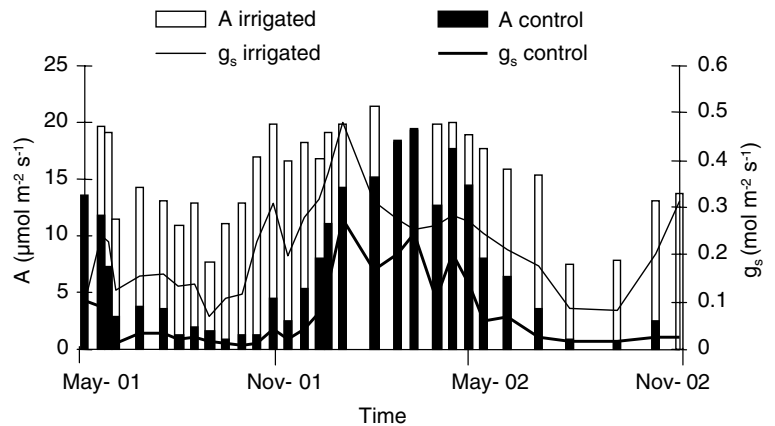
irrigation period, 15 May 2001 to 15 November 2002. The most significant correlations were observed between environmental variables and control plot $\delta^{13}\text{C}_n$ and $\delta^{13}\text{C}_t$. For stems, strong positive correlations were noted between control $\delta^{13}\text{C}_t$ and PAR (0.68, $P < 0.01$), VPD (0.59, $P < 0.01$) (Fig. 4a) and air T (0.48, $P < 0.01$), and a negative correlation with 10–20 cm SWC (-0.48 , $P < 0.01$). $\delta^{13}\text{C}_n$ of the control trees was significantly correlated with 10–20 cm SWC (-0.79 , $P < 0.01$) (Fig. 4b) and air T (0.51, $P < 0.01$). For trees in the irrigated plot, however, $\delta^{13}\text{C}_t$ and $\delta^{13}\text{C}_n$ significantly correlated only with PAR (0.58, $0.01 < P < 0.05$ and -0.55 , $0.01 < P < 0.05$, respectively). Insignificant correlations were observed between irrigated trees and other environmental parameters, such as VPD, air T and SWC.

Leaf gas exchange

Throughout the irrigation period, average A and g_s values of irrigated trees were 220% and 290% more ($A = 15.5 \mu\text{mol m}^{-2} \text{s}^{-1}$, $g_s = 0.22 \text{ mol m}^{-2} \text{s}^{-1}$) than those measured on control trees ($A = 7.1 \mu\text{mol m}^{-2} \text{s}^{-1}$, $g_s = 0.08 \text{ mol m}^{-2} \text{s}^{-1}$). Higher rates of A and g_s for irrigated trees occurred during the entire seasonal cycle, although differences between irrigated and control trees were larger during the summer, and the high values for irrigated trees were prolonged into the spring and summer (Fig. 5). Both A and g_s showed clear seasonal cycles, with maxima during winter, and wide, relatively stable, minima during summer.

During both irrigation years, the average $\delta^{13}\text{C}_{\text{gx}}$ for the periods of tree-ring and needle formation, weighted

Fig. 5 Assimilation rates and stomatal conductance in needles of control and irrigated trees. Measurements were taken at late morning (around 11 a.m.), and at about ~bi-weekly intervals, during the irrigation period



by A (see **Materials and methods**), were significantly lower, by $\sim 2.8\text{‰}$, in irrigated trees than in controls (Table 2). Also, average $\delta^{13}\text{C}_{\text{gx}}$ values were lower, by $\sim 1.0\text{‰}$, in both irrigated and control trees during the period of tree-ring formation, compared to corresponding values during needle formation. Between 2001 and 2002 the average $\delta^{13}\text{C}_{\text{gx}}$ values of all trees decreased by $\sim 2.0\text{‰}$. Despite considerable variability between measurements, a general seasonal cycle, of lower $\delta^{13}\text{C}_{\text{gx}}$ during winter and higher in the summer, was observed for all trees, although, very low $\delta^{13}\text{C}_{\text{gx}}$ values (-28.0‰ and -29.8‰) were also produced on 14 July 2002 and 30 August 2002 only in control trees.

Comparison of $\delta^{13}\text{C}$ values measured and derived from gas exchange

Average $\delta^{13}\text{C}_{\text{gx}}$ values of control and irrigated trees and their seasonal trends were generally comparable with the measured $\delta^{13}\text{C}_{\text{C}_i}$ and $\delta^{13}\text{C}_t$ values (Table 2, Fig. 3). The most significant differences, of 2.0‰ , were observed between average $\delta^{13}\text{C}_{\text{gx}}$ and $\delta^{13}\text{C}_t$ in 2002, and the most similar values were noted between $\delta^{13}\text{C}_{\text{gx}}$ and $\delta^{13}\text{C}_n$ of the same year. Even when average $\delta^{13}\text{C}_{\text{gx}}$ differed significantly from the corresponding $\delta^{13}\text{C}_n$ and $\delta^{13}\text{C}_t$ values, offsets between control and irrigated trees were still similar, especially in 2002.

A major difference between the $\delta^{13}\text{C}_{\text{gx}}$ values and $\delta^{13}\text{C}_n$ and $\delta^{13}\text{C}_t$ was the significantly larger standard deviation (SD) of $\delta^{13}\text{C}_{\text{gx}}$. Irrigated and control tree $\delta^{13}\text{C}_t$ had SD's of 1.3 and 1.1, respectively, and for $\delta^{13}\text{C}_n$ of irrigated and control trees SD were 0.4 and 0.8, respectively, compared with corresponding SD's of 2.0 and 2.5 for the irrigated and control $\delta^{13}\text{C}_{\text{gx}}$ time series.

Notably, throughout the season $\delta^{13}\text{C}_n$ and $\delta^{13}\text{C}_t$ series followed the same general trends as $\delta^{13}\text{C}_{\text{gx}}$, although, between June and October 2002, $\delta^{13}\text{C}_{\text{gx}}$ values, from the control trees only, were considerably lower, by 5–9‰, than either $\delta^{13}\text{C}_n$ or $\delta^{13}\text{C}_t$.

Discussion

The significant relationships in the timing and magnitude of $\delta^{13}\text{C}_{\text{gx}}$ variations compared with $\delta^{13}\text{C}_n$ and $\delta^{13}\text{C}_t$ values suggest that there is a close link between average leaf-level CO_2 exchange and carbon deposition in the leaf and stem over seasonal and inter-annual time scales. This experimental verification under field conditions is of significance since it provides evidence for widely accepted, but less examined, assumptions based on variations in tree-rings $\delta^{13}\text{C}$, and, notably, extends the $\delta^{13}\text{C}$ fractionation model to seasonal time scales. It is clear, from the high variability of $\delta^{13}\text{C}_{\text{gx}}$ compared with the relatively conservative range in $\delta^{13}\text{C}_n$ and $\delta^{13}\text{C}_t$ values, that gas-exchange measurements provide snapshots of information that are much more sensitive to short-term variations in conditions, and these can be very different

from the integrated signals provided by structural material. This is particularly highlighted in the results of control plot trees between 18 June and 14 July 2001 (Figs. 6a, 7a), when $\delta^{13}\text{C}_{\text{gx}}$ decreased from -19.8‰ to -28.1‰ , but no such variations were observed in the $\delta^{13}\text{C}_n$ and $\delta^{13}\text{C}_t$ values, probably because low assimilation rates during this period meant that little, if any, structural material was formed. Provided that recently assimilated photosynthates are utilised for needle and stem growth, differences between $\delta^{13}\text{C}_{\text{gx}}$ and $\delta^{13}\text{C}_n$ or $\delta^{13}\text{C}_t$ should be reduced by weighting individual $\delta^{13}\text{C}_{\text{gx}}$ values by the corresponding A values. Indeed, weighted average $\delta^{13}\text{C}_{\text{gx}}$ values, and their differences between years and irrigation conditions, compared reasonably well with corresponding average $\delta^{13}\text{C}_n$ and $\delta^{13}\text{C}_t$ values (Table 2). In accordance with A , the $\delta^{13}\text{C}$ signal of tree-rings was sensitive to time and rate of deposition. In 2003, $\delta^{13}\text{C}$ values of control trees were clearly higher, and their cumulative growth was lower than the previous 2 years, which was in contrast to the expected influence of increased precipitation during that year. We could explain this only by the unusual precipitation patterns in 2003, which showed markedly lower precipitation during the peak cambial activity period (as reflected by the soil water content in Fig. 4).

In most cases, $\delta^{13}\text{C}_n$ and $\delta^{13}\text{C}_t$ variations occurred shortly (in the first or second sampling) after a significant environmental change, such as initiation or cessation of irrigation, and the magnitude of the variations

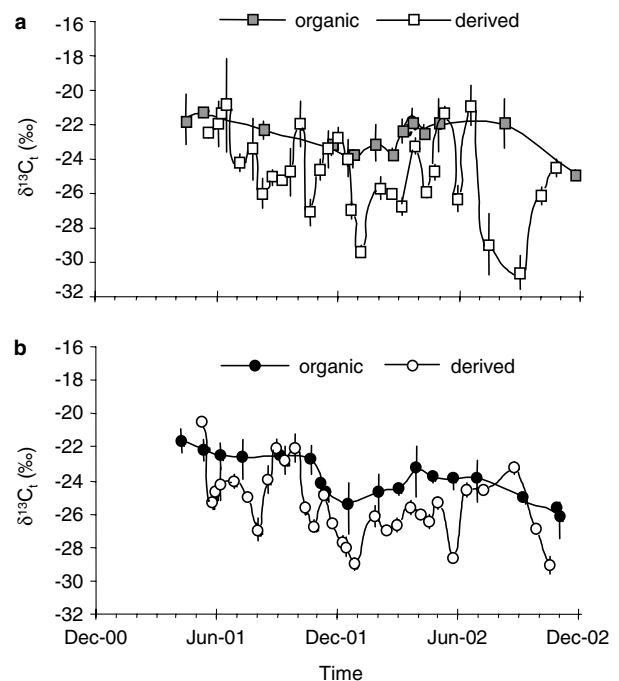


Fig. 6 Mean $\delta^{13}\text{C}$ values measured and calculated from gas exchange measurements of C_i and using Eq. 1, for control (a) and irrigated (b) stems. These values were not weighted by the net photosynthesis fraction, unlike in Table 2. Error bars indicate the standard error of the means ($n=1-3$)

were similar to $\delta^{13}\text{C}_{\text{gx}}$ variations. Notably, this provides evidence for a relatively small contribution or effect of stored carbohydrates on the $\delta^{13}\text{C}_n$ and $\delta^{13}\text{C}_t$ values. This is significant, particularly in the light of unusual phenological timings of leaf extension, during the stressful summer, and the very low corresponding assimilation rates. Yet, the results do not provide a clear picture of storage utilisation in these trees. Some clues indicate that storage might be used to buffer fluctuations in carbon supply to growing tissue, both on seasonal and inter-annual time-scales. Such speculations are consistent with (1) significantly lower $\delta^{13}\text{C}_t$ values observed in irrigated plot trees as compared to control plot trees, during the summer of 2003, 10 months after irrigation was stopped, and (2) very stable $\delta^{13}\text{C}_n$ values compared with corresponding highly variable $\delta^{13}\text{C}_{\text{gx}}$ values and extremely low A rates during the same periods.

Gas exchange results supported predictions derived using Eq. 1 that higher g_s was usually translated, via higher C_i concentrations, into lower $\delta^{13}\text{C}_{\text{gx}}$ values, and vice versa. These results also support the needle growth and BAI data, by confirming that irrigated trees experienced considerably higher rates of A and g_s during the whole year, and particularly in the summer, compared with non-irrigated trees. The rapid response of leaf and stem growth, and $\delta^{13}\text{C}$ to irrigation, and the very low levels of A and g_s in control trees during the summer,

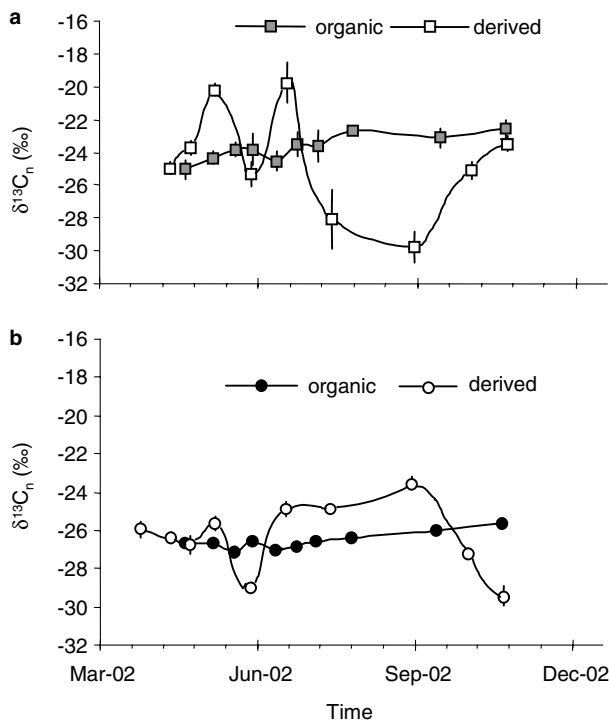


Fig. 7 Mean $\delta^{13}\text{C}$ values measured and calculated from gas exchange measurements of C_i and using eq. 1 for control (a) and irrigated (b) needles during needle growth period of 2002. These values were not weighted by the net photosynthesis fraction, unlike in Table 2. Error bars indicate the standard error of the means ($n = 1-3$)

backs up other results which indicated that Aleppo pine is extremely adaptive to environmental variations (Liphschitz and Lev-Yadun 1986; Schiller 2000), and is a drought-avoiding species (Ferrio et al. 2003). Further, it indicates that needle growth during the summer is predominantly the product of concurrent assimilation rather than utilisation of storage carbohydrates. This notion has recently got additional evidence from measurements on respired CO_2 in a Mediterranean beech forest (Scartazza et al. 2004). Interestingly, in spite of higher soil moisture, the peak in leaf gas-exchange activity remained during winter, indicating that factors other than soil water supply limited A and g_s in the summer. Some of the likely reasons for this are; (1) Low hydraulic conductivity of pine trees coupled with extremely high VPD during the summer at this location (up to 6.5 kPa on some days; see Fig. 4a for monthly means), resulting in stomatal closure, (2) Other acclimatizations to the harsh summer conditions occurred, such as decreases in the pigment content and increases in leaf reflectance (Lin et al., in preparation).

The results presented here, demonstrate the expected significant influence of water supply on tree growth and leaf gas exchange of Aleppo pine growing in the naturally semi-arid climatic regime at Yatir forest. The higher soil moistures provided by irrigation enabled trees to assimilate more carbon, and extend the dominant period of A and trunk growth well into the dry summer. Such results also indicate that water is, at least in the short term, the major limiting factor for productivity in this ecosystem, and not e.g. nutrients. Division of tree-rings into early and late wood is equally evident in both treatments (Fig. 1b), though annual tree-rings of irrigated trees were up to 400% wider.

Although total needle length varied according to environmental conditions, it was interesting to note that even major changes in soil moisture did not influence the timing of needle extension, which appears to be genetically predetermined, commencing near 1 April and finishing around 15 August. This is significant, as it encompasses the driest and the most stressful period of the year, and it was not clear whether this growth relied on concurrent photosynthetic activity or on mobilisation of stored photosynthates. Either way, it provides the trees a clear advantage for rapidly reaching maximal activity during the following wet period. We measured similar needle lengths for both control and irrigation treatments in 2002, and even shorter needles on post-irrigated trees as compared to control trees, in 2003. Interestingly, these needle extension measurements were from needles of all years collected at one time at the end of the study, however, other growth measurements made during the irrigated years, in 2001–2002, showed 33% longer needles for irrigated trees as compared to controls. It is likely that the longer needles of 2002 were shed after irrigation was stopped (as a response to the immediate stress situation), possibly resulting in a bias towards shorter needles in the final estimates. This explanation is consistent with the observation of relatively short needles on the

trees in 2003, and with the notion of total leaf area regulation through needle width and density, branching rate, etc. (Melzack et al. 1985; Noormets et al. 2001).

Conclusions

We presented results from a 4-year study that compared inter- and intra-annual variations in leaf gas-exchange properties, growth, and time-resolved $\delta^{13}\text{C}$ signals along needles and across tree-rings. The trees studied were Aleppo pine growing in a semi-arid region under natural conditions or with supplemental summer irrigation. The results provide experimental verification under field conditions for the association between leaf gas-exchange and leaf and tree-ring $\delta^{13}\text{C}$. They also broaden this association to seasonal and inter-annual time scales. Specifically, we show:

1. A close association between average, assimilation-weighted $\delta^{13}\text{C}_{\text{gx}}$ and $\delta^{13}\text{C}_{\text{n}}$ and $\delta^{13}\text{C}_{\text{t}}$
2. Indications that needle growth during the stressful dry season was mainly supported by concurrently assimilated carbon
3. Stored photosynthates did not significantly affect the dynamic and robust links between $\delta^{13}\text{C}_{\text{gx}}$ and $\delta^{13}\text{C}_{\text{n}}$ and $\delta^{13}\text{C}_{\text{t}}$ values under the conditions of this study
4. Large enhancements in growth rate with supplemental irrigation, and an associated reduction in sensitivity of the $\delta^{13}\text{C}$ signal to environmental conditions under irrigation
5. Tree-ring and needle $\delta^{13}\text{C}$ measurements were sensitive proxies of integrated gas-exchanges, indicating the significance of plant material from semi-arid conditions as a sensitive indicator of environmental fluctuations.

Acknowledgements We thank Emanuela Negreanu for technical expertise and help with the $\delta^{13}\text{C}$ analyses. Funding of this project was provided by the Israel Science Foundation (ISF), the European Union (Carboeuroflux), the US-Israel Binational Science Foundation (BSF) and the International Arid Land Consortium (IALC). DH and DY acknowledge the financial support provided through the European Community's Human Potential Programme under contract HPRN-CT-1999-00059, NETCARB. (Declaration: all experiments in this research comply with the current law of Israel.)

References

- Coplen TB (1994) Reporting of stable hydrogen, carbon, and oxygen isotopic abundances. *Pure Appl Chem* 66:273–276
- Dawson TE, Ward JK, Ehleringer JR (2004) Temporal scaling of physiological responses from gas exchange to tree rings: a gender-specific study of *Acer negundo* (Boxelder) growing under different conditions. *Funct Ecol* 18:212–222
- Ehleringer JR, Hall AE, Farquhar GD (1993) Stable isotopes and plant carbon-water relations. Academic, San Diego
- Evans JR, Sharkey TD, Berry JA, Farquhar GD (1986) Carbon isotope discrimination measured concurrently with gas exchange to investigate CO_2 diffusion in leaves of higher plants. *Aust J Plant Phys* 13:281–292
- Farquhar GD, O'Leary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and intercellular carbon dioxide concentration in leaves. *Aust J Plant Phys* 9:121–137
- Feng X, Epstein S (1995) Carbon isotopes of trees from arid environments and implications for reconstructing atmospheric CO_2 concentration. *Geochim Cosmochim Acta* 59:2599–2608
- Ferrio JP, Florit A, Vega A, Serrano L, Voltas J (2003) $\Delta^{13}\text{C}$ and tree-ring width reflect different drought responses in *Quercus ilex* and *Pinus halepensis*. *Oecologia* 144:512–518
- Fritts HC (1976) Tree rings and climate. Academic, London
- Gleixner G, Danier HJ, Werner RA, Schmidt HL (1993) Correlations between the ^{13}C content of primary and secondary plant products in different cell compartments and that in decomposing basidiomycetes. *Plant Physiol* 102:1287–1290
- Grunwald C, Schiller G (1988). Needle xylem water potential and water saturation deficit in provenances of *Pinus halepensis* Mill. and *P. brutia* Ten. *Forêt méditerranéenne* 10:407–414
- Grünzweig JM, Lin T, Rotenberg E, Schwartz A, Yakir D (2003) Carbon sequestration in arid-land forest. *Global Change Biol* 9:791–799
- Hemming D, Fritts HC, Leavitt SW, Wright W, Long A, Shashkin A (2001) Modelling tree-ring $\delta^{13}\text{C}$. *Dendrochronologia* 19(1):23–38
- IPCC (2001) Climate change: the scientific basis. Cambridge University Press, Cambridge
- Leavitt SW, Long A (1989) The atmospheric $\delta^{13}\text{C}$ record as derived from 56 pinyon trees at 14 sites in the southwestern United States. *Radiocarbon* 31:469–474
- Lev-Yadun S (2000) Cellular patterns in dicotyledonous woods: their regulation. In: Savidge R, Barnett J and Napier R (eds) Cell and molecular biology of wood formation, vol 25. BIOS Scientific Publishers, Oxford, pp 315–324
- Lev-Yadun S, Liphshitz N, Waisel Y (1981) Dendrochronological investigations in Israel: *Pinus halepensis*—the oldest living pines in Israel. *La-Yaaran* 31:1–8, 49–52 (in Hebrew)
- Liphshitz N, Lev-Yadun S (1986) Cambial activity of evergreen and seasonal dimorphics around the Mediterranean. *IAWA Bull* 7(2):145–153
- Loader NJ, Switsur VR, Field EM (1995) High-resolution stable isotope analysis of tree rings: implications of 'microdendroclimatology' for palaeoenvironmental research. *Holocene* 5(4):457–460
- Marshall JD, Monserud RA (1996) Homeostatic gas-exchange parameters inferred from $^{13}\text{C}/^{12}\text{C}$ in tree rings of conifers. *Oecologia* 105:13–21
- Melzack RN, Bravdo B, Riov J (1985) The effect of water stress on photosynthesis and related parameters in *Pinus halepensis*. *Physiol Plant* 64:295–300
- Noormets A, McDonald EP, Dickson RE, Kruger EL, Sober A, Isebrands JG, Karnosky DF (2001) The effect of elevated carbon dioxide and ozone on leaf- and branch-level photosynthesis and potential plant-level carbon gain in aspen. *Trees* 15:262–270
- Ogle N, McCormac FG (1994) High-resolution $\delta^{13}\text{C}$ measurements of oak show a previously unobserved spring depletion. *Geophys Res Lett* 21(22):2373–2375
- Robertson I, Switsur VR, Carter AHC, Barker AC, Waterhouse JS, Briffa KF, Jones PD (1997) Signal strength and climate relationships in $^{13}\text{C}/^{12}\text{C}$ ratios of tree ring cellulose from oak in east England. *J Geophys Res* 102(D16):19507–19516
- Saurer M, Siegenthaler U (1989) $^{13}\text{C}/^{12}\text{C}$ isotope ratios in trees are sensitive to relative humidity. *Dendrochronologia* 7:9–13
- Scartazza A, Mata C, Matteucci G, Yakir D, Moscatello S, Brugnoli E (2004) Comparisons of $\delta^{13}\text{C}$ of photosynthetic products and ecosystem respiratory CO_2 and their responses to seasonal climate variability. *Oecologia* 140:340–351
- Schiller G, Cohen Y (1998) Water balance of a *Pinus halepensis* Mill. afforestation in an arid region. *For Ecol Manag* 105:121–128
- Schleser GH (1985) Parameters determining carbon isotope ratios in plants. In: Frenzel B (ed) Problems of stable isotopes in tree-rings, lake-sediments and peat-bogs as climatic evidence for the Holocene. *Palaeoclim Res* 15:71–96

- Schleser GH, Frielingsdorf J, Blair A (1999) Carbon isotope behaviour in wood and cellulose during artificial aging. *Chem Geol* 158:121–130
- Schweingruber FH (1996) Tree rings and environment dendroecology. Paul Haupt, Bern
- Switsur VR, Waterhouse J (1989) Stable isotopes in tree ring cellulose. In: Griffiths H (ed) *Stable Istopes: Integration of biological, ecological and geochemical processes*, vol 18. BIOS Scientific Publishers, Oxford, pp 303–321
- Tcherkez G, Nogues S, Bleton J, Cornic G, Badeck F, Ghashghaie J (2003) Metabolic origin of carbon isotope composition of leaf dark-respired CO₂ in French bean. *Plant Physiol* 131(1):237–244
- Terwilliger VJ, Huang J (1996) Heterotrophic whole plant tissues show more C-13 enrichment than their carbon sources. *Phytochemistry* 43(6):1183–1188
- Van der Water PK, Leavitt SW, Betancourt JL (1994) Trends in stomatal density and ¹³C/¹²C ratios of *Pinus flexilis* needles during last glacial-interglacial cycle. *Science* 264:239–243