

Variation in the degree of coupling between $\delta^{13}\text{C}$ of phloem sap and ecosystem respiration in two mature *Nothofagus* forests

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Summary

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- Day-to-day variability in the carbon isotope composition of phloem sap ($\delta^{13}\text{C}_{\text{hd}}$) and ecosystem respiratory CO_2 ($\delta^{13}\text{C}_{\text{R}}$) were measured to assess the tightness of coupling between canopy photosynthesis ($\delta^{13}\text{C}_{\text{hd}}$) and ecosystem respiration ($\delta^{13}\text{C}_{\text{R}}$) in two mature *Nothofagus solandri* (Hook. f.) forests in New Zealand.
- Abundant phloem-tapping scale insects allowed repeated, nondestructive access to stem phloem sap 1–2 m above ground. $\delta^{13}\text{C}_{\text{hd}}$ was compared with $\delta^{13}\text{C}$ predicted by an environmentally driven, process-based canopy photosynthesis model. Keeling plots of within-canopy CO_2 were used to estimate $\delta^{13}\text{C}_{\text{R}}$.
- By including a lag of 3 d, there was good agreement in the timing and direction of variation in $\delta^{13}\text{C}_{\text{hd}}$ and predictions by the canopy photosynthesis model, suggesting that $\delta^{13}\text{C}_{\text{hd}}$ represents a photosynthesis-weighted, integrative record of canopy photosynthesis and conductance.
- Significant day-to-day variability in $\delta^{13}\text{C}_{\text{R}}$ was recorded at one of the two forests. At this site, $\delta^{13}\text{C}_{\text{R}}$ reflected variability in $\delta^{13}\text{C}_{\text{hd}}$ only on days with <2 mm rain. We conclude that the degree of coupling between canopy photosynthesis and ecosystem respiration varies between sites, and with environmental conditions at a single site.

Key words: carbon-13 discrimination, ecosystem respiration, Keeling plot, *Nothofagus*, phloem sugar $\delta^{13}\text{C}$.

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Introduction

An understanding of the exchange of energy, carbon and water between the atmosphere and the biosphere is vital for successful predictions of ecosystem response to environmental change. Ecosystem carbon exchange is commonly measured using micrometeorological techniques such as eddy covariance. However, carbon-exchange measurements are unable to separate photosynthetic and respiratory fluxes, and cannot distinguish autotrophic from heterotrophic respiration. The stable isotope composition of CO_2 is of particular interest, as it provides information about the components of gross carbon

exchange (Yakir & Sternberg, 2000). Further, models using mass balance of stable isotopes of carbon and oxygen are an important tool in constraining the global carbon budget, and determining the size and location of terrestrial carbon sources and sinks (Cias *et al.*, 1995; Battle *et al.*, 2000; Canadell *et al.*, 2000; Randerson *et al.*, 2002).

Carbon isotope discrimination during C_3 photosynthesis is reflected in the isotopic composition of plant organic material ($\delta^{13}\text{C}_{\text{p}}$, expressed relative to the Vienna Pee Dee Belemnite standard, VPDB), and provides an integrative record of CO_2 supply relative to demand. When this plant material is later respired, the $\delta^{13}\text{C}$ of CO_2 released is expected to reflect the

isotope composition resulting from photosynthetic discrimination, at least to some extent (Duranceau *et al.*, 1999; Xu *et al.*, 2004). While the processes regulating $\delta^{13}\text{C}_p$ are generally well understood at the leaf level (Farquhar *et al.*, 1989), and scaling procedures allow leaf-level processes to be extrapolated to canopy level (Hanson *et al.*, 2004), knowledge of the processes determining the carbon isotope composition of ecosystem-respired CO_2 ($\delta^{13}\text{C}_R$) is lacking.

By invoking conservation of mass, it follows that the isotope composition of carbon respired by an ecosystem should reflect the isotope composition of carbon fixed by photosynthesis (Pataki *et al.*, 2003) over the entire life of the ecosystem. $\delta^{13}\text{C}_R$ has been shown to vary consistently with changes in $\delta^{13}\text{C}_p$ between sites with trees of differing age (Fessenden & Ehleringer, 2002). Variation in $\delta^{13}\text{C}_R$ has also been found to reflect environmental variability, such as changes in air saturation deficit (D) during a growing season, at single sites (Ekblad & Högberg, 2001; Bowling *et al.*, 2002). The relationship between D and $\delta^{13}\text{C}_R$ is suggested to be driven by the influence of D on stomatal conductance, and hence on $\delta^{13}\text{C}$ of recently fixed carbohydrate (McDowell *et al.*, 2004b), coupled with the observation that current photosynthesis strongly drives ecosystem respiration (Högberg *et al.*, 2001). Recent work (Scartazza *et al.*, 2004) demonstrates a strong link between the carbon isotope composition of photosynthetic products and $\delta^{13}\text{C}_R$ during a growth season in a northern hemisphere beech forest. In contrast, other studies have shown that $\delta^{13}\text{C}_R$ varies over only a small range during a season, suggesting that environmentally driven variation in $\delta^{13}\text{C}_p$ is not reflected in $\delta^{13}\text{C}_R$ (Flanagan *et al.*, 1996; Buchmann *et al.*, 1998). Decoupling of $\delta^{13}\text{C}_R$ from $\delta^{13}\text{C}_p$ can also occur over daily timescales. For example, when $\delta^{13}\text{C}_R$ was measured on consecutive nights over 2 wk, McDowell *et al.* (2004a) found little variability in $\delta^{13}\text{C}_R$, despite large changes in D which caused a wide range in canopy conductance (g_c).

The decoupling of $\delta^{13}\text{C}_R$ from $\delta^{13}\text{C}_p$ may be caused by variable time lags between carbon fixation and respiration by different carbon pools (Bowling *et al.*, 2002). McDowell *et al.* (2004a) list a number of factors that could contribute to variability in the time lag between fixation and respiration, including: distance from source leaves to the respiring tissue, phloem temperature, source and sink strengths, size of ecosystem carbon pools, soil microbial turnover rates, fungal transport rates, nutrient availability, and soil moisture and temperature effects on soil microbial respiration rates. CO_2 respired by an ecosystem is derived from a number of sources. If stem and branch respiration is assumed to be negligible, the dominant sources are leaves, roots, litter decomposition and soil microbial respiration. Respiration by microbes in the rhizosphere is assumed to be dependent on plant-derived carbon, so may be isotopically indistinguishable from root respiration (Formanek & Ambus, 2004). The time taken from carbon fixation to CO_2 release during respiration is expected to vary considerably between leaves (days) and soil (months to

years). Ecosystems in which heterotrophic respiration forms a large proportion of total ecosystem respiration may be expected to display loose coupling between $\delta^{13}\text{C}_p$ and $\delta^{13}\text{C}_R$ if heterotrophs do not use recently fixed carbon as a substrate.

It is also possible that decoupling of $\delta^{13}\text{C}_R$ from $\delta^{13}\text{C}_p$ results from isotopic fractionation between carbon fixation and respiration. CO_2 respired by isolated protoplasts showed no shift in isotopic composition compared with source carbon (Lin & Ehleringer, 1997), but CO_2 respired by whole leaves has been found to be consistently less depleted than that of carbon sources (Duranceau *et al.*, 1999; Ghashghaie *et al.*, 2001; Xu *et al.*, 2004). Further, isotopic fractionation has been demonstrated during litter decomposition (Fernandez *et al.*, 2003) and during uptake of carbon by fungi, resulting in CO_2 released during fungal respiration being 1–8‰ more enriched than the carbon supplied to the fungi (Henn & Chapela, 2001).

The low variability in $\delta^{13}\text{C}_R$ observed by McDowell *et al.* (2004a) may result simply from a lack of variation in $\delta^{13}\text{C}_p$ (not measured in their experiment). Applying mechanistic models (Farquhar *et al.*, 1989), variation in g_c may not have resulted in variation in $\delta^{13}\text{C}_p$ if this was accompanied by changes in canopy photosynthetic rate (A_{can}) that maintained a constant ratio of leaf intercellular to ambient CO_2 concentration (c_i/c_a). Simultaneous measurement of $\delta^{13}\text{C}$ of recently fixed carbon and $\delta^{13}\text{C}_R$ has allowed this possibility to be tested. Scartazza *et al.* (2004) observed extremely tight coupling between $\delta^{13}\text{C}$ of phloem sap and $\delta^{13}\text{C}_R$ over a seasonal timescale in an Italian beech forest. The carbon isotope composition of carbohydrate is known to reflect c_i/c_a (Brugnoli *et al.*, 1988, 1998); and more recently, variability in the stable isotope composition of phloem sap bled from mature trees has been shown to reflect relative rates of canopy photosynthesis and conductance (Pate & Arthur, 1998; Keitel *et al.*, 2003; Cernusak *et al.*, 2005).

Current understanding suggests that photosynthetic discrimination varies considerably from day to day as environmental conditions vary. Insofar as plant respiration uses recently fixed carbon as a substrate, $\delta^{13}\text{C}$ of CO_2 respired by plants is expected to be quite dynamic at timescales of a few weeks, although root respiration may be out of phase with leaf respiration because of the time taken to transport photosynthate from leaves to roots. $\delta^{13}\text{C}$ of litter- and soil-respired CO_2 may be less variable than plant-respired CO_2 over a few weeks if soil carbon substrates remain constant. The contribution of respiration by each component to total ecosystem respiration is likely to vary with environmental conditions (e.g. temperature and water availability, for which there is some evidence: McDowell *et al.*, 2004b), suggesting that the tightness of coupling between canopy photosynthesis and ecosystem respiration may also vary in time.

In this paper we investigate coupling between canopy photosynthesis and ecosystem respiration in two mature *Nothofagus solandri* (Hook. f.) forests on a daily timescale.

Carbon isotope discrimination during photosynthesis ($\delta^{13}\text{C}_p$) was modelled with an environmentally driven process-based model of canopy photosynthesis. Carbon in phloem sap was sampled for $\delta^{13}\text{C}$ analysis using the honeydew scale insect (*Ultracoelostoma* spp.) common on *Nothofagus* trees in New Zealand and abundant on the sampled trees. The phloem-tapping scale insects allowed repeated, nondestructive access to current photosynthate via excretion of excess carbohydrates at the end of long anal threads (honeydew). By comparing modelled $\delta^{13}\text{C}_p$ with measured $\delta^{13}\text{C}$ in honeydew, we test the hypothesis that day-to-day variation in $\delta^{13}\text{C}_p$ (driven by changes in canopy photosynthesis and conductance) is reflected in $\delta^{13}\text{C}$ of stem phloem sap, and estimate the time taken for carbon fixed in the canopy to move to the sampling point on the stem 1.5 m above the ground. By comparing $\delta^{13}\text{C}$ of honeydew ($\delta^{13}\text{C}_{\text{hd}}$) with $\delta^{13}\text{C}$ of within-canopy CO_2 , we test the hypothesis that $\delta^{13}\text{C}_R$ is related to variation in $\delta^{13}\text{C}$ of recent photosynthate. Finally, results from two forests are compared to test the hypothesis that the degree of coupling between discrimination during carbon fixation and ecosystem respiration varies with environmental conditions (specifically, rainfall before sampling).

Materials and Methods

Description of sites

Samples were collected at two sites in the South Island of New Zealand with contrasting long-term environmental conditions. The first site is at Craigieburn Forest Park (CFP), c. 100 km west of Christchurch at 740 m elevation (43.1° S, 171.4° E). A meteorological station within 6 km of the site recorded average annual rainfall of 1470 mm over the past 40 yr. Mean annual air temperature at the same station is 8.0°C, with an average seasonal range of 11.8°C and an average daily range of 10.7°C. The forest is dominated by *Nothofagus solandri* [var. *cliffortioides* (Hook. f.) Poole: mountain beech] trees 15–20 m tall, with basal areas between 0.016 and 0.126 m². The second site is within Ashley Forest Park (AFP), c. 60 km north of Christchurch (43.1° S, 172.3° E) and at 400 m elevation. Meteorological measurements made at the site during 2003 recorded 1170 mm of rainfall. Average temperature during 2003 was 11.2°C with a seasonal range of 8.6°C and an average daily range of 9.1°C. The forest is dominated by *Nothofagus solandri* [var. *solandri* (Hook. f.) Oerst.: black beech] trees 15–25 m tall, with basal areas between 0.016 and 0.229 m². Forest structure at both sites is similar, with a canopy dominated by beech and a very sparse understorey consisting mainly of small shrubs of the genus *Coprosma*. Both sites were on moderately steep slopes.

Half-hourly average air temperature, relative humidity, incident irradiance and total rainfall were measured in clearings and recorded using standard techniques and data loggers (CR10X and 21X; Campbell Scientific, Logan, UT, USA) at

the existing meteorological station near the CFP site, and at a station installed in December 2002 at the AFP site.

Phloem sap sampling and measurement of carbon isotope composition

Nothofagus forests in the central and northern South Island of New Zealand support large populations of the sooty beech scale insect (*Ultracoelostoma* spp., Hemiptera: Margarodidae) that live within the bark and tap the phloem sap. Excess carbohydrates are excreted by the insect from long waxy anal threads that extend from the bark, producing droplets of sugar-rich honeydew (Morales, 1991). The rate of honeydew production measured in winter at both sites was 2 mg sugar m⁻² bark h⁻¹, but was up to 80 mg sugar m⁻² bark h⁻¹ at AFP in summer (Dungan & Kelly, 2003; RJD, unpublished data). We found that ≈25% of trees at CFP and 60% of trees at AFP were heavily infested with scale insects. Preliminary photosynthesis measurements in January and April at AFP suggest that upper-canopy leaves of infested and uninfested trees were not significantly different in c_i/c_a . (RJD and MHT, unpublished data). This suggests that the $\delta^{13}\text{C}$ of honeydew ($\delta^{13}\text{C}_{\text{hd}}$) from the sampled trees is representative of carbon fixed recently by all canopy trees within the forest. The extra sink for carbon created by the presence of the insects does not appear to have a negative impact on the carbon balance of the tree (RJD, unpublished data), with neighbouring, similarly sized, uninfested and highly infested trees showing no differences in rates of growth (Chew, 2003) or health (Wardle, 1984).

Honeydew was sampled using 10 µl capillaries, either by drawing the liquid up into the tube, or by allowing more concentrated and viscous honeydew to adhere to the end of the tube (the viscosity of honeydew is related to air temperature, D , and wind speed; Dungan & Kelly, 2003). Honeydew was then placed in weighed tin capsules, returned to the laboratory and evaporated at 60°C for 24 h. Samples ranged in volume from 0.5 to 10 µl and yielded between 0.5 and 2.5 mg C, depending on honeydew concentration. Samples were divided into 0.5 mg C mass classes and analysed for $\delta^{13}\text{C}$ composition with standards of similar mass. Carbon isotope analysis was performed on an isotope ratio mass spectrometer (Europa Scientific 20/20) interfaced to a Dumas elemental analyser (Europa Scientific ANCA-SL, Europa Scientific Ltd., Crewe, UK). Isotope ratios are presented in parts per thousand in delta notation as:

$$\delta^{13}\text{C}_p = (R_{\text{sample}}/R_{\text{standard}}) - 1 \quad \text{Eqn 1}$$

where R is the isotope ratio ($^{13}\text{C}/^{12}\text{C}$), and the standard used is CO_2 from VPDB. The standard deviation for the repeated analysis of an internal standard, commercial sugar, was $\pm 0.14\%$. Calibration vs VPDB was achieved using a certified secondary standard from CSIRO, Canberra, Australia.

Honeydew from *Ultracoelostoma* feeding on *Nothofagus* has been found to consist of 42% fructose, 23% sucrose, 1%

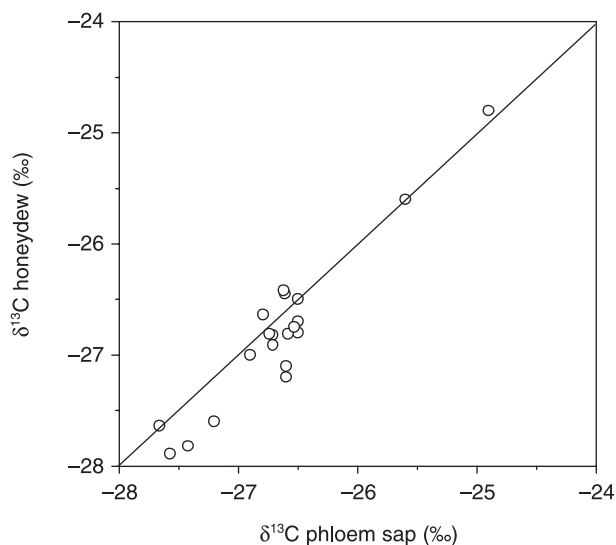


Fig. 1 Relationship between the carbon isotope composition of phloem sap and honeydew excreted by adjacent phloem-tapping scale insects on *Nothofagus solandri* trees. Line represents 1 : 1 relationship.

glucose and 33% oligosaccharides (probably tetrasaccharides) with trace amounts (<0.05%) of protein (Grant & Beggs, 1989). *Nothofagus* species are thought to transport sucrose (Zimmerman & Ziegler, 1975; Grant & Beggs, 1989), so isotopic fractionation during conversion of sucrose into fructose and oligosaccharides by the insect is possible. To be certain that changes in $\delta^{13}\text{C}$ of honeydew represent changes in $\delta^{13}\text{C}$ of phloem sap, it was important to establish if the insects change the isotopic ratio of sampled sugars. Insects were removed from the bark, taking care to leave their phloem-tapping stylets in place. Phloem sap continued to bleed from the stylet for 1–3 d, allowing phloem sap to be sampled and compared with honeydew excreted by adjacent insects. Honeydew and phloem sap samples were taken for $\delta^{13}\text{C}$ analysis over a range of environmental conditions. A paired *t*-test showed no significant difference between $\delta^{13}\text{C}$ of phloem sap and honeydew ($\delta^{13}\text{C}_{\text{hd}} = \delta^{13}\text{C}_{\text{sap}}$; $P = 0.004$), confirming that there was no significant fractionation of ^{13}C through the insect (Fig. 1).

Another methodological issue to resolve was the degree of variation in $\delta^{13}\text{C}$ of honeydew over time, i.e. variation between $\delta^{13}\text{C}$ of 'standing' honeydew droplets and those freshly formed within an hour of clearing all droplets from a defined area. In November 2003, $\delta^{13}\text{C}$ of fresh droplets was compared with $\delta^{13}\text{C}$ of standing honeydew at both sites, samples being collected between 00:30 and 04:30 h (New Zealand standard time, NZST). No significant difference was found between standing and fresh honeydew $\delta^{13}\text{C}$ (Fig. 2). In subsequent sampling, only standing droplets were collected.

At each site three or four trees were selected with naturally high levels of insect infestation and honeydew production. Basal area of sampled trees ranged between 0.038 and

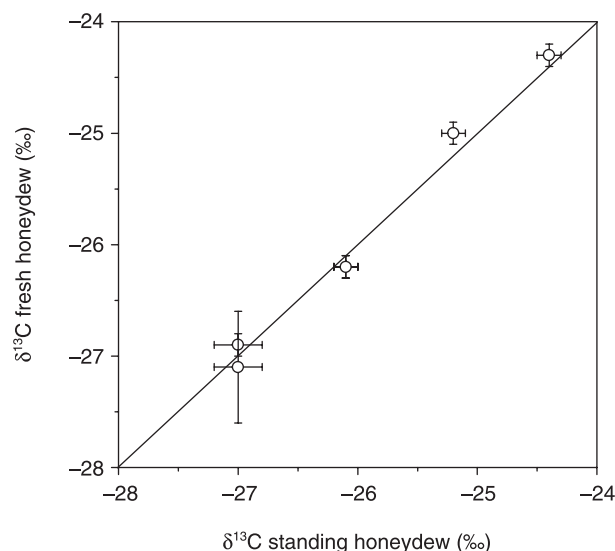


Fig. 2 Relationship between the carbon isotope composition of standing and freshly formed honeydew droplets. Line represents 1 : 1 relationship.

0.096 m² at CFP and 0.024 and 0.118 m² at AFP. Three replicate honeydew samples were collected from insects on the stem between 1 and 2 m above the ground (a vertical displacement 5–15 m from source leaves) either between 00:30 and 01:40 h NZST over four nights at AFP in November, or between 1 and 2 h after sunset over six nights at CFP in January and AFP in March. Samples were collected on consecutive nights in summer between 27 January and 1 February at CFP (at 21:30 h NZST), and on alternate nights between 20 and 27 November (at approximately 01:00 h NZST), and 8 and 17 March at AFP (at 20:30 h NZST).

Five replicate samples of soil, from within the upper 0.1 m, and leaf litter were collected at each site in March and dried at 80°C for 48 h. At the same time, leaves from three trees were sampled from 2.5 m above the ground at both sites, and from the sunlit upper canopy at AFP (≈17 m above the ground, with access via a cherry picker not available at CFP). Also sampled at AFP was the abundant black sooty fungus (a number of species of the genera *Trichopeltthea*, *Capnocybe* and *Capnodium* are present; Wardle, 1984) occurring as thick layers coating tree stems and branches. The fungus was also present at the higher-elevation site at CFP, but was not as thick or abundant. Sieved soil (<500 μm) and leaves, litter and fungus were ground and analysed for $\delta^{13}\text{C}$ as described above for honeydew.

Within-canopy air sampling and estimation of $\delta^{13}\text{C}_R$

Keeling (1958, 1961) suggested use of a simple mixing model to calculate the $\delta^{13}\text{C}$ of CO_2 respired by the ecosystem ($\delta^{13}\text{C}_R$):

$$\delta^{13}\text{C}_f = [(c_a/c_p)(\delta^{13}\text{C}_a - \delta^{13}\text{C}_R)] + \delta^{13}\text{C}_R \quad \text{Eqn 2}$$

where c is CO_2 concentration, $\delta^{13}\text{C}$ is the stable carbon isotope ratio of CO_2 , and the subscripts a and f refer to CO_2 in the atmosphere above and within the canopy, respectively. From equation 2 it can be seen that a plot of $1/c_f$ vs $\delta^{13}\text{C}_f$ gives a straight line with an intercept $\delta^{13}\text{C}_R$. Estimates of $\delta^{13}\text{C}_R$ were obtained from geometric mean linear regressions as described by Pataki *et al.* (2003). We assumed no change in $\delta^{13}\text{C}_R$ and $\delta^{13}\text{C}_a$ during sampling for each Keeling plot.

Within-canopy air was sampled into pre-evacuated 60 ml glass flasks between 0.01 and 7 m above the soil surface from within a 4 m² plot at each site on the same nights as honeydew collection. Samples were taken during the night to avoid confounding effects of photosynthesis on the carbon isotope composition of the sampled air. Air was pumped through a magnesium perchlorate trap before sampling to remove water vapour. The gas intake was not fixed to a solid structure, as described for most studies using the Keeling plot technique (e.g. Fessenden & Ehleringer, 2002; McDowell *et al.*, 2004a; Scartazza *et al.*, 2004). Rather, the intake tube was moved between heights within the sampling plot to ensure a sufficient range in CO_2 concentration (>75 $\mu\text{mol mol}^{-1}$; Pataki *et al.*, 2003) to minimize errors in $\delta^{13}\text{C}_R$ estimation. An infrared gas analyser (LiCor 6262, Lincoln, NE, USA) was placed in the sampling line to check the CO_2 concentration before sampling, but this concentration was not used in Keeling plot analysis. Keeling plots were constructed with the CO_2 concentration measured during isotopic analysis, as described below. Care was taken to avoid sample contamination with human breath. Five samples were taken, with the lowest sample intake and highest CO_2 concentration taken at 0.02 m below the surface of the litter and each set of samples taking $c.$ 15 min to collect. The range in CO_2 concentration for a single Keeling plot varied between 60 and 382 $\mu\text{mol mol}^{-1}$.

Sampling air within the litter layer raises the possibility that the CO_2 within the sampled footprint was very localized and dominated by the $\delta^{13}\text{C}$ of below-ground respiration. Unfortunately this is a limitation of the Keeling plot approach; a wide range in CO_2 concentration is required for Keeling plot construction, and high CO_2 concentrations are only found very close to the forest floor. We acknowledge that the value of $\delta^{13}\text{C}_R$ calculated here is unlikely to be a true representation of whole-ecosystem $\delta^{13}\text{C}_R$, and likely to be biased by below-ground respiration. To partially assess this issue, we constructed Keeling plots with samples collected only above the litter layer, and compared intercepts with those estimated from all samples. The two intercepts were not significantly different for any sampling night ($P > 0.05$, Student's t -test; Bailey, 1981), suggesting that the sampled footprint within the litter layer was large enough to be somewhat integrative.

Nothofagus cover was continuous within the small watersheds and for at least 2 km uphill of the sampling locations at both sites, and observations of air movement at night suggest that the sampled footprint was dominantly upslope within the *Nothofagus* forests. Vegetation cover changed beyond the

edge of the sampled watersheds (to conifer plantation at AFP and to pasture at CFP), and may have contributed to the sampled CO_2 . However, the highest sample was collected at 7 m (one-third to one-half canopy height), and 80% of samples were taken within 100 mm of the soil surface, suggesting that the sampled footprint was small and well within the *Nothofagus* forests. Further, Keeling plots would deviate significantly from linearity if CO_2 was sourced from ecosystems with differing $\delta^{13}\text{C}$ of respiratory CO_2 , and this was not the case.

Air samples were analysed for CO_2 concentration and $\delta^{13}\text{C}$ using a stable isotope mass spectrometer (MAT 252 IRMS, Finnigan, Bremen, Germany) interfaced to a gas chromatograph (HP5890 series II, Hewlett Packard, Avondale, PA, USA) as described by Ferretti *et al.* (2000), at the National Institute of Water and Atmospheric Research, Wellington, New Zealand. Isotope ratios are also presented relative to the VPDB standard. The standard deviation for the repeated analysis of two internal standards (clean air samples collected at Baring Head, New Zealand) were ± 0.03 and $\pm 0.02\text{‰}$ for $\delta^{13}\text{C}$, and ± 0.09 and ± 0.13 ppm for CO_2 concentration. Calibration of internal standards to VPDB was achieved by measurement of internal standards at CSIRO Atmospheric Research, Melbourne, Australia. $\delta^{13}\text{C}$ values were assigned using the CSIRO CG2003 reference scale, which is calibrated to PDB via the National Bureau of Standards reference scale (NBS-19).

Keeling plots constructed from within-canopy air samples generally had very high r^2 values (between 0.994 and 0.999 at CFP and between 0.979 and 0.999 at AFP), producing estimates of $\delta^{13}\text{C}_R$ with low standard errors (between 0.2 and 0.5‰ at CFP; between 0.1 and 1.3‰ at AFP), confirming our assumption of constant $\delta^{13}\text{C}_R$ and $\delta^{13}\text{C}_a$ during sampling.

Modelled canopy c_i/c_a and $\delta^{13}\text{C}_p$

We estimated canopy intercellular CO_2 concentration using a one-dimensional multilayer canopy model described by Leuning (1995) and applied by Whitehead *et al.* (2002) and Richardson *et al.* (2005). The model scales leaf-level measurements of photosynthesis, respiration and stomatal conductance to the canopy level, using submodels for radiative transfer, leaf energy balance, evaporation and photosynthesis. The canopy is divided into 12 layers based on assumed vertical distribution of cumulative canopy leaf area index. Values for the parameters required by the model are presented in Table 1. Leaf energy balance and coupled photosynthesis, stomatal conductance and leaf c_i (Leuning, 1995) were calculated simultaneously for sunlit and shaded foliage in each layer using biochemical models of photosynthesis (Farquhar *et al.*, 1980). Canopy c_i is calculated as a photosynthesis-weighted average of sunlit and shaded leaves from each canopy layer.

Carbon isotope discrimination ($\Delta^{13}\text{C}$, expressed relative to $\delta^{13}\text{C}$ of atmospheric CO_2) was calculated from daily photosynthesis-weighted canopy c_i/c_a by (Farquhar *et al.*, 1989):

Table 1 Values for parameters in the integrated canopy gas model used to estimate canopy c_i for *Nothofagus solandri* at Craigieburn Forest Park (CFP) and Ashley Forest Park (AFP), New Zealand

Parameter	Definition	Reference	CFP	AFP	Units
V_{cmax}	Maximum rate of carboxylation at top of canopy	1, 2, 3	48.1	78.1	$\mu\text{mol m}^{-2} \text{s}^{-1}$
J_{max}	Maximum rate of electron transport at top of canopy	1, 2, 3	90.6	154.5	$\mu\text{mol quanta m}^{-2} \text{s}^{-1}$
R_{d}	Light-independent rate of respiration	4	0.416	0.416	$\mu\text{mol m}^{-2} \text{s}^{-1}$
α	Quantum yield of electron transport	2	0.11	0.11	$\text{mol mol quanta}^{-1}$
τ	Convexity of light response curve	2	0.66	0.66	–
a_1	Parameter related to intercellular CO_2 concentration	2	6.3	6.3	–
g_{sc0}	Residual stomatal conductance to CO_2 transfer	2	0.0025	0.0025	$\text{mol m}^{-2} \text{s}^{-1}$
D_{so}	Sensitivity of stomatal conductance to air saturation deficit	2	817.6	817.6	Pa
D_{smin}	Minimum value of air saturation deficit for decreasing g_{sc}	2	250	250	Pa
L	Leaf area index	3	3.5	3.5	$\text{m}^2 \text{m}^{-2}$

1, Hollinger (1989); 2, Richardson *et al.* (2004); 3, R.J.D. and co-workers (unpublished data); 4, Benecke & Nordmeyer (1982). Values for photosynthesis parameters are estimated from measurements made at 20°C.

$$\Delta^{13}\text{C} = a + (b - a)(c_i/c_a) \quad \text{Eqn 3}$$

where a is diffusional fractionation (4.4‰); b is biochemical fractionation (27‰ after taking wall resistance effects into account, Bruognoli & Farquhar, 2000) during carbon fixation; and c_a is assumed to be 370 $\mu\text{mol mol}^{-1}$. Modelled $\Delta^{13}\text{C}$ values were compared with the carbon isotope composition of honeydew ($\delta^{13}\text{C}_{\text{hd}}$) by conversion to the VPDB scale:

$$\delta^{13}\text{C}_{\text{p}} = (\delta^{13}\text{C}_a - \Delta^{13}\text{C}) / (1 + \Delta^{13}\text{C}) \quad \text{Eqn 4}$$

assuming $\delta^{13}\text{C}$ of source CO_2 for carbon fixation is -7.8‰ and invariable during the season (Walcroft *et al.*, 1997). Equation 3 does not include biochemical processes downstream of carbon fixation that could alter the $\delta^{13}\text{C}$ of carbohydrates transported in the phloem (Jäggi *et al.*, 2002). For example, sucrose is often more enriched than the initial products of photosynthesis, but less enriched than starch (Schmidt & Gleixner, 1998). Recent work (Gessler *et al.*, 2004) suggests that observed differences between leaf and phloem sucrose may be related to temporal variation in discrimination during photosynthesis, rather than fractionation during transport, as has been suggested (Damesin & Lelarge, 2003; Hobbie & Werner, 2004).

Statistical analyses

Correlation analysis was used to test our first two hypotheses, that canopy c_i/c_a is reflected in $\delta^{13}\text{C}_{\text{hd}}$ and $\delta^{13}\text{C}_{\text{R}}$. Pearson correlation coefficients were calculated using Microsoft EXCEL. The distance between the location of canopy assimilation and respiring tissue suggests that correlations between $\delta^{13}\text{C}_{\text{p}}$, $\delta^{13}\text{C}_{\text{hd}}$, and $\delta^{13}\text{C}_{\text{R}}$ are likely to be lagged in time. The time lag between assimilation and transport in the stem ($\delta^{13}\text{C}_{\text{p}}$ and $\delta^{13}\text{C}_{\text{hd}}$) has been found to be ≈ 2 d for European beech trees (Keitel *et al.*, 2003), while the time lag between assimilation and respiration ($\delta^{13}\text{C}_{\text{p}}$ and $\delta^{13}\text{C}_{\text{R}}$) varies between

1 d (McDowell *et al.*, 2004a) and 5–10 d (Bowling *et al.*, 2002). Accordingly, we calculated A_{can} -weighted average $\delta^{13}\text{C}_{\text{p}}$ for each individual day as well as A_{can} -weighted average $\delta^{13}\text{C}_{\text{p}}$ for two successive days, then conducted correlation analyses between modelled $\delta^{13}\text{C}_{\text{p}}$ and measured $\delta^{13}\text{C}_{\text{hd}}$ and $\delta^{13}\text{C}_{\text{R}}$ with lag times of 1–10 d (a subset of these results are presented). Analysis of variance in $\delta^{13}\text{C}_{\text{hd}}$ was conducted in GENSTAT version 6.

Results

Weather conditions and modelled $\delta^{13}\text{C}_{\text{p}}$

A cold front brought 20.9 mm of rain to AFP on 17 and 18 November (days 321 and 322; Fig. 3) before the start of sampling. After the passage of the front, conditions were increasingly warm, dry and sunny until a low pressure system brought cooler, overcast conditions with patchy rain from 22 November (day 326) to the end of the sampling period. A significant rain event (15.7 mm) was recorded on the day before the last samples being taken. Modelled A_{can} varied between 0.54 and 1.27 $\text{mol m}^{-2} \text{d}^{-1}$ (average 0.92 $\text{mol m}^{-2} \text{d}^{-1}$), driven by variation in irradiance. Coupled with stomatal response to variation in D , variation in A_{can} resulted in more negative modelled $\delta^{13}\text{C}_{\text{p}}$ on days 322 and 323 (18 and 19 November) and less negative modelled $\delta^{13}\text{C}_{\text{p}}$ on 325 (21 November).

During the January sampling at CFP, two cold fronts moved across the site on 26 and 31 January, bringing 25.8 and 31.0 mm of rain, respectively (days 26 and 31, Fig. 4). Days between 22 and 25 January were warm and sunny with moderately high D . After the first front had passed (28–30 January), conditions remained partly cloudy, with lower temperatures and values of D than those before the front. Variation in irradiance resulted in a range in modelled total canopy assimilation rate (A_{can}) of between 0.31 and 0.78 $\text{mol m}^{-2} \text{d}^{-1}$, with an average of 0.65 $\text{mol m}^{-2} \text{d}^{-1}$. Variation in A_{can} and D resulted in modelled $\delta^{13}\text{C}_{\text{p}}$ being less negative in the warm,

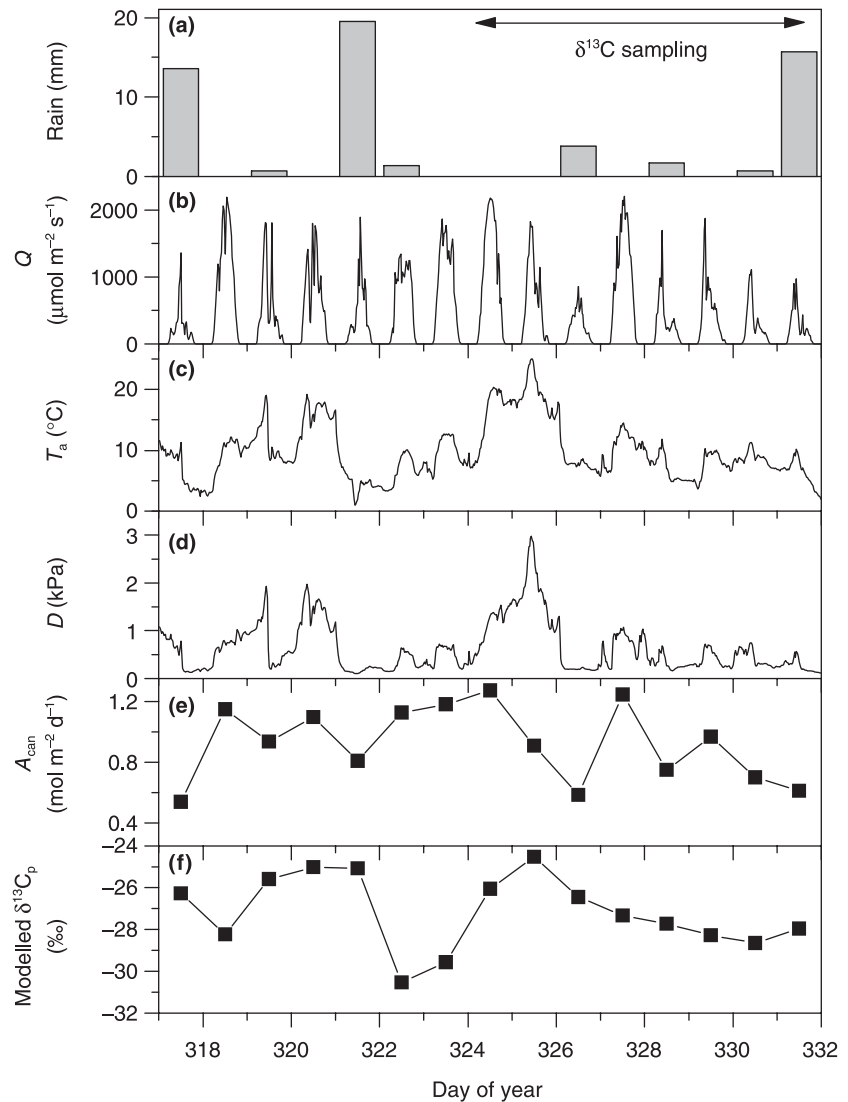


Fig. 3 Variation in weather variables and in modelled canopy photosynthetic rate (A_{can}) and modelled photosynthesis-weighted carbon isotope discrimination ($\delta^{13}\text{C}_p$) for Ashley Forest Park (New Zealand) between 13 and 27 November (days 217–231). (a) daily rainfall; (b) incident irradiance (400–700 nm; Q); (c) air temperature (T_a); (d) vapour pressure deficit (D); (e) A_{can} ; (f) modelled $\delta^{13}\text{C}_p$.

sunny conditions before the first front, more negative during the passage of both fronts, and intermediate on the partly cloudy days between the two fronts (Fig. 4).

Two cold fronts also moved across the AFP site during the March sampling period. The first brought 38.5 mm of rain to AFP between 1 and 3 March, and the second 22.3 mm on 16 and 17 March (days 61 and 76, respectively, Fig. 5). Between the fronts the days were mostly clear and sunny. Variation in irradiance and D resulted in a range in modelled A_{can} of between 0.41 and 1.05 mol m⁻² d⁻¹, with an average of 0.76 mol m⁻² d⁻¹. Air temperature and air saturation deficit were quite variable during this time, with no clear diurnal pattern, but generally higher than in January at CFP. This wide variability in D probably results from differences in wind direction. Hot, dry föhn winds from the northwest are often interspersed with cool, moist easterlies from the Pacific Ocean east of the Southern Alps in the South Island. Variable D resulted in considerable variation in modelled $\delta^{13}\text{C}_p$.

Modelled $\delta^{13}\text{C}_p$ reflected changes in D , with less negative $\delta^{13}\text{C}_p$ associated with warm, dry conditions and more negative $\delta^{13}\text{C}_p$ with cool, moist conditions (Fig. 5).

Values of $\delta^{13}\text{C}_{\text{hd}}$ and $\delta^{13}\text{C}_R$

At AFP in November, there was a general decline in the carbon isotope composition of honeydew ($\delta^{13}\text{C}_{\text{hd}}$) over consecutive nights, from -26.0 to -26.9‰. Two trees (trees 1 and 3) followed similar trends in $\delta^{13}\text{C}_{\text{hd}}$, while the third tree samples (tree 4) showed little variation (Fig. 6a). ANOVA revealed highly significant ($P < 0.001$) differences between trees and between nights and a highly significant ($P < 0.001$) tree \times night interaction. Significant variation in $\delta^{13}\text{C}_R$ (between -23.5 and -26.0‰) was observed between nights at AFP in November (Fig. 6b).

A general decline in $\delta^{13}\text{C}$ of honeydew with time was also found at CFP in January, from -25.4 to -26.5‰. Considerable

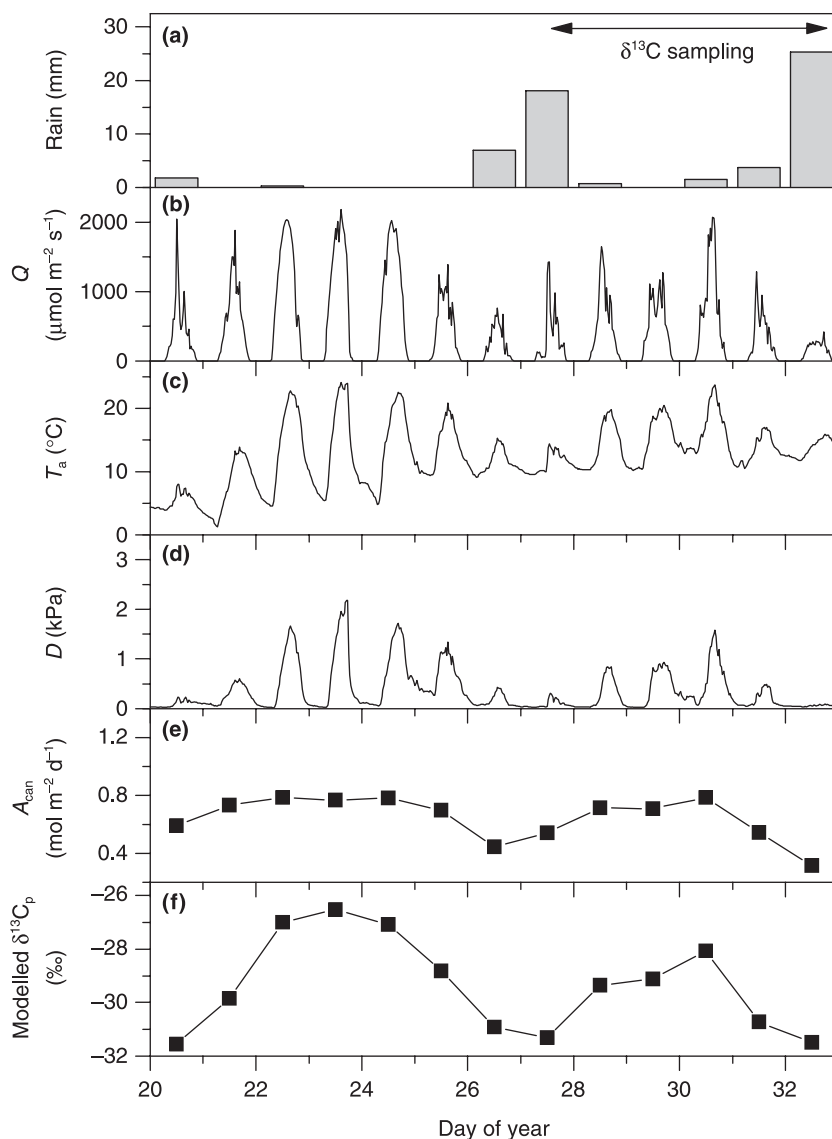


Fig. 4 Variation in weather variables and in modelled canopy photosynthetic rate (A_{can}) and modelled photosynthesis-weighted carbon isotope discrimination ($\delta^{13}\text{C}_p$) for Craigieburn Forest Park (New Zealand) between 20 January and 1 February (days 20–32). (a) daily rainfall; (b) incident irradiance (400–700 nm; Q); (c) air temperature (T_a); (d) vapour pressure deficit (D); (e) A_{can} ; (f) modelled $\delta^{13}\text{C}_p$.

variation in $\delta^{13}\text{C}_{\text{hd}}$ was observed between trees on the same night, but variation between replicate samples from a single tree was generally small. Three of the four trees showed similar patterns in $\delta^{13}\text{C}_{\text{hd}}$, but the fourth tree showed very little variation in $\delta^{13}\text{C}_{\text{hd}}$ between nights (Fig. 6c). ANOVA revealed highly significant ($P < 0.001$) differences between trees and between nights and a significant ($P = 0.017$) tree \times night interaction. Values of $\delta^{13}\text{C}_R$ varied between -24.1 and -25.1‰ , although differences between nights were not statistically significant (Fig. 6d).

At AFP in March, average values of $\delta^{13}\text{C}$ of honeydew varied between -27.0 and -27.8‰ . Variation between nights for a single tree was larger, up to 1.7‰ . As at CFP, three of the four trees showed similar patterns in $\delta^{13}\text{C}_{\text{hd}}$ (Fig. 6e). ANOVA revealed significant ($P < 0.01$) tree and night effects, and a highly significant ($P < 0.001$) tree \times night interaction. Signif-

icant variation in $\delta^{13}\text{C}$ of ecosystem respiration was observed between nights at AFP in March, with a range in $\delta^{13}\text{C}_R$ of 3.3‰ (Fig. 6f).

Relationships between modelled $\delta^{13}\text{C}_p$ and measured $\delta^{13}\text{C}_{\text{hd}}$ and $\delta^{13}\text{C}_R$

Significant positive correlations were found between modelled $\delta^{13}\text{C}_p$ (averaged over a single day) and $\delta^{13}\text{C}_{\text{hd}}$ for all three trees sampled at AFP in November when $\delta^{13}\text{C}_{\text{hd}}$ at 1.5 m above the ground lagged 3 d (trees 1 and 3) or 5 d (tree 4) behind canopy carbon fixation (Fig. 7a). Average $\delta^{13}\text{C}_{\text{hd}}$ across all trees was most strongly correlated with modelled $\delta^{13}\text{C}_p$ with a 3 d lag time and photosynthesis-weighted average $\delta^{13}\text{C}_p$ over a single day (Fig. 7b). No significant correlation was found between $\delta^{13}\text{C}_R$ and modelled $\delta^{13}\text{C}_p$

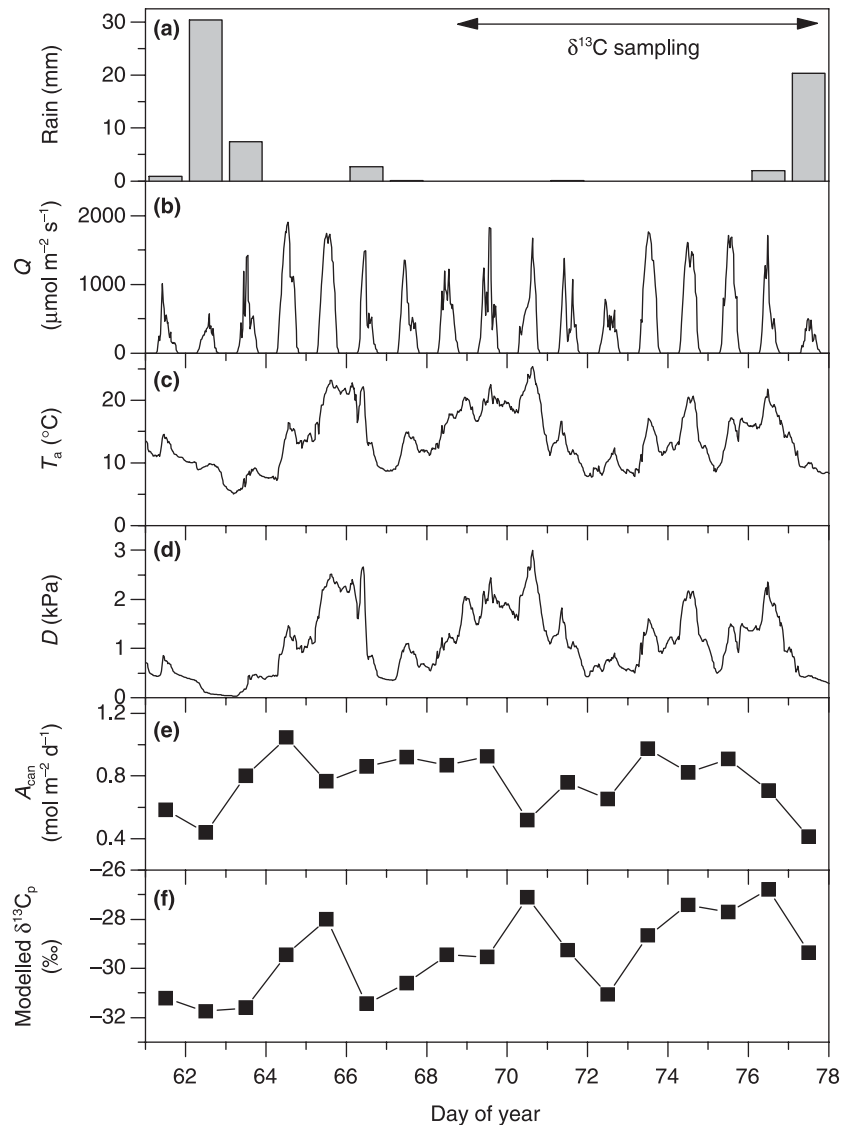


Fig. 5 Variation in weather variables and in modelled canopy photosynthetic rate (A_{can}) and modelled photosynthesis-weighted carbon isotope discrimination ($\delta^{13}\text{C}_p$) for Ashley Forest Park (New Zealand) between 1 and 17 March (days 61–77). (a) daily rainfall; (b) incident irradiance (400–700 nm; Q); (c) air temperature (T_a); (d) vapour pressure deficit (D); (e) A_{can} ; (f) modelled $\delta^{13}\text{C}_p$.

with any time lag between 0 and 10 d (Fig. 7b). Correlation coefficients were not improved significantly for $\delta^{13}\text{C}_{\text{hd}}$ of individual trees or the average across all trees, or $\delta^{13}\text{C}_R$, when a photosynthesis-weighted 2 d average modelled $\delta^{13}\text{C}_p$ was used (data not shown).

At the CFP site, significant positive correlations were found between modelled $\delta^{13}\text{C}_p$ (averaged over a single day) and $\delta^{13}\text{C}_{\text{hd}}$ for three of the four trees when $\delta^{13}\text{C}_{\text{hd}}$ lagged 3 d (trees 1 and 2) or 4 d (tree 4) behind canopy carbon fixation. No significant correlation was found between $\delta^{13}\text{C}_{\text{hd}}$ from tree 3 and modelled $\delta^{13}\text{C}_p$ with any time lag between 0 and 10 d, where $\delta^{13}\text{C}_p$ was averaged over either 1 or 2 d (a subset of correlation coefficients is presented in Fig. 7c). Average $\delta^{13}\text{C}_{\text{hd}}$ across all trees was most strongly correlated with modelled $\delta^{13}\text{C}_p$ with a 3 d lag time, and photosynthesis-weighted average $\delta^{13}\text{C}_p$ over a single day (Fig. 7d). No significant correlation was

found between $\delta^{13}\text{C}_R$ and modelled $\delta^{13}\text{C}_p$ with any time lag between 0 and 10 d (Fig. 7d). Correlation coefficients were not significantly improved for $\delta^{13}\text{C}_{\text{hd}}$ of individual trees or the average across all trees, or $\delta^{13}\text{C}_R$, when a photosynthesis-weighted 2 d average modelled $\delta^{13}\text{C}_p$ was used (data not shown). The relationship between modelled $\delta^{13}\text{C}_p$, with a 3 d lag time, and measured $\delta^{13}\text{C}_{\text{hd}}$ is presented in Fig. 8a and shows that, while the timing and direction of variation in $\delta^{13}\text{C}_{\text{hd}}$ was well predicted, absolute values and the magnitude of day-to-day variation were poorly predicted (the modelled range was 4.2‰, while the measured range in $\delta^{13}\text{C}_{\text{hd}}$ was just 1.3‰).

At AFP in March, significant positive correlations were also found between modelled $\delta^{13}\text{C}_p$ and $\delta^{13}\text{C}_{\text{hd}}$ for three of the four trees when $\delta^{13}\text{C}_p$ lagged 3 d (trees 1 and 4) or 6 d (tree 3) behind canopy photosynthesis. No significant correlation was found between $\delta^{13}\text{C}_{\text{hd}}$ from tree 2 and modelled $\delta^{13}\text{C}_p$

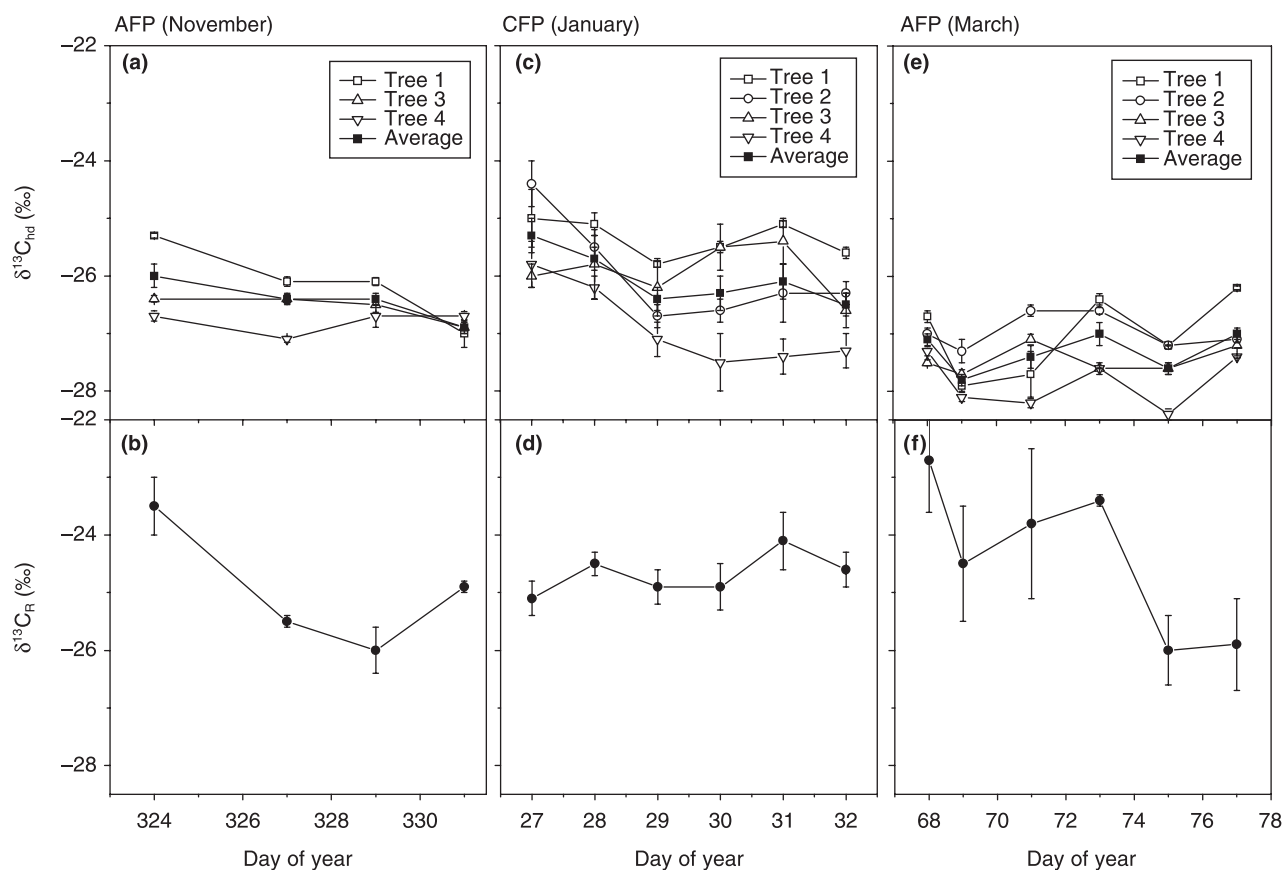


Fig. 6 Temporal variation in the carbon isotope composition of honeydew from four *Nothofagus solandri* trees (a,c,d) and ecosystem-respired CO_2 (b,d,f), at Craigieburn Forest Park (c,d) and Ashley Forest Park in November (a,b) and in March (e,f). Error bars represent (a,c,e) SE of the mean value; (b,d,f) SE of the Keeling plot geometric mean regression.

with any time lag between 0 and 10 d, although the highest correlation coefficient was found with a 3 d lag (Fig. 7e). When $\delta^{13}\text{C}_{\text{hd}}$ was averaged across all trees, a highly significant correlation was observed with modelled $\delta^{13}\text{C}_{\text{p}}$ including a 3 d lag time (Fig. 7f). No significant correlation was found between $\delta^{13}\text{C}_{\text{R}}$ and modelled $\delta^{13}\text{C}_{\text{p}}$ at AFP, but the highest correlation coefficient was found with a 3 d lag (Fig. 7f). Correlation coefficients were not significantly improved when a photosynthesis weighted 2 d average $\delta^{13}\text{C}_{\text{p}}$ was used (data not shown). As at CFP, the canopy photosynthesis model accurately predicted the timing and direction of changes in $\delta^{13}\text{C}_{\text{hd}}$ (with a 3 d lag), but did not predict the absolute values and amplitude of changes in $\delta^{13}\text{C}_{\text{hd}}$ (the modelled range in $\delta^{13}\text{C}_{\text{p}}$ over both November and March sampling periods was 6.3‰, while the measured range in $\delta^{13}\text{C}_{\text{hd}}$ was just 1.8‰; Fig. 8b).

Coupling between $\delta^{13}\text{C}_{\text{hd}}$ and $\delta^{13}\text{C}_{\text{R}}$

At both sites there was no significant relationship between $\delta^{13}\text{C}_{\text{hd}}$ and $\delta^{13}\text{C}_{\text{R}}$ when all days were included in the analysis. As suggested in the Introduction, the degree of coupling

between $\delta^{13}\text{C}_{\text{p}}$ and $\delta^{13}\text{C}_{\text{R}}$ may vary with environmental conditions. With this in mind, the sampling days were divided into 'wet days' (>2 mm rain fell within 12 h of sampling) and 'dry days' (all other days). When wet days were excluded from the analysis, $\delta^{13}\text{C}_{\text{R}}$ tended to increase with increasing $\delta^{13}\text{C}_{\text{hd}}$ at both sites and both sampling times at AFP, although the relationships were not statistically significant (Fig. 9). Further, significant seasonal variation in $\delta^{13}\text{C}_{\text{hd}}$ between spring and autumn sampling periods at AFP was not reflected in $\delta^{13}\text{C}_{\text{R}}$ (Fig. 9; Table 2).

Respired CO_2 was more enriched than recently fixed carbon at both sites, by 1.4, 2.0 and 3.3‰ on average at CFP and AFP in November and March, respectively. At AFP in March, the difference between $\delta^{13}\text{C}_{\text{R}}$ and $\delta^{13}\text{C}_{\text{hd}}$ tended to decrease during the sampling campaign ($P = 0.016$), from 4.4 to 1.1‰. Respired CO_2 was also more enriched than carbon from any measured pool at both sites, and the difference was greater at AFP than at CFP (Table 2). Carbon in ecosystem pools was less depleted in ^{13}C at CFP than at AFP, by between 0.6‰ for soil and 1.5‰ for leaf litter, suggesting that leaves of trees at CFP had lower c_i/c_a over the long term than did those at AFP.

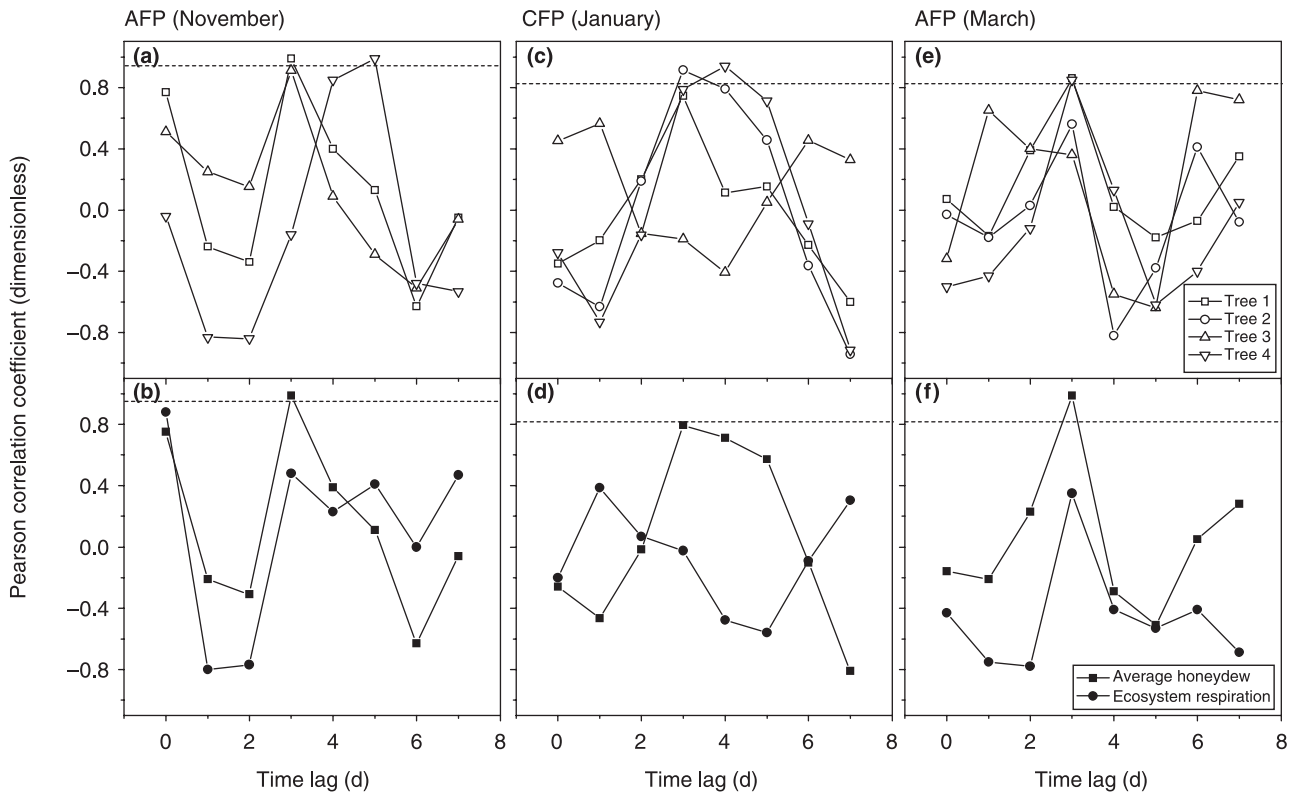


Fig. 7 Pearson product-moment correlation coefficients for linear regressions of the carbon isotope composition of honeydew from individual *Nothofagus solandri* trees (a,c,e); site average honeydew and ecosystem-respired CO_2 (b,d,f) vs photosynthesis-weighted daily modelled canopy carbon isotope discrimination time-lagged by 0–7 d for two forests (Craigieburn Forest Park, CFP; Ashley Forest Park, AFP, New Zealand). A 0 d shift corresponds to the photosynthesis-weighted average modelled canopy carbon isotope composition for the day immediately preceding sampling. Dashed line represents a statistically significant positive relationship ($P < 0.05$; $n = 4$ (a,b); $n = 6$ (c–f)).

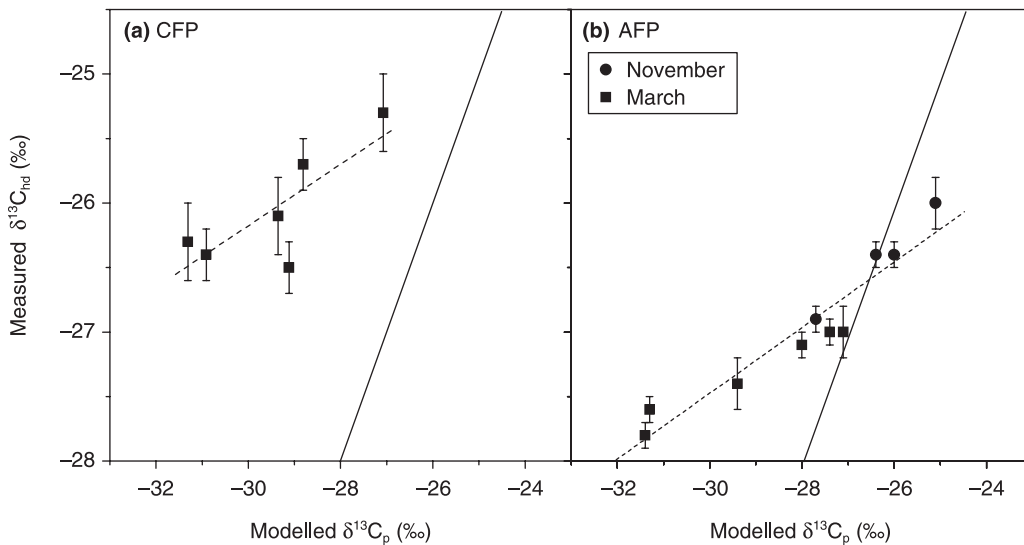


Fig. 8 Relationships between measured and modelled carbon isotope composition of honeydew at two forests (Craigieburn Forest Park, CFP; Ashley Forest Park, AFP, New Zealand), including a 3 d lag time at both sites. Dashed line represents linear regression; solid line the 1 : 1 relationship. (a) Measured $\delta^{13}\text{C} = 0.24 \times \text{modelled } \delta^{13}\text{C} - 18.98$, $r^2 = 0.63$, $P = 0.058$. (b) Measured $\delta^{13}\text{C} = 0.25 \times \text{modelled } \delta^{13}\text{C} - 19.84$, $r^2 = 0.98$; $P < 0.0001$. Error bars represent SE of mean values ($n = 12$).

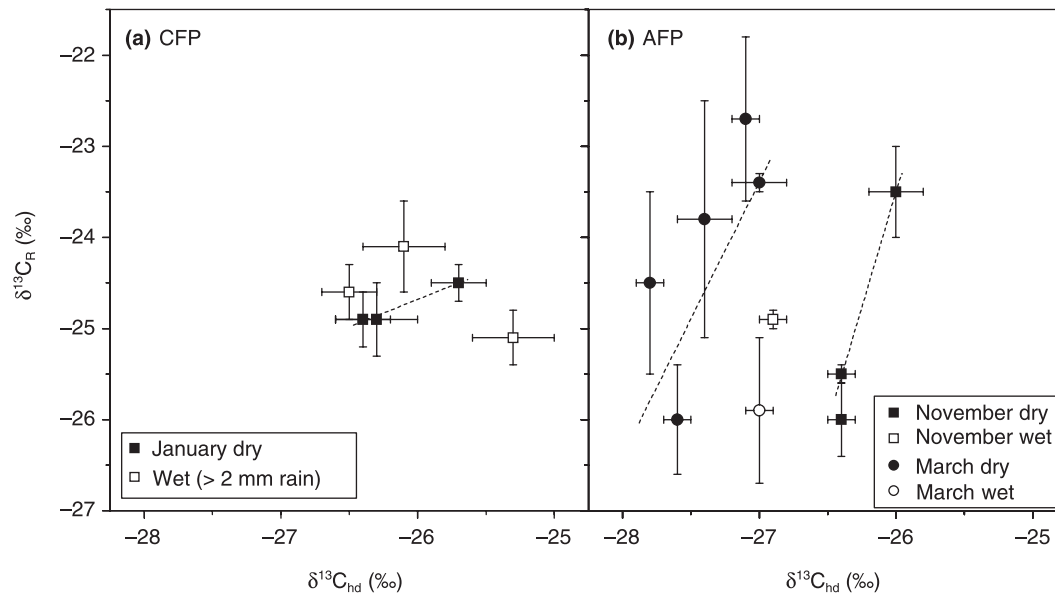


Fig. 9 Relationships between the carbon isotope composition of honeydew and ecosystem-respired CO₂ at Craigieburn Forest Park (CFP, a) and Ashley Forest Park (AFP, b) (New Zealand) for wet (open symbols) and dry (closed symbols) nights. Dashed lines represent linear regressions for dry nights (a, $P = 0.06$; b, $P = 0.19$ for November data, $P = 0.06$ for March data).

Component	$\delta^{13}C$ (‰)		
	AFP (Nov)	CFP	AFP (Mar)
Sunlit leaves (17 m)	nd	nd	-26.6 ± 0.2
Leaves (2.5 m)	nd	-28.8 ± 1.1	-29.4 ± 0.7
Honeydew (overall average)	-26.5 ± 0.1	-26.0 ± 0.1 (Jan)	-27.3 ± 0.1
Sooty mould	nd	nd	-27.9 ± 0.2
Leaf litter	nd	-27.4 ± 0.1	-28.9 ± 0.1
Soil (0–100 mm)	nd	-26.5 ± 0.2	-27.1 ± 0.2
Ecosystem-respired CO ₂ (all samples on a single plot)	-24.5 ± 0.3	-24.7 ± 0.1 (Jan)	-23.5 ± 0.3

Table 2 Carbon isotopic composition of ecosystem components at Craigieburn Forest Park (CFP) and Ashley Forest Park (AFP), New Zealand

Mean \pm SE; nd, not determined. Leaves were sampled either 17 or 2.5 m above ground.

Discussion

The range in weather conditions over the sampling periods provided an ideal opportunity to test the hypotheses that short-term changes in canopy $\delta^{13}C_p$ determine the isotopic composition of phloem sap, and influence $\delta^{13}C$ of ecosystem-respired CO₂ ($\delta^{13}C_R$, but likely to be biased towards below-ground respiration caused by the CO₂ sampling strategy). Isotope analysis of phloem sap, sampled via the honeydew scale insect, provided evidence of significant day-to-day variation in canopy $\delta^{13}C_p$, the timing and direction of which were predicted with an integrated canopy photosynthesis model. On dry days (< 2 mm rain within 12 h of sampling), $\delta^{13}C_R$ tended to increase with increasing $\delta^{13}C$ of phloem sap at both sites, although the relationships were not statistically significant (probably because of the small number of

samples). Overall, the data suggest that $\delta^{13}C_R$ was influenced, to some extent, by variation in discrimination during carbon fixation. However, the relationships between $\delta^{13}C$ of phloem sap and $\delta^{13}C_R$ are much weaker than those reported by Scartazza *et al.* (2004) in a northern hemisphere beech forest, suggesting that the tightness of coupling between canopy photosynthesis and ecosystem respiration varies considerably between ecosystems. The data also provide support for the third hypothesis: that environmental conditions influence the degree of coupling between canopy $\delta^{13}C_p$ and $\delta^{13}C_R$.

Time lags between canopy carbon fixation, phloem transport and ecosystem respiration

An integrated canopy model accurately predicted the direction of changes in $\delta^{13}C_{hd}$ and, with a 3 d lag time, the timing

of changes in $\delta^{13}\text{C}_{\text{hd}}$ was also well predicted. A lag of 3 d between canopy photosynthesis and $\delta^{13}\text{C}$ of phloem sugars collected near the ground fits well with previous observations in European beech trees (Keitel *et al.*, 2003). While the timing and direction of changes in $\delta^{13}\text{C}_{\text{hd}}$ was well predicted by the canopy photosynthesis model, the magnitude of the changes was poorly predicted. Modelled variation in $\delta^{13}\text{C}_{\text{p}}$ of carbon fixed by leaves was 3.2 and 3.5 times higher than measured variation at CFP and AFP, respectively. This may be caused by incorrect values for model parameters (no attempt was made to 'tune' the model), but may also be caused by mixing of carbon fixed by different source leaves. For example, if we assume that carbohydrates are loaded into the phloem immediately after fixation, and phloem transport velocities are constant throughout the tree (to simplify the calculation) at 0.75 m h^{-1} (Zimmermann & Braun, 1971), we can estimate the time taken for carbon to reach the base of the stem. Carbon fixed by the highest leaves of a 17 m tall tree would reach phloem at 1.5 m above the ground (total path length is 15.5 m, with a transport time of 20.7 h), 13.4 h after carbon fixed at the end of a 3 m long branch that was 4 m above the ground (total path length is 5.5 m, with a transport time of 7.3 h). Further mixing of carbon fixed at different times is likely to be caused by variation in the timing of phloem loading resulting from variation in the soluble carbohydrate content of leaves (i.e. rate of phloem loading is related to leaf carbohydrate concentration; Moing *et al.*, 1994). Laboratory experiments (Grodzinski *et al.*, 1998) and modelling simulations (Thompson & Holbrook, 2004) suggest that phloem transport velocity and assimilate supply are tightly coupled, via osmotic pressure/concentration waves that move much more quickly than the solution itself, so phloem transport velocity (and so lag times) may vary considerably between trees, and could even vary widely over a day within a single tree.

Interestingly, differences in lag times between November and March at AFP were observed for two of the three trees sampled at both times. Tree 1, a dominant tree in an exposed canopy position, showed a 3 d lag for both spring and autumn sampling times. In contrast, trees 3 and 4 (both smaller, subdominant trees) had variable lag times: 3 and 6 d for tree 3 and 5, and 3 d for tree 4, in November and March, respectively. The limited data do not allow firm conclusions to be drawn, but it seems possible that seasonal differences in carbon source and sink strengths could change the rate of carbon transport to the roots. Future work with the *Nothofagus*–honeydew system will include sampling at higher temporal resolution (both on daily and seasonal scales), more replication of dominant and subdominant trees and at a number of positions along the stem.

There was no evidence of an additional lag between phloem sugars sampled 1.5 m above the ground and ecosystem respiration. A possible explanation for this observation is that the honeydew insect provides a 'shortcut' for carbon

between the phloem and the microbial community in the litter and soil, via honeydew droplets falling or being washed from branches and stem. The amount of honeydew-derived carbon arriving at the forest floor has not been quantified, so the importance of this shortcut is unknown. A 3 d lag between environmental drivers of variation in $\delta^{13}\text{C}_{\text{p}}$ and $\delta^{13}\text{C}_{\text{R}}$ compares well with previously published data in a number of forests. For example, Horwath *et al.* (1994) found a peak in $^{14}\text{CO}_2$ respired by the soil 2 d after *Populus* trees were radiolabelled; and Mikan *et al.* (2000) found a lag of 3–4 d in a similar experiment. A lag of 2–4 d in $\delta^{13}\text{C}_{\text{R}}$ following a change in D was found in a boreal forest by Ekblad & Högberg (2001), while Bowling *et al.* (2002) found peaks in the correlation coefficients between D and $\delta^{13}\text{C}_{\text{R}}$ with a lag of 5–10 d in several coniferous forests, and McDowell *et al.* (2004b) found a lag of 0–3 d between $\delta^{13}\text{C}_{\text{R}}$ and meteorological and physiological variables at two coniferous sites.

Coupling between $\delta^{13}\text{C}_{\text{p}}$ and $\delta^{13}\text{C}_{\text{R}}$

Scartazza *et al.* (2004) report very tight coupling ($r^2 = 0.99$) between seasonal variability in canopy discrimination and $\delta^{13}\text{C}_{\text{R}}$ in a northern hemisphere beech forest. Such tight coupling was not found on a daily temporal scale in our study. Further, seasonal variability in canopy discrimination was not reflected in $\delta^{13}\text{C}_{\text{R}}$ at AFP. This suggests that the degree of coupling between canopy photosynthesis and ecosystem respiration varies between ecosystems. It seems possible that, while recently fixed carbon was the main substrate for respiration in the northern hemisphere beech forest, other carbon pools with less temporally variable $\delta^{13}\text{C}$ were the main substrate in the *Nothofagus* forests studied here. A trend of increasing $\delta^{13}\text{C}_{\text{R}}$ with increasing $\delta^{13}\text{C}$ of phloem sugar (as reported by Scartazza *et al.*, 2004) was observed for all three sampling periods in our study when wet days (for which >2 mm of rain was recorded) were excluded, although the relationships were not significant statistically. This suggests that the degree of coupling between $\delta^{13}\text{C}_{\text{p}}$ and $\delta^{13}\text{C}_{\text{R}}$ may vary with environmental conditions at a single site.

Differences between $\delta^{13}\text{C}_{\text{R}}$ and $\delta^{13}\text{C}$ of respiratory sources

Ecosystem-respired carbon was found to be significantly more enriched in ^{13}C than any measured pool of carbon in the ecosystem (Table 2). A discrepancy between $\delta^{13}\text{C}$ of carbon sources within the ecosystem and $\delta^{13}\text{C}$ of respired CO_2 has been reported in a number of ecosystems (Pataki *et al.*, 2003), and $\delta^{13}\text{C}_{\text{R}}$ has been reported to be both less depleted (Bowling *et al.*, 2003a), and more depleted (Scartazza *et al.*, 2004), than ecosystem carbon pools. These disequilibria are still poorly understood. Clearly, conservation of mass dictates that fractionation between input (photosynthates) and output (respired CO_2) carbon cannot be sustained over the entire

life of the ecosystem (Pataki *et al.*, 2003). Further, Lin & Ehleringer (1997) have shown that no ^{13}C fractionation occurs during mitochondrial respiration in isolated leaf protoplasts. However, there is growing evidence of isotopic fractionation during biochemical transformation of carbohydrates before respiration (Duranceau *et al.*, 1999; Ghashghaie *et al.*, 2001; Tcherkez *et al.*, 2003); during respiration itself (Fernandez *et al.*, 2003; Xu *et al.*, 2004); and during carbon uptake by microbes (Henn & Chapela, 2001). Leaf-respired CO_2 is often more enriched in ^{13}C than bulk leaf carbon under normal (well watered and not carbon-limited; Tcherkez *et al.*, 2003) conditions because the carbohydrate substrates for respiration are enriched compared with total leaf carbon (Schmidt & Gleixner, 1998), and because the carbon released as CO_2 comes from atoms in positions 3 and 4 of the hexose phosphate substrates, which are more enriched than the average $\delta^{13}\text{C}$ for the molecule (Tcherkez *et al.*, 2003). That is, the nonstatistical (nonrandom) distribution of ^{13}C in the hexose phosphate substrates results in fragmentation fractionation during respiration (Tcherkez *et al.*, 2004). Future studies into variation in $\delta^{13}\text{C}_R$ should quantify the carbon isotope composition of CO_2 respired by the various components, rather than the source carbon (although even when this is done, there are problems with interpretation; McDowell *et al.*, 2004a).

Conclusions and recommendations

Data presented here suggest that the degree of coupling between $\delta^{13}\text{C}_p$ and $\delta^{13}\text{C}_R$ varies between ecosystems and with environmental conditions at a single site. A likely explanation for variation in the tightness of the link between environmentally driven variability in $\delta^{13}\text{C}_p$ and $\delta^{13}\text{C}_R$ found in this and previous studies (Bowling *et al.*, 2003a; McDowell *et al.*, 2004a, 2004b; Scartazza *et al.*, 2004) is variation in the proportional contribution to $\delta^{13}\text{C}_R$ by recently fixed carbon substrates both in time at a single site, and between ecosystems. We suggest that future studies either compare ecosystems with very different plant vs soil respiratory contributions, or compare times at a single site when the proportional contribution of different ecosystem components are widely and predictably variable. For example, respiration from an ecosystem with very low soil organic carbon content should be strongly plant-derived, so $\delta^{13}\text{C}_R$ may be highly temporally variable and closely linked to $\delta^{13}\text{C}_p$, while an older ecosystem with high soil organic carbon content may display loose coupling between short-term variation in $\delta^{13}\text{C}_p$ and $\delta^{13}\text{C}_R$. An environmental effect that should produce a clear $\delta^{13}\text{C}_R$ signal is the rapid and transient increase in litter decomposition following rewetting of dry litter. Eddy covariance measurements in a number of ecosystems have revealed a large, short-lived increase in ecosystem respiration immediately after the break of a drought (CO_2 exchange rates nearly an order of magnitude higher than those before rain;

Xu & Baldocchi, 2004), attributed to microbial litter decomposition (Kelliher *et al.*, 2004; Lee *et al.*, 2004). In this scenario, $\delta^{13}\text{C}$ of litter decomposition will dominate $\delta^{13}\text{C}_R$ immediately after rain, then become increasingly less important as the litter dries. The development of 'real-time' methods to measure the $\delta^{13}\text{C}$ of CO_2 using tunable diode laser absorption spectroscopy (Bowling *et al.*, 2003b; Griffis *et al.*, 2004) will greatly increase the temporal resolution of $\delta^{13}\text{C}_R$ estimation, and should allow significant advances in mechanistic understanding of the link between $\delta^{13}\text{C}_p$ and $\delta^{13}\text{C}_R$.

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