Foliage, fine-root, woody-tissue and stand respiration in *Pinus radiata* in relation to nitrogen status

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**Summary** We measured respiration of 20-year-old *Pinus radiata* D. Don trees growing in control (C), irrigated (I), and irrigated + fertilized (IL) stands in the Biology of Forest Growth experimental plantation near Canberra, Australia. Respiration was measured on fully expanded foliage, live branches, boles, and fine and coarse roots to determine the relationship between CO₂ efflux, tissue temperature, and biomass or nitrogen (N) content of individual tissues. Efflux of CO₂ from foliage (dark respiration at night) and fine roots was linearly related to biomass and N content, but N was a better predictor of CO₂ efflux than biomass. Respiration (assumed to be maintenance) per unit N at 15 °C and a CO₂ concentration of 400 µmol mol⁻¹ was 1.71 µmol s⁻¹ mol⁻¹ N for foliage and 11.2 µmol s⁻¹ mol⁻¹ N for fine roots. Efflux of CO₂ from stems, coarse roots and branches was linearly related to sapwood volume (stems) or total volume (branches + coarse roots) and growth, with rates for maintenance respiration at 15 °C ranging from 18 to 104 µmol m⁻³ s⁻¹. Among woody components, branches in the upper canopy and small diameter coarse roots had the highest respiration rates. Stem maintenance respiration per unit sapwood volume did not differ among treatments.

Annual C flux was estimated by summing (1) dry matter production and respiration of aboveground components, (2) annual soil CO₂ efflux minus aboveground litterfall, and (3) the annual increment in coarse root biomass. Annual C flux was 24.4, 25.3 and 34.4 Mg ha⁻¹ year⁻¹ for the C, I and IL treatments, respectively. Total belowground C allocation, estimated as the sum of (2) and (3) above, was equal to the sum of root respiration and estimated root production in the IL treatment, whereas in the nutrient-limited C and I treatments, total belowground C allocation was greater than the sum of root respiration and estimated root production, suggesting higher fine root turnover or increased allocation to mycorrhizae and root exudation. Carbon use efficiency, the ratio of net primary production to assimilation, was similar among treatments for aboveground tissues (0.43–0.50). Therefore, the proportion of assimilation used for construction and maintenance respiration on an annual basis was also similar among treatments.

**Keywords:** biomass, carbon use efficiency, CO₂ efflux, dry matter production, fertilization, irrigation, nitrogen content.

**Introduction**

Little is known about the mechanisms underlying the large variability in measured shoot and leaf respiration rates, or about the relationship between respiration rates and other components of leaf physiology (e.g., photosynthetic activity, enzyme content) or the environment. A better understanding of respiratory physiology will enable us to improve models of leaf-level carbon exchange and aid in scaling fluxes from the leaf to the canopy. At the whole-plant and plant community levels, we need more detailed information about the magnitude of respiration fluxes, and we also need to to know whether the fraction of assimilation used for respiration varies over a relevant time scale (e.g., a year for a forest). Knowing the size of respiratory fluxes is important for deriving carbon budgets, validating models and comparing scaled chamber measurements with ecosystem-scale flux measurements. Knowing whether the fraction of assimilation used for production (carbon use efficiency, CUE) varies is also important for validating models and potentially for simplifying models of regional and global carbon balance.

Respiration, especially maintenance respiration (Rₘ), has been linked with tissue nitrogen content (Jones et al. 1978, Ryan 1991, 1995), because typically 90% of the nitrogen (N) in plant cells is in protein, which needs energy for replacement and repair (about 20% of Rₘ, Bouma et al. 1994) and which is tightly linked with cellular activity. However, respiration rates per unit tissue N can vary (McCree 1982, 1983, Szaniawski and Kielkiewicz 1982, Ryan 1995), and the relationship fails occasionally (Byrd et al. 1992). Understanding how respiration rates vary with nutrition within a single species may help explain the plasticity of respiration rates with respect to nutrition. Such information may be critical for managing the many ecosystems that are currently receiving high N inputs from atmospheric deposition (Wright et al. 1995).
There is evidence that the annual costs of respiration are between 30 and 70% of assimilation (Edwards et al. 1980, Ryan et al. 1994), suggesting that small changes in annual fluxes could have a large impact on net production, particularly if some components (such as wood production) are more plastic than others. Because of the strong dependence of respiration rates on temperature, many models of ecosystem response to climate warming show a decrease in CUE (Ryan et al. 1996). Other analyses suggest that CUE might decrease with increasing woody biomass (Ryan et al. 1994). However, because growth, cellular activity and respiration are tightly linked in plants (Amthor 1994), changes in CUE may be fairly conservative (Gifford 1994). A conservative CUE would simplify models, particularly for deriving global scale carbon budgets.

Because nutrition affects carbon allocation and tissue nutrient concentrations in forests (Gower et al., unpublished observations), and because of the strong link between respiration and tissue nutrient contents, we hypothesized that changes in stand nutrient alter CUE. In this study, we determined how induced changes in nutrient status influence respiration rates of individual tissues (foliage, fine roots and woody tissue). We then developed relationships between respiration and nutrient content or biomass to scale chamber measurements to annual CO$_2$ efflux from respiration. Finally, we calculated how changes in tissue nutrient content and allocation affect CUE for Pinus radiata D. Don stands.

**Methods**

**Study area**

The Biology of Forest Growth experimental plantation (Benson et al. 1992, Raison and Myers 1992) is located at 630 m elevation in the Pierce’s Creek Forest about 20 km west of Canberra in the Australian Capital Territory (35°21′ S, 148°56′ E). The site was cleared of the original eucalypt woodland in 1934, broadcast burned and planted with P. radiata in 1935. The pine crop was clear-felled in 1972, residues burned, and the second pine stand planted in 1973.

The site experiences mild winters (July average minimum and maximum temperatures are 1 and 11 °C) and warm summers (January average minimum and maximum temperatures are 14 and 28 °C). Summer maxima may reach 35–40 °C when relative humidity is low (< 10%). Annual rainfall averages 791 mm, but is highly variable, and summer droughts of 2–3 months are common.

The soil is a yellow podzolic (Stace et al. 1968) or Typic Albaqualf (Soil Survey Staff 1975) derived from granite. It has a duplex profile comprising a sandy A horizon extending to about 0.40 m depth, and a dense (bulk density 1.7 Mg m$^{-3}$) B horizon of clay and gravel overlying a C horizon of weathering granite (> 1 m). The soil has a low N-supplying capacity (Raison et al. 1992b) but provides adequate P for tree growth (Raison et al. 1990). Soil water storage capacity is 75 mm in the A horizon where most fine roots are concentrated, and 250 mm to a depth of 2 m (Myers and Talsma 1992). Because of low soil water storage and a high probability of summer drought, trees often experience severe water stress during the late summer and early autumn (Myers and Talsma 1992).

In winter 1983, several irrigation and fertilization treatments were established on 0.25-ha plots. The treatments we used were control (C), irrigated (I) and irrigated + liquid fertilized (IL). The irrigation treatment maintained soil water near field capacity, and approximately doubled annual rainfall. The IL treatment, which commenced in spring (September) 1984, provided a balanced nutrient input weekly in the irrigation water and was varied seasonally to match tree demand; annual inputs of N totaled 300 kg ha$^{-1}$. After 1990, mineralization of N in the soil and forest floor of the IL treatment was considered to be in excess of tree requirements, and fertilization was halted. The stand was thinned in October 1988, removing 30% of the basal area for the IL treatment and 50% of the basal area for the I and C treatments.

We measured respiration in the C, I and IL treatments in February–June 1993, when the trees were 20 years old. Characteristics of the sample plots are given in Table 1.

**Respiration measurements**

At night in February and March 1993, foliar maintenance respiration ($R_m$, see Amthor 1989) was determined by measuring CO$_2$ efflux from fully expanded foliage on about 30 samples per treatment. Samples were distributed among three canopy positions (canopy thirds) and three foliage age classes (current whorl, current + 1, and older than current + 1), with about three samples for each age class at each canopy position. In the C and I treatments, shoots typically flushed once per year, whereas shoots typically flushed more than once per year in the IL treatment. Measurements were taken from scaffolding towers adjacent to three to five trees.

In March 1993, CO$_2$ efflux from fine roots was measured on 15 samples of roots (< 2 mm in diameter) per treatment in the litter layer or upper A horizon. Roots were isolated by gently digging the soil, removing loose organic matter, and moistening the root mat before placing roots in the measuring cuvette. Cuvette CO$_2$ concentration ([CO$_2$]) averaged 400 ppm during respiration measurements. The respiration from these root

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C</th>
<th>I</th>
<th>IL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density ha$^{-1}$</td>
<td>275</td>
<td>304</td>
<td>412</td>
</tr>
<tr>
<td>Basal area (m$^2$ ha$^{-1}$)</td>
<td>23.3</td>
<td>28.3</td>
<td>50.3</td>
</tr>
<tr>
<td>Projected LAI (m$^2$ m$^{-3}$)</td>
<td>3.1</td>
<td>3.0</td>
<td>4.6</td>
</tr>
<tr>
<td>Average diameter (m)</td>
<td>0.325</td>
<td>0.338</td>
<td>0.390</td>
</tr>
<tr>
<td>Average height (m)</td>
<td>21.2</td>
<td>23.3</td>
<td>23.9</td>
</tr>
<tr>
<td>Stem biomass (Mg ha$^{-1}$)</td>
<td>83.9</td>
<td>107</td>
<td>170</td>
</tr>
<tr>
<td>Branch biomass (Mg ha$^{-1}$)</td>
<td>18.9</td>
<td>18.0</td>
<td>24.7</td>
</tr>
<tr>
<td>Bark biomass (Mg ha$^{-1}$)</td>
<td>8.1</td>
<td>10.4</td>
<td>18.2</td>
</tr>
<tr>
<td>Foliage biomass (Mg ha$^{-1}$)</td>
<td>9.3</td>
<td>7.8</td>
<td>13.3</td>
</tr>
<tr>
<td>Coarse root biomass (Mg ha$^{-1}$)</td>
<td>25.9</td>
<td>33.0</td>
<td>64.0</td>
</tr>
<tr>
<td>Fine root biomass (Mg ha$^{-1}$)</td>
<td>2.6</td>
<td>3.3</td>
<td>1.8</td>
</tr>
</tbody>
</table>
samples was considered to be maintenance respiration, because only 5% of the samples contained root tips. The average soil [CO\textsubscript{2}] of pore space around the sample roots was determined by collecting, with a syringe, twenty 15-ml samples per treatment and analyzing the samples with an infrared gas analyzer (IRGA) (CI-301, CID Inc., Moscow ID) in absolute mode.

The value of \( R_m \) for woody tissues (stems, branches and coarse roots) was estimated either from CO\textsubscript{2} efflux in autumn or winter (stems, coarse roots), or by subtracting construction respiration (estimated from volume growth) from CO\textsubscript{2} efflux measured in growing tissues. Stem CO\textsubscript{2} efflux was measured on 15 stems in each of the three treatment plots as described by Ryan (1990) and Ryan et al. (1995). We used a fixed plate (to ensure a gas-tight seal and resampling at the same location) and a removable chamber (to allow normal ventilation of the bore between sampling). A 12-mm-wide aluminum plate (inside dimension of 100 × 250 mm) was attached to the bark with putty after removing loose bark. A neoprene gasket was attached to the outer surface of the plate. Plates were located on the south side of the tree 1.4 m above the ground. To measure CO\textsubscript{2} flux, we attached a Plexiglas mixing chamber to the plate and connected the chamber to the IRGA. Stem respiration was measured on February 27, March 8 and 17, May 3–6, and July 22–24, 1993. Additionally, CO\textsubscript{2} efflux was measured on March 12, at elevations of 1.4, 12 and 16 m for two trees per treatment adjacent to the scaffolding towers.

On March 12–14, CO\textsubscript{2} efflux from branches was measured at night on nine to ten branches per treatment. Samples were distributed evenly among canopy thirds. Chambers were made of a hollow, split Plexiglas block and were clamped onto the branch over neoprene gaskets at each end of the chamber. Efflux of CO\textsubscript{2} from coarse roots was measured on five samples per treatment on March 17 using chambers similar to those used for branches.

For all tissues, CO\textsubscript{2} efflux was measured with an open system IRGA (LCA3, Analytical Development Corp., Hoddesdon, Herts, U.K., or CI-301, CID Inc.), with flow rates through the chamber of 140–300 µmol s\textsuperscript{-1}. Internal volumes of the chambers ranged from 0.00005–0.0002 m\textsuperscript{3}; air inside the chambers was mixed by a small fan. For all measurements, reference air was drawn through a 0.02 m\textsuperscript{2} mixing chamber to maintain a stable CO\textsubscript{2} concentration. Flux data were recorded after the difference in [CO\textsubscript{2}] between reference air and chamber air was stable for 3 min. Tissue temperature was measured for all samples with copper-constantan thermocouples.

Temperature response

The temperature responses of foliar and fine root respiration were measured in a temperature-controlled cuvette (Hubbard et al. 1995) with measurements at 5, 15 and 25 °C. Foliage temperature response was measured on current-year foliage for three samples per treatment. Fine root temperature response was measured on three samples in the control plot. Temperature responses of stems, branches and coarse roots were estimated by continuously monitoring CO\textsubscript{2} efflux and temperature of one stem, midcanopy branch, or coarse root for 20–50 h. Individual flux measurements were standardized to 15 °C using tissue-specific values of temperature response (\( Q_{10} \)).

Tissue analysis

Foliage was dried at 70 °C for 24 h and weighed to ±0.01 g. Fine roots were sorted into two size classes (> 1 mm, < 1 mm) before drying. Samples were ground in a Wiley mill to pass a 40-mesh screen, digested in H\textsubscript{2}SO\textsubscript{4}-H\textsubscript{2}O\textsubscript{2}, and analyzed for N and P colorimetrically (Heffernan 1985). For stems, we estimated sapwood volume from diameter outside the bark, using a local relationship between diameter and bark thickness (R.J. Raison, unpublished data) and assuming sapwood was 95% of the total volume (based on a sample of felled trees from an adjacent stand). Sapwood volume assigned to the chamber was estimated by multiplying sapwood volume determined for the cylinder under the chamber by the ratio of chamber width to the cylinder’s circumference (Ryan 1990). Volume growth was estimated similarly, using the difference between diameter outside the bark for successive measurements. For branches and coarse roots, we measured diameter outside the bark and estimated bark thickness using samples of similar size from nearby recently felled trees.

Statistical analysis

We used regression analyses to determine how \( R_m \) of foliage and fine roots at 15 °C varied with N or P concentration, and how \( R_m \) of stems, branches and coarse roots at 15 °C varied with sapwood or total volume and wood growth. Regression slopes for the relationship between \( R_m \) at 15 °C and N concentration ([N]) or volume were also compared among treatments (all tissues), canopy position (foliage, branches), and foliage age (foliage) by the MANOVA procedure in SPSS/PC. Differences in respiration with tree height were assessed by a repeated measures analysis of variance and the MANOVA procedure in SPSS/PC. If the intercept in a linear regression was not significant, we estimated a zero-intercept slope and calculated \( R^2 \) as 1 – \( \sum (Y_i - \bar{Y})^2 / \sum (Y_i - \bar{Y})^2 \) (Kvalseth 1985).

Scaling to an annual budget

We estimated an annual budget for respiration for each treatment from measured or estimated biomass (foliage, fine roots, branches, stems, bark and coarse roots), tissue-specific respiration rates, tissue-specific temperature response, and daily maximum and minimum temperatures. Foliage maintenance respiration in the day was estimated as 0.6 times the dark respiration rate (Kirschbaum and Farquhar 1984). Construction respiration for all tissues was estimated using dry matter production and a construction constant of 0.25 (Penning de Vries 1975, Sprugel et al. 1995).

Stem and branch biomass were estimated by allometric relationships with stand basal area, height and density (Magwick 1994). Bark biomass was estimated with a tree-level allometric relationship using individual tree diameter and height (Snowdon and Benson 1992). Coarse root biomass was estimated by an allometric relationship with stem diameter.
(Jackson and Chittenden 1981); the relationship was developed using data from a wide range of *P. radiata* sites and was conservative with respect to site fertility. Biomass estimates for bark and coarse roots were calculated for each tree in a treatment, summed and extrapolated to a hectare basis. Annual growth for a component was calculated as the difference in biomass at the beginning and end of the year. Foliage biomass was estimated from the leaf area index (LAI) determined with a DEMON leaf area analyzer (CSIRO, Canberra, ACT, Australia) and treatment-specific values for leaf area per unit mass (Raison et al. 1992c). Foliar N content was estimated from the N content of the respiration samples.

Fine root biomass was estimated for soil layers 0–0.05, 0.05–0.10 and 0.10–0.20 m based on 12 cores (0.1 m diameter) per treatment in August 1992 and March 1993 (S. Pongracic, unpublished data). Fine root production was estimated from fine root biomass by assuming an annual turnover of 1.45 (cf. Santantonio and Grace 1987, Santantonio and Santantonio 1987a). Fine root respiration was estimated from fine root N content by assuming (1) that soil CO$_2$ concentrations reduce respiration (Qi et al. 1994), and (2) that the measured respiration was maintenance respiration. Efflux of CO$_2$ from fine roots measured at a [CO$_2$] of 400 µmol mol$^{-1}$ was adjusted to the [CO$_2$] (µmol mol$^{-1}$) of the upper mineral soil using a relationship between CO$_2$ efflux ($R_s$) and soil [CO$_2$] developed from other studies of pine roots (M.G. Ryan and R.M. Hubbard, unpublished data):

$$R_s \text{ at } [\text{CO}_2 = x] = R_s \text{ at } [\text{CO}_2 = y] \times 1.00924\exp(-0.00136(y - x)).$$  

(1)

If soil and forest floor organic matter and coarse and fine root biomass are in approximately steady state, total belowground carbon allocation ($B_l$) can be estimated as the difference between annual soil CO$_2$ efflux and aboveground litterfall (Raich and Nadelhoffer 1989). For the treatment stands, soil and forest floor organic matter and fine root biomass were approximately in steady state (S. Pongracic, unpublished data), but coarse root biomass increased substantially over the sample year. Therefore, we added coarse root annual increment to the difference between annual soil CO$_2$ efflux and annual aboveground litterfall to estimate $B_l$. Soil CO$_2$ efflux was measured every 2 weeks with a CO$_2$ absorbent (soda-lime) in a static chamber (0.4 m diameter, 0.1 m headspace, inserted 0.02 m into mineral soil). Annual soil CO$_2$ efflux was estimated by extrapolating the biweekly measurements using soil temperature. The static chamber fluxes were within 20% of fluxes measured in the same chambers with an IRGA (LI-6250, Li-Cor, Inc., Lincoln, NE) across a wide range of soil CO$_2$ fluxes (S. Pongracic, unpublished data).

We estimated canopy assimilation from winter 1992 to winter 1993 using a process-oriented model developed from earlier studies at the same site (BIOMASS, McMurtrie et al. 1990, 1992, McMurtrie and Landsberg 1992). Climate forcing was obtained from a weather station located on site, and simulations were run using treatment-specific values of LAI, woody biomass and foliar N. Canopy assimilation from biomass was compared with the sum of fluxes of respiration and productivity. Standing crops for each component are given in Table 1.

### Results

#### Foliage

Foliar N, P and K concentrations differed significantly among treatments ($P < 0.01$), and foliar [N] differed significantly among foliage age classes and canopy positions ($P < 0.01$) (Table 2). Nitrogen concentration followed the usual pattern of decreases with leaf age and depth in the canopy (data not shown). Phosphorus concentration was significantly lower in the I treatment than in the C or IL ($P < 0.01$) treatments, but did not vary significantly with foliage age ($P = 0.32$) or canopy position ($P = 0.07$). Potassium concentration was significantly greater in the IL treatment than in the C or I treatments ($P = 0.01$), but did not vary significantly with foliage age ($P = 0.63$) or canopy position ($P = 0.12$).

Foliar dark respiration per unit leaf mass for fully expanded foliage at 15 °C varied from 0.45 to 2.7 µmol kg$^{-1}$ s$^{-1}$ and was strongly correlated with foliar [N] (Figure 1). Regression slopes between respiration and [N] did not differ significantly among treatments ($P = 0.28$). However, the relationship between respiration and [N] was more variable for the IL treatment than for the C and I treatments (Figure 1). Because the intercept for a regression that combined the three treatments was not significant ($P = 0.13$), we fitted a zero-intercept linear regression to data from all treatments combined: $R_n = 1.71N (R^2 = 0.38, P < 0.01, n = 93)$, where $R_n$ is CO$_2$ efflux from foliar maintenance respiration at 15 °C (µmol kg$^{-1}$ s$^{-1}$), and N is foliar [N] (mol kg$^{-1}$). Foliar P concentration ([P]) was correlated with foliar [N] ($r = 0.59, P < 0.01$), but [P] did not explain significant additional variation in $R_n$ ($P = 0.53$). There was no difference among treatments in the response of respiration to temperature. Regression to data from all treatments combined:

$$R_n = \frac{1.71}{N}$$

(2)

### Table 2. Mean (SE) foliar N, P and K concentrations as a percent of oven-dry mass by foliage age class for three treatments. Values are averages of all canopy positions.

<table>
<thead>
<tr>
<th>Foliage age</th>
<th>Control</th>
<th>Irrigated</th>
<th>Irrigated + fertilized</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nitrogen</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.34 (0.04)</td>
<td>1.08 (0.03)</td>
<td>1.43 (0.02)</td>
</tr>
<tr>
<td>C + 1</td>
<td>1.18 (0.05)</td>
<td>0.94 (0.06)</td>
<td>1.32 (0.04)</td>
</tr>
<tr>
<td>&gt; C + 1</td>
<td>0.96 (0.04)</td>
<td>0.88 (0.04)</td>
<td>1.07 (0.05)</td>
</tr>
<tr>
<td>All</td>
<td>1.22 (0.04)</td>
<td>0.99 (0.03)</td>
<td>1.35 (0.03)</td>
</tr>
<tr>
<td><strong>Phosphorus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.16 (0.01)</td>
<td>0.11 (0.01)</td>
<td>0.14 (0.01)</td>
</tr>
<tr>
<td>C + 1</td>
<td>0.14 (0.01)</td>
<td>0.10 (0.01)</td>
<td>0.14 (0.01)</td>
</tr>
<tr>
<td>&gt; C + 1</td>
<td>0.14 (0.01)</td>
<td>0.08 (0.01)</td>
<td>0.12 (0.01)</td>
</tr>
<tr>
<td>All</td>
<td>0.15 (0.01)</td>
<td>0.10 (0.01)</td>
<td>0.14 (0.01)</td>
</tr>
<tr>
<td><strong>Potassium</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.87 (0.02)</td>
<td>0.77 (0.03)</td>
<td>0.87 (0.02)</td>
</tr>
<tr>
<td>C + 1</td>
<td>0.77 (0.05)</td>
<td>0.74 (0.03)</td>
<td>0.88 (0.03)</td>
</tr>
<tr>
<td>&gt; C + 1</td>
<td>0.64 (0.05)</td>
<td>0.80 (0.07)</td>
<td>0.91 (0.09)</td>
</tr>
<tr>
<td>All</td>
<td>0.80 (0.03)</td>
<td>0.77 (0.03)</td>
<td>0.88 (0.01)</td>
</tr>
</tbody>
</table>
temperature \( P = 0.57 \); the \( Q_{10} \) (5–25 °C) was 2.5 \( \left( R^2 = 0.99, P < 0.01, n = 9 \right) \).

**Fine roots**

Fine root [N] varied significantly among the three treatments \( P < 0.01 \) and reflected the nutrient status of the stands (Table 3). Fine root [P] was significantly lower in the IL treatment than in the C treatment \( \left( P = 0.04 \right) \), whereas fine root K concentration ([K]) was significantly higher in the IL treatment than in the C and I treatments \( \left( P < 0.01 \right) \). Other treatment comparisons were not significant.

Fine root respiration per unit mass at 15 °C varied from 2.1 to 13.9 \( \mu \text{mol kg}^{-1} \text{s}^{-1} \) and was negatively correlated with sample mass \( (r = -0.63, P < 0.01) \). Fine root respiration was strongly correlated with fine root [N] (Figure 2), and respiration per unit N was only weakly correlated with sample mass \( (r = -0.29, P = 0.06) \). Slopes for a regression between respiration and [N] were not statistically different among treatments \( (P = 0.06) \). Fitting a zero-intercept linear regression to data from all treatments combined gave: \( R_m = 11.2N \) \( (R^2 = 0.51, P < 0.01, n = 44) \), where \( R_m \) is CO\(_2\) efflux from maintenance respiration of fine roots at 15 °C \( (\mu \text{mol kg}^{-1} \text{s}^{-1}) \), and N is fine root [N] \( (\text{mol kg}^{-1}) \). Average soil [CO\(_2\)] in the vicinity of the sampled roots was 1500 \( \mu \text{mol mol}^{-1} \) \( (\text{SD} = 500, n = 60) \), whereas average [CO\(_2\)] of the fine root measurements was 400 \( \mu \text{mol mol}^{-1} \). Fine root respiration increased exponentially with temperature over 5–25 °C, with a \( Q_{10} \) of 2.0 \( \left( R^2 = 0.99, P < 0.01, n = 3 \right) \).

**Table 3. Mean (SE) fine root N, P and K concentrations as a percent of oven-dry mass for three treatments.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>P</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.68</td>
<td>0.14</td>
<td>0.55</td>
</tr>
<tr>
<td>Irrigated</td>
<td>0.50</td>
<td>0.13</td>
<td>0.51</td>
</tr>
<tr>
<td>Irrigated + fertilized</td>
<td>1.03</td>
<td>0.11</td>
<td>0.76</td>
</tr>
</tbody>
</table>

**Figure 1.** Carbon dioxide efflux of fully expanded foliage at night, corrected to 15 °C versus foliage N concentration for *Pinus radiata* growing in three treatments. For all treatments combined, dark CO\(_2\) efflux \( (\mu \text{mol kg}^{-1} \text{s}^{-1}) \) = 1.71N \( (\text{mol kg}^{-1}) \); \( R^2 = 0.38 \). The correlation between CO\(_2\) efflux and [N] was 0.71 for the control and irrigated treatments combined and 0.49 for the irrigation plus fertilization treatment.

**Figure 2.** Efflux of CO\(_2\) from fine roots (< 2 mm in diameter) corrected to 15 °C versus root N concentration for *Pinus radiata* growing in three treatments. For all treatments combined, dark CO\(_2\) efflux \( (\mu \text{mol kg}^{-1} \text{s}^{-1}) \) = 11.2N \( (\text{mol kg}^{-1}) \); \( R^2 = 0.51 \).

**Stems**

Neither CO\(_2\) efflux per unit area \( (P = 0.10) \) nor estimated maintenance respiration \( (P = 0.78) \) varied consistently with tree height (Figure 3). Stem respiration at 15 °C was highest in the IL treatment and lowest in the C treatment, but varied little within treatments from late summer to late winter (Figure 4), perhaps because an average of 74% of the annual growth had occurred before the first respiration measurement. For each...
sample period, stem CO₂ efflux was significantly correlated with chamber sapwood volume, chamber annual volume growth, and chamber volume growth from March to July. Correlation coefficients for the sample periods ranged from 0.44 to 0.67 for sapwood volume and from 0.57 to 0.75 for annual growth ($P < 0.01$). However, sapwood volume was correlated with annual volume growth ($r = 0.60$) and with volume growth from March to July ($r = 0.72$).

Estimated maintenance respiration was also positively correlated with sapwood volume and, in some cases, annual volume growth. For March 17, when measurements made using the upper chambers were included in the analysis, $R_m$ at $15°C$ ($\mu$mol s$^{-1}$) = $18.1V$ ($R^2 = 0.43$, $P < 0.01$, $n = 57$), where $V$ is sapwood volume (m$^3$) (Figure 5). For this sample, March–July volume growth did not explain significant additional variability in $R_m$ ($P = 0.56$). For the May 3–6 sample (no measurements made on upper chambers), $R_m$ at $15°C$ ($\mu$mol s$^{-1}$) = $39.2V$ ($R^2 = 0.39$, $P < 0.01$, $n = 45$). For May 3–6, annual volume growth for the chamber explained significant ($P < 0.01$) additional variability in $R_m$ ($R^2$ increased by 0.11). There were no significant differences among treatments in the regression slopes for $R_m$ and sapwood volume for either March 17 ($P = 0.28$) or May 3–6 ($P = 0.91$). The lower value of $R_m$ (18.1 $\mu$mol s$^{-1}$ m$^{-3}$) was used to develop the annual budget, because measurements on May 3–6 were substantially greater than those taken on the other four sampling dates (Figure 4) and may include growth respiration.

**Branches and coarse roots**

Branch CO₂ efflux rate at night and estimated maintenance respiration rate were linearly related to branch volume, but branches from the upper two thirds of the canopy had substantially greater rates than branches in the lower one third of the canopy (Figure 6). For branches in the upper two thirds of the canopy, $R_m$ at $15°C$ ($\mu$mol s$^{-1}$) = $104V$ ($R^2 = 0.57$, $P < 0.01$, $n = 16$), where $V$ is branch volume (m$^3$). For branches in the lower one third of the canopy, $R_m$ at $15°C$ ($\mu$mol s$^{-1}$) = $33.2V$ ($R^2 = 0.64$, $P < 0.01$, $n = 12$). Regression slopes between $R_m$ and $V$ did not differ among treatments for branches in either the upper two thirds of the canopy ($P = 0.97$) or the lower one third of the canopy ($P = 0.12$). The $Q_{10}$ of the temperature response was 1.4.

Carbon dioxide efflux per unit root volume decreased exponentially with root size (Figure 7). Respiration rates for roots greater than 0.06 m in diameter were substantially lower than for roots less than 0.06 m in diameter. For coarse roots less than 0.06 m in diameter, CO₂ efflux was linearly related to root volume ($R_m$ at $15°C$ ($\mu$mol s$^{-1}$) = $67.8V$ ($R^2 = 0.69$, $P < 0.01$, $n = 10$), where $V$ is root volume (m$^3$)). The $Q_{10}$ of the temperature response was 1.5.

**Annual budget**

Table 4 shows the annual carbon balance for the C, I and IL treatments. The budget uses fine root respiration rates adjusted
Figure 7. Efflux of CO$_2$ per unit root volume at 15 °C versus root diameter ($D$) for Pinus radiata coarse roots growing in three treatments. The equation is: ln(CO$_2$ efflux) = 5.27 − 0.333D; $R^2 = 0.73$.

Table 4. Annual carbon budget (1992–1993) for three Pinus radiata stands. Respiration was partitioned into construction ($R_c$) and maintenance ($R_m$) respiration. Total belowground carbon allocation ($B_c$) was estimated as the difference between annual soil respiration and aboveground litterfall plus coarse root increment. The unknown sink was estimated as the difference between $B_c$ and root respiration and production. All values are given in Mg C ha$^{-1}$ year$^{-1}$; C = control, I = irrigated, and IL = irrigated + fertilized.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C</th>
<th>I</th>
<th>IL</th>
</tr>
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<tr>
<td>Foliage production</td>
<td>0.84</td>
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<td>2.13</td>
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<tr>
<td>Foliage $R_c$</td>
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<td>0.28</td>
<td>0.53</td>
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<tr>
<td>Foliage $R_m$</td>
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<td>6.28</td>
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<td>0.22</td>
<td>0.27</td>
</tr>
<tr>
<td>Branch $R_c$</td>
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<td>0.05</td>
<td>0.07</td>
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<tr>
<td>Branch $R_m$</td>
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<td>1.20</td>
<td>1.87</td>
</tr>
<tr>
<td>Stem wood + bark production</td>
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<td>6.15</td>
<td>10.51</td>
</tr>
<tr>
<td>Stem wood + bark $R_c$</td>
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<td>1.54</td>
<td>2.63</td>
</tr>
<tr>
<td>Stem wood $R_m$</td>
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<td>Total belowground carbon allocation</td>
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<td>Coarse root production</td>
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<td>Coarse root $R_c$</td>
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<td>Fine root production</td>
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<td>Fine root $R_m$</td>
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<td>1.47</td>
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<tr>
<td>Unknown sink</td>
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<td>−0.63</td>
</tr>
<tr>
<td>Total</td>
<td>24.2</td>
<td>25.3</td>
<td>34.4</td>
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<tr>
<td>Assimilation estimated with BIOMASS</td>
<td>29.5</td>
<td>26.9</td>
<td>36.9</td>
</tr>
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</table>

for our measured soil [CO$_2$] of 1500 µmol mol$^{-1}$ using Equation 1. Because of uncertainties in fine root respiration and production in the belowground budget, we consider the sum of dry matter production and respiration of aboveground components and total belowground carbon allocation (i.e., $B_c$ estimated as annual soil CO$_2$ efflux minus aboveground litterfall plus coarse root production) to be the best estimate of annual carbon flux ($F_a$). This calculated total flux is conservative because we did not estimate losses to herbivory or leaching of dissolved carbon. Total annual flux of carbon was 82% of assimilation estimated with BIOMASS in the C treatment, and 93–94% in the I and IL treatments.

The sum of production and construction + maintenance respiration for coarse and fine roots nearly equaled $B_c$ in the IL treatment, but was only 57 and 68% of $B_c$ in the C and I treatments, respectively. If fine root respiration is not adjusted for soil CO$_2$ (i.e., rates measured at 400 ppm CO$_2$ are used), fine root respiration would be 4.4 times greater. In this case, the sum of production and construction + maintenance respiration for coarse and fine roots would be 6, 16 and 74% greater than $B_c$ in the control, irrigated and irrigated + fertilized treatments, respectively. If we use fine root respiration rates adjusted for soil [CO$_2$], the difference between $B_c$ and the total of coarse and fine root production and respiration was 43% of $B_c$ (18% of $F_a$) in the C treatments, 32% of $B_c$ (13% of $F_a$) in the I treatment, and negligible in the IL treatment. This difference between $B_c$ and the total of coarse and fine root production and respiration is an unknown sink that may estimate increased fine root turnover or carbon allocation to root exudation and mycorrhizae, but may also represent cumulative errors in sampling or extrapolation.

The treatments caused shifts in carbon allocation in coarse and fine root production, fine root respiration, foliage production, foliage $R_m$, wood + bark production, and in the unknown belowground sink (Figure 8). Branch production and wood and branch $R_m$ were consistent among treatments. Construction respiration for all tissues was 9–13% of assimilation for all treatments; the fraction of assimilation used for $R_m$ was slightly lower in the I treatment (30%) than in the C and IL treatments (35 and 39%, respectively). Woody-tissue (stem + branch + coarse root) respiration was 22–26% of the annual assimilation, and woody tissue $R_m$ was 15–17% of annual assimilation for all treatments (Figure 8). We did not calculate CUE for the whole plant because of uncertainty in the belowground budget. However, if we subtract $B_c$ from $F_a$ to estimate assimilation allocated to aboveground components and calculate CUE for aboveground respiration, CUE is conservative among treatments (0.43, 0.50 and 0.48 for C, I and IL treatments, respectively).

Discussion

Foliage

Altering nitrogen availability altered foliar $R_m$ and foliar [N] in P. radiata. The fertilization (IL) treatment increased both foliar N and $R_m$ but also increased variability in $R_m$/N. Two explanations could account for the increased variability in $R_m$/N. First, the amount of N in protein could vary if fertilization increased the proportion of foliar N in amino acids (Nasholm and Ericsson 1990, Billow et al. 1994) and also increased variability in the ratio of amino acids to total N within the IL canopy. Second, fertilization could have increased the variability in the activation of Rubisco. Stitt and others (e.g., Stitt and Schulze
It is unclear whether the high rates represent substantially greater metabolic activity in roots than in leaves or whether the measured high rates represent a transient response to a lower CO\textsubscript{2} environment during measurement. However, several factors suggest a transient response to lower CO\textsubscript{2} concentration: (1) elevated CO\textsubscript{2} can suppress CO\textsubscript{2} efflux from foliage (e.g., Amthor 1991) and fine roots (Qi et al. 1994); (2) fine root CO\textsubscript{2} efflux was also strongly inhibited in *Pinus resinosa* Ait., *Pinus strobus* L. and *Tsuga heterophylla* (Raf.) Sarg. (Equation 1); (3) extrapolating fluxes measured at a [CO\textsubscript{2}] of 400 \textmu mol mol\textsuperscript{-1} to a year would yield a much greater annual soil CO\textsubscript{2} efflux than was measured for two of the three treatments; and (4) if the fine roots respired at 23% of the measured rate in situ, \( R_m/N \) at 15 °C would be 2.6 \textmu mol mol\textsuperscript{-1} s\textsuperscript{-1}, or 1.5 times that of foliage. Although fine root respiration was not measured at the CO\textsubscript{2} concentration of the soil in which the root was growing, it seems reasonable to suppose that respiration measurements made at a [CO\textsubscript{2}] far lower than the soil environment would be artificially high. Further work to clarify the role of roots in soil respiration will be especially important, given the high rates at which roots respire.

**Woody tissue**

If it is assumed that the wood in branches and coarse roots was largely sapwood, then stems, coarse roots > 0.06 m in diameter, and lower branches had similar rates of maintenance respiration per unit of sapwood volume (15–39 \textmu mol m\textsuperscript{-3} s\textsuperscript{-1}). These rates are similar to those reported for other conifers. For example, \( R_m \) ranged from 6.4–11.5 \textmu mol m\textsuperscript{-3} s\textsuperscript{-1} at 15 °C for three pines and western hemlock (Ryan et al. 1995), and \( R_m \) in lodgepole pine was 17.1 \textmu mol m\textsuperscript{-3} s\textsuperscript{-1} (Ryan 1990). Respiration rates of branches from the upper two thirds of the canopy were larger than those of coarse roots < 0.06 m in diameter, but rates for both tissues were similar to those reported for young *Abies amabilis* Dougl. ex J. Forbes. trees (86 \textmu mol m\textsuperscript{-3} s\textsuperscript{-1} at 15 °C, Sprugel 1990). The high rates of stem respiration in the May samples in the well-watered trees in the I and IL treatments may be associated with late-autumn growth because the average temperature for the 2 weeks before sampling was 14 °C. The trees in the C treatment may have been drought stressed because only 19 mm precipitation fell in the 30 days before measurement and, therefore, they may not have been able to respond to the higher temperatures.

Differences in \( R_m/volume \) between branch positions and with coarse root size might have been caused by differences in sapwood volume among the samples or by differences in the activity of the ray parenchyma cells. Additionally, the correlation between stem \( R_m \) and annual growth suggests physiological differences in \( R_m \) related to growth. Differences in tissue N content might explain some of the differences in rates because \( R_m \) has been shown to increase with wood N content in *Pinus taeda* L. (Maier and Dougherty 1994).

**Annual budget**

Differences in N availability among treatments (Raison et al. 1990, 1992a) caused differences in carbon allocation patterns,
particularly in allocation to wood + bark and in total belowground allocation. Of the 42% increase in assimilation in the IL treatment compared with the control, roughly half was allocated to increased wood production.

Increased nutrient availability in the IL treatment decreased fine root biomass and, hence, allocation to fine root production and respiration. These results are consistent with observed decreases in absolute and relative allocation to fine root production in response to fertilization (Keys and Grier 1981, Gower et al., unpublished observations). In our study, the decreased allocation to fine roots in the IL treatment was offset by increased allocation to coarse roots. Therefore, the sum of production and respiration of coarse and fine roots was greater in the IL treatment (8.0 Mg C ha\(^{-1}\) year\(^{-1}\)) than in the C treatment (5.8 Mg C ha\(^{-1}\) year\(^{-1}\)). Total root production and respiration as a fraction of \(F_i\) were similar for all treatments.

The belowground portion of the carbon budget was based on several assumptions. Calculation of \(B_i\) depends on robust estimates of annual soil CO\(_2\) efflux, annual litterfall, and annual changes in soil and litter carbon and root biomass. Annual soil CO\(_2\) efflux was estimated from fluxes measured twice each month with a static chamber technique and a CO\(_2\) absorbent, and static fluxes were within 20% of fluxes measured with an IRGA (S. Pongracic, unpublished data). Soil and litter carbon were measured and found to be roughly constant from year to year. Coarse root increment was estimated with an allometric equation developed from a wide range of \(P.\) radiata sites, with very different site fertilities; coarse root increment is likely to be accurate for the different treatments. Therefore, we conclude that \(B_i\) is a reasonably accurate estimate of total belowground carbon allocation.

Fine root biomass measurements were similar in the spring and fall. However, turnover may differ with treatment. If turnover rates were greater in trees in the infertile C and I treatments than in trees in the fertilized IL treatment, allocation to fine root production would increase in the C and I treatments and lower the estimate of the unknown sink. Fine root turnover would need to be 4.2 times biomass in the C treatment and 3.1 times biomass in the I treatment to balance the belowground budget.

We assumed that fine root respiration was affected by soil CO\(_2\) concentration and that the soil [CO\(_2\)] (1500 \(\mu\)mol mol\(^{-1}\)) was constant throughout the year. These assumptions seem reasonable, given the known response of root respiration to [CO\(_2\)] (Qi et al. 1994). However, if we used the response of fine root respiration to soil [CO\(_2\)] reported by Qi et al. (1994), respiration at 1500 \(\mu\)mol mol\(^{-1}\) would be 67% of that at 400 \(\mu\)mol mol\(^{-1}\) (compared with 23% using Equation 1). If we estimate annual budgets by assuming fine root respiration was not affected by soil [CO\(_2\)], the annual total of root production and respiration would exceed \(B_i\) in the C and I treatments. Additionally, the fraction of root respiration to total soil respiration for unadjusted rates would be larger than that reported for other studies. In this study, root/total soil respiration was 28–60% for fine root respiration adjusted for soil CO\(_2\) versus 79–137% for unadjusted root respiration. Other conifer studies estimate root/total soil respiration at 33 (Bowden et al. 1993), 47–51 (Nakane et al. 1983) and 62% (Ewel et al. 1987). We did not adjust coarse root respiration for soil [CO\(_2\)] because the internal [CO\(_2\)] of sapwood is high (1.5–8%, Hari et al. 1991).

An increase in fine root turnover or a decrease in the response of fine root respiration to soil [CO\(_2\)] would lower the unknown sink in the belowground budget. However, allocation to mycorrhizae and root exudation might also differ among treatments and provide an alternative explanation for the unknown sink. Mycorrhizae use a substantial fraction of belowground carbon allocation in nutrient-poor ecosystems (Vogt et al. 1982). Additionally, fertilization has been shown to reduce mycorrhizal infection and activity (Blaise and Garbaye 1983), and mycorrhizal infection appeared to be lowest in trees in the IL treatment.

Annual budgets estimated by summing aboveground production and respiration and total belowground allocation agreed well with annual assimilation estimated by the BIOMASS model. However, the agreement between model prediction and field measurements is not surprising because the model was developed from data at the Biology of Forest Growth site (see Ryan et al. 1996).

Carbon use efficiency appears to be conservative with respect to nutrition. Nutrition altered the fraction of annual assimilation used for foliage and fine root respiration, but increased foliar respiration in the IL treatment was offset by a decrease in fine root respiration. The canopy in the I treatment appeared to be slightly more efficient than the canopies in the C and IL treatments, i.e., foliar \(R_m\) used only 11% of annual assimilation in the I treatment, compared with 17–18% in the C and IL treatments. As a fraction of total assimilation, construction and maintenance respiration were both conservative across treatments (9–13% for construction, and 30–39% for maintenance).

The strong link between photosynthesis, cellular activity, growth and respiration (Amthor 1994) may explain the conservative CUE. Photosynthesis supplies the substrate for respiration, and photosynthetic capacity is linked with foliar enzyme levels. Growth and respiration are linked, and growth is limited to some extent by the supply of carbohydrates produced by photosynthesis. Therefore, more rapid growth is linked with an increased supply of carbohydrates, the maintenance respiration required to support higher cellular activity, and higher growth respiration at greater growth rates. Additionally, the low cost for maintenance of structural tissue indicates that support costs are unlikely to change much with treatment differences in woody biomass. The lack of change in CUE with large changes in assimilation, standing biomass, tissue N concentrations and annual allocation suggests that, at a given annual temperature, CUE may be conservative for forests.

Acknowledgments

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