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RESEARCH PAPER

Storage versus substrate limitation to bole respiratory potential in two coniferous tree species of contrasting sapwood width

Michele L. Pruyn[1,*], Barbara L. Gartner[1,2] and Mark E. Harmon[1]

[1] *Department of Forest Science, Oregon State University, Corvallis, OR 97331-5752, USA*

[2] *Department of Wood Science and Engineering, Oregon State University, Corvallis, OR 97331-5751, USA*

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Abstract

Two coniferous tree species of contrasting sapwood width (*Pinus ponderosa* L., ponderosa pine and *Pseudotsuga menziesii* Mirb., Douglas-fir) were compared to determine whether bole respiratory potential was correlated with available storage space in ray parenchyma cells and/or respiratory substrate concentration of tissues (total nitrogen content, N; and total non-structural carbohydrate content, TNC). An increment core-based, laboratory method under controlled temperature was used to measure tissue-level respiration (termed respiratory potential) from multiple positions in mature boles (>100-years-old). The most significant tissue-level differences that occurred were that N and TNC were two to six times higher for inner bark than sapwood, TNC was about two times higher in ponderosa pine than Douglas-fir and there was significant seasonal variation in TNC. Ray cell abundance was not correlated with sapwood respiratory potential, whereas N and TNC often were, implying that respiratory potential tended to be more limited by substrate than storage space. When scaled from cores to whole boles (excluding branches), potential net CO_2 efflux correlated positively with live bole volume (inner bark plus sapwood), live bole ray volume, N mass, and TNC mass (adjusted $R^2 \geqslant 0.4$). This relationship did not differ between species for N mass, but did for live bole volume, live bole ray volume, and TNC mass. Therefore, N mass appeared to be a good predictor of bole respiratory potential. The differences in net CO_2 efflux between the species were largely explained by the species' relative amounts of whole-bole storage space or substrate mass. For example, ponderosa pine's inner bark was thinner than Douglas-fir's, which had the greater concentration of ray cells and TNC compared with the sapwood. This resulted in ponderosa pine boles having 30–60% less ray volume and 10–30% less TNC mass, and caused ponderosa pine net CO_2 efflux/ray volume and net CO_2 efflux/TNC mass to be 20–50% higher than Douglas-fir. In addition, because inner bark respiratory potential was 2–25 times higher than that of sapwood, ponderosa pine's thinner inner bark and deeper sapwood (relative to Douglas-fir) caused its bole net CO_2 efflux/live bole volume to be 20–25% lower than that of similarly-sized Douglas-fir trees.

Key words: Bole respiration, nitrogen, non-structural carbohydrates, ray parenchyma, sapwood width, xylem anatomy.

Introduction

Autotrophic respiration is a key physiological process. Along with gross primary production, it determines the net input of carbon to plants (net primary production). The controls for autotrophic respiration are not completely understood. One possible respiratory determinant in trees is the amount and vitality of living wood in the bole (i.e. inner bark and sapwood). Tree boles with greater volumes of living tissue will possibly have more respiring parenchyma cells, providing more sites for storage of respiratory substrate (e.g. nitrogen, proteins, and total non-structural carbohydrates) and potentially greater respiratory losses from the main bole. However, the frequency of parenchyma cells alone does not indicate respiratory activity, because it also depends on their vitality and longevity, which may be

* To whom correspondence should be addressed. Fax: +1 541 737 3385. E-mail: Michele.Pruyn@oregonstate.edu

related to their function (e.g. growth, storage, phloem loading, ion uptake/exchange, and wound response) and/or the concentration of available respiratory substrate.

Ponderosa pine (*Pinus ponderosa* L.) has wide sapwood (often about 15–20 cm in the radial direction with 50–200 annual rings), whereas Douglas-fir (*Pseudotsuga menziesii* Mirb.) has relatively narrow sapwood (often about 5 cm in the radial direction with 15–40 rings). Because ray parenchyma occupies 6.8% and 7.4% of the sapwood volume in ponderosa pine and Douglas-fir, respectively (Panshin and de Zeeuw, 1980), ponderosa pine may have 2–4 times more sites for respiration and storage than a similarly-sized Douglas-fir tree. Thus, if sapwood amount correlates positively with respiratory loss, ponderosa pine's losses should be greater than those of Douglas-fir. Instead, the opposite was shown (Pruyn *et al*., 2003): in a species sample including Douglas-fir and ponderosa pine, sapwood respiratory potential (a woody tissue-based, laboratory estimate of respiration) was 1.3–3 times greater in species with small sapwood volumes than in those with large volumes for large, old-growth trees. Those results suggested that sapwood respiration was not limited by sapwood quantity alone, but by other factors, such as frequency, vitality, and content of the sapwood parenchyma.

Literature results on patterns of parenchyma size and abundance within conifer sapwood vary considerably, showing ray homogeneity in the sapwood; pith-to-bark increases in ray size, but decreases in ray frequency; or increases/decreases in only specific annual rings near the bark or pith (e.g. pith-to-bark trends in Douglas-fir, Gartner *et al*., 2000; and in other conifer species, Bannan, 1937, 1954; Gregory and Romberger, 1975; Lev-Yadun, 1998; tree base-to-tree top trends in conifers, Jaccard, 1915; Bannan, 1965; Gartner *et al*., 2000). Thus, the general impression of conifer sapwood is that storage sites are available throughout boles and not particularly concentrated to specific areas. Despite this apparent uniformity in sapwood storage site availability, ray metabolism varies with bole position. On a dry mass basis, respiratory potential of the outer sapwood of *P. ponderosa* was 30–60% higher than that of the middle or inner sapwood, depending on which heights within the tree and/or which tree ages were being compared (Pruyn *et al*., 2002*a*). In the same study, sapwood rings produced in the same calendar year released over 50% more CO_2 in treetops than in tree bases. Similar within-tree patterns were found within boles of *P. menziesii* (Pruyn *et al*., 2002*b*). One possible explanation for these patterns is that ray cell vitality may vary within the bole. In fact, ray cell vitality (indicated by changes in nuclear morphology) has been shown to decline from outer to inner sapwood in various conifer species (Frey-Wyssling and Bosshard, 1959; Yang, 1993; Gartner *et al*., 2000). Ray vitality was also found to be highest at the base of the live crown and to decrease from there towards both the tree base and the tree top (Yang *et al*., 1994).

If parenchyma abundance is homogenous throughout boles, then differences in vitality among sapwood parenchyma locations suggest that bole respiration may instead be controlled by the availability of substrates for respiration. Woody tissue nitrogen and total non-structural carbohydrate (TNC) levels serve largely as a proxy for proteins, and for sugars and starches, respectively. Thus, both nitrogen and TNC may be used to predict local respiration in plant tissues. As a caveat, however, their abundance may not relate to respiration if they are stored and then used at other times or locations, or in different processes. Tissue-level, respiratory activity (O_2 uptake) correlated positively with stem nitrogen concentration in *Fraxinus nigra* L. (Goodwin and Goddard, 1940) and with respiratory enzyme activity in *Pinus radiata* D. Don (Shain and Mackay, 1973). Studies on the organ-level have shown that leaves, stems, and root respiration are closely related to their Kjeldahl nitrogen contents (Ryan, 1991, 1995; Ryan *et al*., 1996; Pregitzer *et al*., 1998).

In contrast to nitrogen, there are few direct links between TNC concentrations and actual tree respiration in the literature. Reduced soluble carbohydrates from bark to pith were identified for *Pinus sylvestris* L. (Saranpää and Höll, 1989) and significant seasonal variation in the storage and mobilization of starch, sugars, protein, and fat occurred in the sapwood of *Populus*×*canadensis* Moench '*robusta*' (Sauter and van Cleve, 1993, 1994), but the possible correlation to respiration was not investigated. Strong correlations between respiration and carbohydrate concentration have been identified for growing tissue (Ryle *et al*., 1976; Penning de Vries *et al*., 1979), but not for mature tissues or organs, especially those involved in storage (Coggeshall and Hodges, 1980; Farrar, 1985): an observation that is consistent with theoretical predictions from respiration models (Dewar, 2000). More research is restricted to herbaceous species (e.g. *Glycine* spp., *Lolium multiflorum*, *Zea* spp., citations in previous sentence), woody shrubs (e.g. *Larrea tridentata*, Cunningham and Syvertsen, 1977) and leaves (*Festuca arundinacea* Schreb, Moser *et al*., 1982), suggesting a need for tissue-level studies on the correlation between respiration and TNC contents in tree boles, especially since tree bole tissues are devoted to multiple functions, e.g. translocation (phloem), growth (cambium), storage (sapwood), and development (transition from sapwood to heartwood).

The objectives of the current study were first to investigate the relationships between respiration and abundance of ray cells (as a proxy for sites and storage for respiration), nitrogen and/or TNC content (as a proxy for respiratory substrate) on the tissue level; and second, to scale-up to determine whether the tissue level trends persisted on the whole bole level. This research was undertaken in ponderosa pine and Douglas-fir to compare the results in coniferous species with differing quantities of live bole (inner bark plus sapwood) and to test the following

two hypotheses. (i) Tissue-level respiration is not limited by physical storage capacity of the bole tissue (rays), but rather by enzyme (nitrogen) and metabolic substrate availability (total non-structural carbohydrates). (ii) At the whole-bole level, there is a strong positive correlation between respiratory demand (the total respiration expenditure for the tree's bole tissue, or whole-bole, potential net CO_2 efflux) and ray and substrate abundance; inter-species differences in this relationship indicate different storage and metabolic strategies for the live bole.

Materials and methods

Study areas and species characteristics

The ponderosa pine trees were located just east of the Cascade Range in central Oregon, near Gilchrist (43° 28′ N, 121° 41′ W, elevation 1355 m). Samples were collected from trees more than 240 years old in early September of 1999. The Douglas-fir trees were located just east of the Coast Range in southern Oregon, near Riddle (42° 57′ N, 123° 22′ W, elevation 215 m). Samples were collected from trees more than 100 years old in September of 1998. The size and age characteristics of the trees are presented in Table 1. Measurements were not taken during the growing season (March–September) to ensure that measured respiration rates represented only maintenance respiration and not growth respiration. Growth respiration is more likely than maintenance respiration to depend on hormonal or other stimuli and on carbohydrate supply from outside the immediate xylem stores (McCree, 1970; Thornley, 1970). Ponderosa pine and Douglas-fir trees in the current study were selected from sites used in previous studies (Pruyn *et al*., 2002*a*, *b*), however, different trees were used and none of the previously published data appears here.

Cores were also collected once monthly at breast height from four mature trees of both species (100–250-years-old, with stem diameter-at-breast-height and height similar to the harvested individuals) from 1998–2000 at the same sites as the harvested trees. Respiratory potential was measured for each month, and a subsample of months (five) was chosen for chemical analysis. Months that were selected for the subsample were those with significantly higher respiratory potentials and the months before and after the higher-respiration month for comparison.

Tree felling and sampling

Twenty trees were selected randomly for each species that were free of broken tops, bole deformities, or visible disease. Six trees of each species (from the randomly selected 20) were chosen for study to avoid individuals that were clumped or would be hard to fell later. The diameter at 1 m was recorded (Table 1). After felling the ponderosa pine trees, 20-cm-tall discs were sawed from boles at node 240 years from the treetop (base), and just above (to avoid branch whorls within the crown) nodes 65 (lower), 50 (upper), and 15 (top). The same method was used for the Douglas-fir trees, except that bole discs were sampled from nodes 100 (base), 95 (lower), 35 (upper), and 15 (top). Tree height measurements were taken from tree base to base of the live crown (first bole position above ground level with three live branches), to treetops (Table 1), and to each sampled node. Discs for respiration measurements were transported to the laboratory wrapped in extra-strength black plastic bags with moist paper towelling inside to reduce desiccation, and then stored in these bags at 4 °C. Within 1 week after harvesting, three 12 mm diameter increment cores were extracted in the radial direction from each node, wrapped in plastic bags and returned to cold storage.

Tree leaf area above each sampled node was determined as described by Pruyn *et al*. (2002*a*). Each tree was divided into four sections. Twenty-five per cent (one of every four leaf bundles) of the leaves were hand-clipped (including attached woody material) from large crown sections (nodes 35–240), and 100% of the leaves from small sections (node 15). The fresh mass of the clipped material was then recorded. Fresh subsamples of the clipped material were weighed, dried, and sorted, leaves from woody twigs and branches, and dry mass for each was recorded. Ten needle fascicles from the clipped material were collected and stored at −10 °C in the

Table 1. *Age and size characteristics for September-sampled ponderosa pine and Douglas-fir trees: means (*n=*6) ±SE*

By tree		Ponderosa pine		Douglas-fir
Age (years old)		243±10		101±1
Bole diameter, breast height (cm)		70±2		61±2
Tree height (m)		34±0.4		44±1
Height to base of live crown (m)		16±1		27±1
Sapwood area at base (cm^2)		2622±162		652±77
Mean foliage area/sapwood area at base ($m^2\ cm^{-2}$)		0.29±0.03		0.50±0.07
By radial position	**Inner bark**	**Outer sapwood**	**Middle sapwood**	**Inner sapwood**
Ponderosa pine				
Age (bark to pith, number of rings)				
Base (node 240)	0	21±1	63±3	105±6
Lower (node 65)	0	12±1	36±4	60±6
Upper (node 50)	0	6±0	19±1	32±2
Top (node 15)	0	3±0	9±1	16±1
Volume (m^3)	0.1±0.01	1.7±0.2	1.4±0.1	1.0±0.1
Mass (kg)	27±3	664±48	526±31	384±22
Douglas-fir				
Age (bark to pith, number of rings)				
Base (node 100)	0	5±1	15±3	25±5
Lower (node 95)	0	4±1	13±2	22±3
Upper (node 35)	0	3±0	8±1	13±2
Top (node 15)	0	2±0	6±1	9±2
Volume (m^3)	0.4±0.03	0.7±0.1	0.6±0.1	0.6±0.1
Mass	218±19	362±45	330±39	281±35

laboratory, until leaf dimensions were measured to calculate leaf area. These leaves were then dried and the mass recorded. The proportion of leaf area to dry leaf mass to fresh leaf mass gave total leaf area for each tree.

Core segment preparation

Cores were divided into four segments: inner bark (phloem and cambium) and outer, middle, and inner sapwood. Any green (photosynthetic phelloderm) tissue that was visible just underneath the bark surface on the inner bark samples was removed with a razor blade. Sapwood was defined as the woody tissue extending from the outermost growth ring to the innermost growth ring before the heartwood. Transition zone rings (one or two lighter coloured rings at the sapwood/heartwood boundary) were not included in the samples. Outer, middle, and inner sapwood samples were obtained by dividing sapwood into three equal radial lengths. The exception was in cores extracted from ponderosa pine trees more than 240 years old, where sapwood was greater than 100 growth rings wide. In these cases, core segments of 10–15 growth rings in length were taken within the sapwood from the outer and inner sapwood boundaries and from the centre of the sapwood. Fresh mass and number of rings (to determine age) were recorded for each core segment. Core segment fresh volume was estimated as the mass (1 g H_2O=1 cm^3) of the water displaced when samples were submerged (D2395, ASTM 2001). Oven-dried masses (60 °C for 48 h) were recorded.

Ray size and distribution

One of the three oven-dried core segments from each sapwood radial and vertical bole position (used in the respiratory analysis) was selected randomly for microscopic analysis for six trees of each species. Inner bark core segments were not analysed because the tissue was needed for the chemical analyses. Tangential sections were made with a sliding microtome in the middle of the earlywood from two (younger tissues) or four growth rings throughout the core segment. Eight tangential sections were made per core segment (two or four per ring, depending on number of rings sampled), stained in safranin, and permanently mounted.

Average ray area (average of individual rays in the tangential view), ray frequency (the number of rays mm^{-2} tangential section) and ray proportion (proportion of sapwood tangential area occupied by rays=sum of all ray areas divided by the sum of the area viewed) were calculated. Assuming that the same ray proportion would be found at any nearby depth, ray volume within the bole was calculated by multiplying the ray proportion by the corresponding tissue volume. Area, frequency, and ray proportion were estimated using an image analysis system and following the protocol of Gartner *et al*. (2000). A compound microscope (Nikon Labophot-2, Nikon Microscopy, Melville, NY, USA) with a ×10 objective lens and a colour video camera (Sony CCD/RGB, Sony Electronics Inc., Park Ridge NJ, USA) were used to view the slides. The microscopic image of the tangential section was projected onto a colour monitor (33 cm diagonal distance, Sony Trinitron, Sony Electronics Inc., Park Ridge NJ, USA) and onto a computer monitor (Apple Macintosh Quadra 800) by way of a digitizing card. One or two fields of view (1.00×0.80 mm each) were randomly selected from each tangential section made, for a total of eight fields per core segment. All rays within each field of view were highlighted using the paint tool in NIH Image v. 1.60 (Rasband, 1996). Entire rays (both simple and fusiform) were highlighted, including cell walls, ray tracheids, and for fusiform rays, epithelial cells. The resin canal itself was excluded from fusiform rays.

Chemical analyses

Oven-dried core segments were ground to pass a 20-mesh screen and then analysed for nitrogen using a LECO CHN-1000 Analyser (LECO, 3000 Lakeview Ave., St Joseph, MN 49085-2396, USA) that measured tissue total carbon content (%) and total nitrogen content (%) via combustion and subsequent gas analysis. Total non-structural carbohydrate content (%) of the ground tissue was determined using a sulphuric acid solution to extract and hydrolyse carbohydrates to reducing sugars, which included the simple sugars, dextrin, starch, and fructusan, but not the structural carbohydrates such as hemicellulose and cellulose (AOAC, 1965). The Nutritional Analysis Laboratory in the Rangeland Ecosystem Science Department at Colorado State University conducted all chemical analyses (Natural Resource Bldg. Rm. 240, Fort Collins, CO 80523-1478, USA).

Tissue chemical contents were compared within and between the ponderosa pine and Douglas-fir trees. The analysed tissue was from a combination of two or three replicates of the oven-dried, core segments from the respiratory analyses. Inner bark, outer sapwood, and inner sapwood core segments were analysed from three bole heights (base, lower or upper, and top) of the ponderosa pine trees and from two heights (base and top) of mature Douglas-fir. To calculate the moles of nitrogen in each sample, core segment dry mass was multiplied by its nitrogen percentage and divided by nitrogen's atomic weight.

Respiratory measurements

Prior to anatomical or chemical analysis, core segment respiration was measured using a previously developed protocol (Pruyn *et al*., 2002*a*, *b*). After core extraction and segment preparation, core segments were wrapped in plastic and stored at 4 °C until the night prior to analysis. Segments were then transferred to a 25 °C incubator and stored overnight to allow metabolic activity in core segments to stabilize (Goodwin and Goddard, 1940; Levy *et al*., 1999). Immediately prior to measurement, core segments were reweighed and placed in vials, which were then sealed with gas-tight rubber septa. To determine a rate of CO_2 production, the CO_2 concentration within vials was measured with a Hewlett-Packard (5700A) gas chromatograph (GC, Hewlett Packard, Rt. 41, Avondale, PA 19311, USA) immediately after closing the vials and again after an incubation period. Core segments were incubated at 25 °C for 6 h between GC measurements. Details of GC analysis and calculation of respiratory potential (nmoles CO_2 s^{-1}, on a core segment volume, core segment dry mass, or moles nitrogen basis) in Pruyn *et al*. (2002*a*, *b*). Immediately following the GC analysis, core segments were weighed a third time. Changes in the three successive wet masses verified that water loss was low (1–3%) between sampling and measurement. Respiratory potential on each basis (mass, volume, ray volume, and moles nitrogen) was plotted against growth ring number from bark. The reported values are referred to as respiratory potential, rather than respiration rate, because measurement conditions of these excised samples were probably different from those within the tree.

Scaling core segment measurements to the whole-bole level

To understand whole-bole level (excluding branches) trends and the effect of the metric used to scale-up, net CO_2 efflux was scaled to whole boles in the September-sampled ponderosa pine and Douglas-fir trees. Core segment respiratory potential was scaled to boles using four cylinders (one for each vertical position sampled) to represent bole segment volumes along the bole heights, as described previously in Pruyn *et al*. (2002*a*). In addition to scaling to whole-bole live volume (inner bark plus sapwood), respiratory potential was also scaled to whole-bole ray volume, nitrogen mass, and total non-structural carbohydrate mass. Ray volume in boles was calculated by multiplying the ray proportion (measured from core segments) to the corresponding volume of tissue at each radial and vertical bole position. Because the ray proportion in the inner bark was not measured, an estimate of 92% was used to represent the living cells of the inner bark for both species. This estimate was based on the

knowledge that non-living elements of inner bark are primarily sclereids (Mauseth, 1988), which in Douglas-fir inner bark approximated 7.5% (Ross and Krahmer, 1971). Total nitrogen and non-structural carbohydrate contents of whole boles were determined by multiplying each radial position's volume at each bole vertical position by its density (oven-dry mass/green volume, determined from core segments) to obtain the oven-dry mass. The proportions of total nitrogen and non-structural carbohydrate from each radial position were multiplied (separately) by the corresponding mass for each bole radial and vertical position. Each radial position's volume or mass for each bole height was then multiplied by the respective respiratory potential on a core segment volume or mass basis to calculate its net CO_2 production. Whole-bole net CO_2 efflux was calculated by summing the volumetric rates of each radial and vertical bole position.

Statistical analyses

All data were analysed in Statistical Analysis Systems software, release 8.0 (SAS Institute Inc., 1998). The Shapiro–Wilk *W*-test was used to determine whether the response variables were distributed normally. A transformation (square-root or logarithmic) was performed when necessary to meet assumptions of normality and constant variance. Least squares means (LSMEANS), generated from the various SAS procedures described below, are reported ±pooled SE, or confidence interval (CI) for transformed variables. Within a specific table or figure, if the CI was required for one variable, it was presented for all. To compare ray anatomical characteristics, tissue chemical contents, and respiratory potential between ponderosa pine and Douglas-fir trees, least squares means (LSMEANS) were generated using PROC MIXED, with a randomized block design and strip-plot (split-block) treatments (Little and Hills, 1978; Milliken and Johnson, 1984). To account for correlation within trees, individual trees were assigned as blocks, with random effects of block and the interactions among block, bole radial and vertical positions. For core anatomy and tissue chemical content, effects of bole radial position and vertical position, and their interaction were tested separately for each species. Significant differences (P <0.05) were identified via pair-wise comparisons (*t*-tests) among bole positions and between species using Fisher's Protected Least Significant Difference (FPLSD) procedure (Fisher, 1966). A comparison of regression lines was used with the random effects accounting for within-bole variation to compare the relationship between average ray area and ray frequency between species. To compare respiratory potential and tissue chemical contents among sampling dates, a repeated measures analysis was used to test each variable separately for each species, with a subject of tree by radial position and an unstructured covariance matrix.

For core segment respiratory potential per unit volume, dry mass, ray volume or nitrogen, the effects of tissue age (growth ring number from bark), species and their interaction were tested using a comparison of regression lines, including random effects to account for within-bole variation. The comparison of regression lines was also used to understand the relationship of core respiratory potential on a mass basis to tissue anatomy or chemical content for each species. These regressions were performed for the inner bark and sapwood combined, and for the sapwood alone. The effects of ray proportion, total nitrogen content, or total non-structural carbohydrate content were tested separately, along with the interaction between species and ray proportion or tissue chemical content. Parameter estimates for these regressions should be interpreted with care because of the lack of independence between inner bark, outer sapwood, and inner sapwood. Although their correlation was accounted for in the regression model, the specified correlation matrix may not entirely reflect the natural variation within trees.

To compare whole-bole level trends in net CO_2 efflux, a comparison of regression lines was used to test the significance of species, scaling index (i.e. live bole volume, ray volume, nitrogen mass, and total non-structural carbohydrate mass), and their interaction. Standard errors were not scaled up with the means from core to whole-bole level for two reasons. (i) Because the ray anatomy and tissue chemical content analysis were only conducted on one core per radial and vertical position, there were no estimates for within tree variation for these explanatory variables. (ii) The error in the response variable was incorporated into the residual error and thus the assumption was that the error was approximately the same for all of the trees. The whole-bole values presented are merely estimates of *in situ* activity, and the variance among the sample size of 12 trees should be interpreted as an approximation for the confines of whole-bole net CO_2 efflux.

Results

Variation in ray anatomy

The most notable significant differences in average ray area (μm^2, average of individual rays in the tangential view), ray frequency (number mm^{-2}), and ray proportion (% of the sapwood volume occupied by rays) occurred with respect to bole vertical position at a specific radial position (Fig. 1). For example, in Douglas-fir trees, ray frequency was higher yet average ray area was smaller in the inner sapwood near treetops than lower in the bole (significant effects of bole vertical position and the interaction of bole radial and vertical positions, P <0.01 from strip-plot in PROC MIXED and FPLSD, Fig. 1a, b). Ponderosa pine sapwood ray frequency was higher near treetops than in the middle and inner sapwood of the lower bole positions (Fig. 1b), and ray area was smaller near treetops than at the base, but the trend was not as strong as in Douglas-fir (Fig. 1a).

There was no difference between either the frequency or ray proportion near the tree base or treetops between ponderosa pine and Douglas-fir (P >0.07, strip-plot in PROC MIXED and FPLSD). Also, the interaction of bole radial position by bole vertical position by species was significant (P <0.03) for the three ray characteristics (i.e. area, frequency, and proportion), indicating that the within-bole variation in ray characteristics differed between species. When the regression of average ray area versus ray frequency was compared between the two species, average ray area was ~20% higher in Douglas-fir than in ponderosa pine for each level of ray frequency (Fig. 1c, P <0.02). This regression confirmed the observation that when sapwood rays are more frequent, they will be smaller, a trend that did not differ between species (insignificant interaction between species and ray frequency, P=0.9).

Variation in tissue chemistry

Total carbon content was fairly constant within boles, between species, and among seasons, ranging from 47–52% (data not shown). Significant differences that did occur were often small. Larger differences occurred between the inner bark and sapwood, with the former being

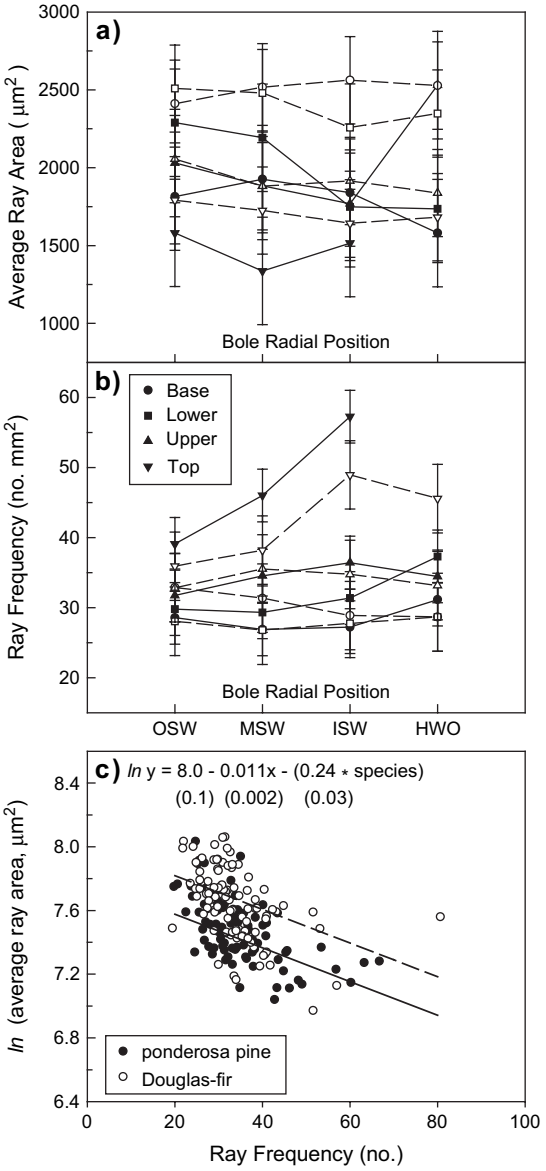

Fig. 1. (a, b) Parenchyma characteristics versus bole radial position and (c) regression of average ray area on ray frequency of mature and young ponderosa pine (PIPO) and Douglas-fir (PSME) trees at different bole heights sampled in September. Bole radial positions: outer sapwood (OSW), middle sapwood (MSW), inner sapwood (ISW), and heartwood (HWO). Bole heights indicated by different symbols. PIPO: black symbols, solid lines; PSME: white symbols, short-dashed lines. *Y*-axis variables versus bole radial position are LSMEANS (strip-plot model in PROC MIXED). Equation parameter estimates with (SE) (P <0.0001). Species effect: ponderosa pine=1 and Douglas-fir=0.

4–8% higher than the latter for most trees. In contrast to tissue carbon content, differences were larger and more frequent for tissue nitrogen and TNC. Both ponderosa pine and Douglas-fir had 1.5–6 times higher nitrogen and TNC contents in the inner bark than in the sapwood (P <0.05; Table 2). At any given vertical position, ponderosa pine sapwood nitrogen and TNC contents were constant with respect to growth ring number. The exception was in September-sampled trees at the lower bole position, which had 20% higher TNC in the inner versus outer sapwood (P <0.05). In Douglas-fir, sapwood nitrogen content near the tree base and treetop was constant from bark to sapwood/heartwood boundary, whereas TNC was significantly higher (14–18%, P <0.05, Table 2) in the inner than the outer sapwood.

There were also significant, yet inconsistent, trends for tissue nitrogen and TNC contents from tree base to treetop, between species and among sampling dates. For the inner bark, ponderosa pine nitrogen and TNC contents decreased by 13–26% from tree base to treetop (Table 3; P <0.05). For sapwood of ponderosa pine and inner bark and sapwood of Douglas-fir, nitrogen and TNC contents significantly increased from tree base to treetop by 20–50% (Table 2). In September, mature ponderosa pine inner bark was higher than mature Douglas-fir in nitrogen (27%) and TNC (1.2–2.2 times) at the tree base, whereas the reverse was true near treetops (Table 2). Sapwood differences between the two species were not evident for nitrogen and small for TNC (if present, 10–25% lower in ponderosa pine). The effect of species alone was not significant for nitrogen or TNC contents (P >0.1), whereas the interaction of species by bole radial and vertical positions was (P <0.0001), indicating that the within-tree trends differed between the two species. Although statistical comparisons were not made between the species for all dates sampled (due to small sample size with respect to number of sampling dates), nitrogen (inner bark) and TNC (inner bark and sapwood) were frequently higher in ponderosa pine than in Douglas-fir (Table 3).

Respiratory potential comparisons between the two species

For the September harvests of mature ponderosa pine and Douglas-fir, core segment respiratory potential (on all indices) varied significantly by tissue age (P <0.0001, comparison of regression lines), decreasing from inner bark, throughout the sapwood to the heartwood/sapwood boundary (Fig. 2). For both volume-based indices (core segment and ray volume), the interaction of species by tissue age was significant (P <0.02), although the main effect of species was not significant (P >0.6, comparison of regression lines). This suggested that the relationship between volume-based respiratory potential and tissue age differed for each species (Fig. 2a, c). For example, inner bark volume-based respiratory potential of ponderosa pine was 1.5–3 times higher than Douglas-fir at all bole vertical positions except near treetops, where ponderosa pine inner bark respiratory potential was 30% lower than that of Douglas-fir (Fig. 2a, inset). In contrast to the inner bark, there were few significant differences between the two species in sapwood volume-based respiratory potential (Fig. 2a, c).

Table 2. *Comparison of core segment chemical composition between mature ponderosa pine and mature Douglas-fir both sampled in September*

Least squares means and confidence intervals (n=6 trees), significant differences among means from Fisher's protected least squares differences (P <0.05). For each tissue radial position (r, row), different lowercase letters represent differences between species. For each species (c, column), different uppercase letters represent differences among tissue and vertical positions.

Bole vertical and radial position	Total nitrogen (%)						Total non-structural carbohydrates (%)					
	Ponderosa pine	r	c	Douglas-fir	r	c	Ponderosa pine	r	c	Douglas-fir	r	c
Tree base												
Inner bark	0.38 (0.35, 0.41)	a	A	0.30 (0.27, 0.32)	b	A	19 (17, 20)	a	A	8.8 (8.1, 9.6)	b	A
Outer sapwood	0.12 (0.09, 0.15)	a	B	0.13 (0.10, 0.16)	a	B	2.5 (2.3, 2.7)	a	B	2.8 (2.6. 3.1)	b	B
Inner sapwood	0.11 (0.08, 0.14)	a	B	0.12 (0.09, 0.15)	a	B	2.4 (2.2, 2.7)	a	B	3.2 (3.0, 3.5)	b	C
Node 15												
Inner bark	0.33 (0.30, 0.36)	a	C	0.44 (0.41, 0.47)	b	C	16 (14, 17)	a	C	13 (12, 14)	b	D
Outer sapwood	0.16 (0.13, 0.19)	a	D	0.17 (0.15, 0.21)	a	D	3.3 (3.0, 3.6)	a	D	3.3 (3.0, 3.5)	a	C
Inner sapwood	0.14 (0.11, 0.17)	a	BD	0.15 (0.12, 0.18)	a	BD	3.5 (3.2, 3.8)	a	D	3.9 (3.6, 4.3)	b	E

Table 3. *Effect of sampling date and bole radial position on the chemical composition and respiratory potential at breast height in mature ponderosa pine and Douglas-fir core segments*

Least squares means and confidence intervals (n=4 trees), significant differences among means for each response variable analysed separately with Fisher's protected least squares differences (P <0.05). Different lowercase roman letters represent significant differences among sampling dates for each bole radial position separately. Abbreviations: N, total nitrogen (%); TNC, total non-structural carbohydrates (%); RP, respiratory potential (nmol CO_2 g^{-1} DW s^{-1}).

Ponderosa pine				Douglas-fir			
Date	N[a]	TNC[c]	RP[b]	Date	N[b]	TNC[c]	RP[c]
Inner bark				Inner bark			
09/99	0.36 a (0.32, 0.40)	19 a (17, 22)	7.2 a (4.4, 12)	08/98	0.29 ac (0.23, 0.35)	8.3 a (6.8, 9.7)	2.2 ac (1.4, 3.2)
12/99	0.37 a (0.34, 0.40)	–	7.4 a (4.5, 12)	09/98	0.30 a (0.27, 0.34)	8.9 a (7.7, 10)	6.4 b (4.2, 9.7)
01/00	0.37 a (0.32, 0.42)	21 a (11, 39)	8.2 a (6.2, 11)	11/98	0.22 b (0.18, 0.26)	10 ab (9.1, 11)	1.4 c (0.9, 2.3)
02/00	0.41 a (0.38, 0.44)	–	7.2 a (5.6, 9.3)	09/99	0.23 bc (0.19, 0.28)	11 b (10, 12)	2.1 a (1.3, 3.3)
03/00	0.38 a (0.34, 0.42)	18 a (16, 21)	6.7 a (5.1, 8.5)	10/99	0.23 b (0.20, 0.26)	10 b (9.7, 11)	3.2 a (2.7, 3.9)
Outer sapwood				Outer sapwood			
09/99	0.13 a (0.09, 0.17)	2.4 a (2.2, 2.8)	0.28 a (0.17, 0.46)	08/98	0.13 a (0.10, 0.16)	3.1 a (1.6, 4.6)	0.41 a (0.27, 0.61)
12/99	0.09 a (0.06, 0.12)	4.5 b (3.6, 5.6)	0.52 ab (0.31, 0.86)	09/98	0.13 a (0.11, 0.15)	2.8 a (1.5, 4.1)	1.1 b (0.76, 1.7)
01/00	0.09 a (0.04, 0.14)	3.8 bc (2.8, 5.2)	0.60 b (0.46, 0.79)	11/98	0.10 a (0.09, 0.12)	2.2 a (1.3, 3.1)	0.55 ac (0.35, 0.87)
02/00	0.11 a (0.09, 0.14)	5.0 b (3.4, 7.4)	0.53 ab (0.41, 0.68)	09/99	0.09 b (0.08, 0.11)	2.9 a (2.0, 3.8)	0.42 a (0.27, 0.67)
03/00	0.14 a (0.10, 0.18)	3.0 ac (2.7, 3.3)	0.53 b (0.41, 0.69)	10/99	0.10 b (0.09, 0.11)	2.0 a (1.4, 2.6)	0.86 bc (0.72, 1.0)
Inner sapwood				Inner sapwood			
09/99	0.11 a (0.07, 0.15)	2.4 a (2.1, 2.7)	0.22 ab (0.14, 0.36)	08/98	0.13 a (0.10, 0.16)	2.5 ab (1.0, 3.9)	0.13 a (0.09, 0.19)
12/99	0.10 a (0.07, 0.12)	4.5 b (3.6, 5.6)	0.28 ab (0.17, 0.47)	09/98	0.12 ab (0.11, 0.13)	3.1 ab (1.8, 4.4)	0.24 ab (0.16, 0.36)
01/00	0.10 a (0.05, 0.15)	4.0 b (2.9, 5.4)	0.39 b (0.39, 0.51)	11/98	0.10 ab (0.09, 0.12)	2.5 ab (1.6, 3.4)	0.16 a (0.10, 0.25)
02/00	0.10 a (0.08, 0.13)	4.0 b (2.7, 5.9)	0.28 ab (0.22, 0.36)	09/99	0.09 b (0.08, 0.11)	3.7 a (2.8, 4.6)	0.15 a (0.10, 0.24)
03/00	0.13 a (0.09, 0.17)	2.7 a (2.5, 3.0)	0.23 a (0.18, 0.30)	10/99	0.08 b (0.07, 0.09)	2.2 b (1.6, 2.8)	0.30 b (0.25, 0.36)

[a] Significant effect of radial position.
[b] Significant effects of date and radial position.
[c] Significant effects of date, radial position, and their interaction.

On a core segment mass basis, ponderosa pine respiratory potential was 1.5–4 times higher (species effect, P=0.0001, strip-plot in PROC MIXED and FPLSD) than Douglas-fir at all bole radial and vertical positions, whereas the relationship between respiratory potential and age did not differ between the two species (P=0.8). When respiratory potential was considered on a core segment nitrogen basis, there was no difference between the two species (P >0.1, Fig. 2d) and respiratory potential varied significantly only by age (P <0.02).

Relation of core segment respiratory potential to ray anatomy and tissue chemical content

The higher concentrations of TNC during the winter months (December, January, February) often corresponded to higher respiratory potentials in ponderosa pine (Table 3). The significant variation of Douglas-fir nitrogen and TNC among sampling dates did not relate consistently to the significant differences of respiratory potential. However, there were isolated examples of positive correlation: TNC increased with respiratory potential from August to

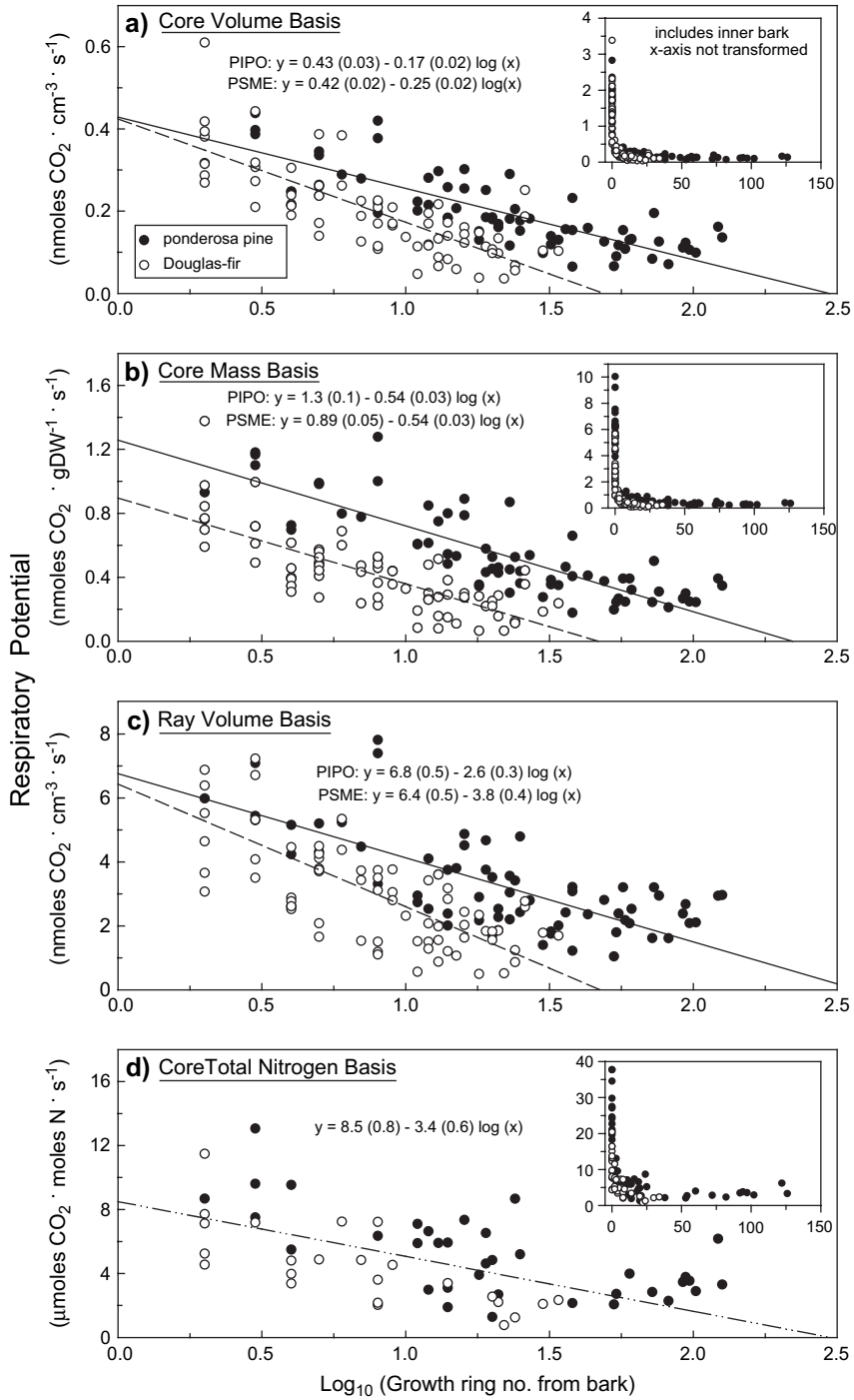

Fig. 2. Respiratory potential at 25 °C on four core-based indices versus approximate growth ring number inward from bark (on a $\log_{10}$ scale) of mature ponderosa pine (PIPO) and Douglas-fir (PSME), sampled in September (data pooled from all four heights). Small inset graphs in (a), (b), and (d) include inner bark. PIPO: black symbols, solid lines; PSME: white symbols, short-dashed lines. Equation parameter estimates with (SE) (P <0.0001).

September in 1998 at all radial positions, and higher TNC in the outer and inner sapwood in September 1999 may have resulted in a delayed effect of increased respiratory potential from September to October in 1999.

There was a significant, positive relationship between respiratory potential on a mass basis and core segment total nitrogen and TNC contents (Fig. 3b, c, P=0.0001, strip-plot in PROC MIXED and FPLSD), whereas there was no effect of ray proportion (Fig. 3a, P=0.3). The effect of species was significant for both total nitrogen and TNC contents (P <0.0001), whereas the interaction between species and tissue chemical content was significant only for TNC

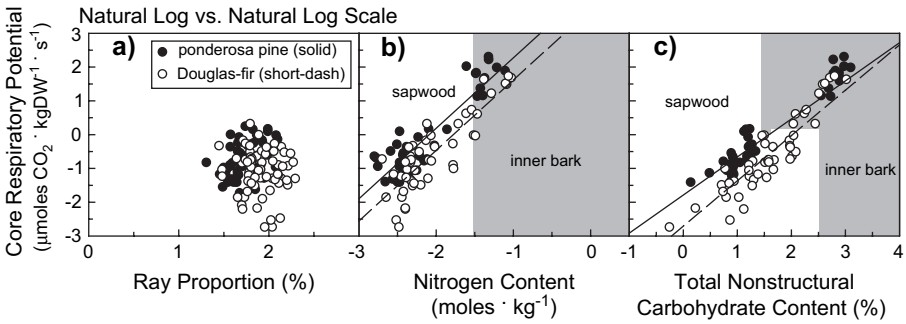

Fig. 3. A log–log relationship between core segment respiratory potential at 25 °C on a mass basis (RP) and (a) ray proportion, (b) total nitrogen content (N), and (c) total non-structural carbohydrate content (TNC) of mature ponderosa pine and Douglas-fir, sampled in September (data pooled from all bole radial and vertical positions). Shaded zones show inner bark data points. Lines show the regressions in sapwood and inner bark (combined) for each species. Parameter estimates (P <0.05) with (SE) for (b) *ln*(RP)=3.7 (0.3)+2.1 (0.1) *ln*(N)+0.65 (0.10) species, and (c) *ln*(RP)= −2.7 (0.2)+1.3 (0.1) *ln*(TNC)+0.92 (0.16) species + −0.21 (0.09) *ln*(TNC) species. For the regressions in sapwood alone (lines not shown): (b) *ln*(RP)=0.36 (0.71)+0.63 (0.31) *ln*(N)+0.44 (0.10) species, and (c) *ln*(RP)= −2.3 (0.2)+0.90 (0.10) *ln*(TNC)+0.66 (0.07) species. Species effect: ponderosa pine=1 and Douglas-fir=0.

(P <0.02), indicating that its relationship to mass-based respiratory potential differed between the two species. The interpretation of the log–log relationship between core segment respiratory potential and tissue chemical content was that for every doubling of nitrogen content, core respiratory potential increased 4.2-fold (95% CI, 2.1–5.2). Moreover, for each level of nitrogen content, core respiratory potential was 1.9 times larger for ponderosa pine than for Douglas-fir (Fig. 3b). Each doubling of TNC resulted in core respiratory potential increasing by a factor of 2.2 (95% CI, 2.0–2.4) for ponderosa pine and by 2.5 (95% CI, 2.2–2.9) for Douglas-fir. For a given TNC content in the range presented (Fig. 3c), ponderosa pine core respiratory potential was estimated to be 1.2–1.9 times larger than that for Douglas-fir.

Scaling to the whole-bole level

Whole-bole level potential net CO_2 efflux correlated positively with live bole volume, ray parenchyma volume in the bole, live bole mass of nitrogen, and live bole mass of TNC (Fig. 4, comparison of regression lines). There was no significant difference in whole-bole net CO_2 efflux/nitrogen mass between ponderosa pine and Douglas-fir (Fig. 4c). By contrast, whole-bole net CO_2 efflux scaled from the three other indices (live bole volume, ray parenchyma volume, and TNC mass) showed significant species effects (P <0.04), but no significant interaction between species and the explanatory variable (P >0.1) (Fig. 4a, b, d). Ponderosa pine whole-bole net CO_2 efflux (mmol s^{-1}) was 0.5 (95% CI, 0.3–0.7) or 0.2 (95% CI, 0.1–0.4) units higher than Douglas-fir for each unit increase in ray volume (m^3) or TNC mass (kg), respectively (Fig. 4b, d), and 0.4 (95% CI, 0.02–0.7) units lower than Douglas-fir for each unit increase in live bole volume (m^3, Fig. 4a).

Discussion

Limitations to tissue-level respiration

As hypothesized, it appeared that tissue-level respiration was not limited by physical storage capacity of the bole tissue (abundance of rays). The ray parenchyma anatomical characteristics in the mature ponderosa pine and Douglas-fir trees were generally constant with respect to bole radial (growth ring) and vertical (node) position, and therefore did not reflect the within-bole respiratory gradients from the current or previous studies (Pruyn *et al*., 2002*a*, *b*). A notable exception was the high frequency of rays at node 15 compared with the lower nodes at all radial positions in mature ponderosa pine and at the inner radial positions of mature Douglas-fir, which corresponded to sapwood respiratory potentials that were 1.3–2 times higher near the treetop compared with the base (current data not shown and Pruyn *et al*., 2002*a*, *b*). The decrease in respiratory potential on a ray volume basis from outer to inner sapwood with relatively constant ray volume in both species suggested a bark-to-pith decrease in ray vitality, a trend that has been reported previously (Goodwin and Goddard, 1940; Frey-Wyssling and Bosshard, 1959; Shain and MacKay, 1973; Yang, 1993; Gartner *et al*., 2000). Decreased ray vitality could result from lower numbers of living rays, or decreased vitality in all rays with increasing proximity to the sapwood/heartwood boundary. To resolve this uncertainty, vital staining techniques should be explored (e.g. triphenyl tetrazolium tri-chloride, Ryan, 1990; or Cooamassie Blue, Stockfors and Linder, 1998).

In contrast to the lack of correlation between bole storage capacity and respiratory potential, both enzyme (nitrogen, N) and metabolic substrate availability (total non-structural carbohydrates, TNC) had significant, positive relationships with volume-based or mass-based, core segment respiratory

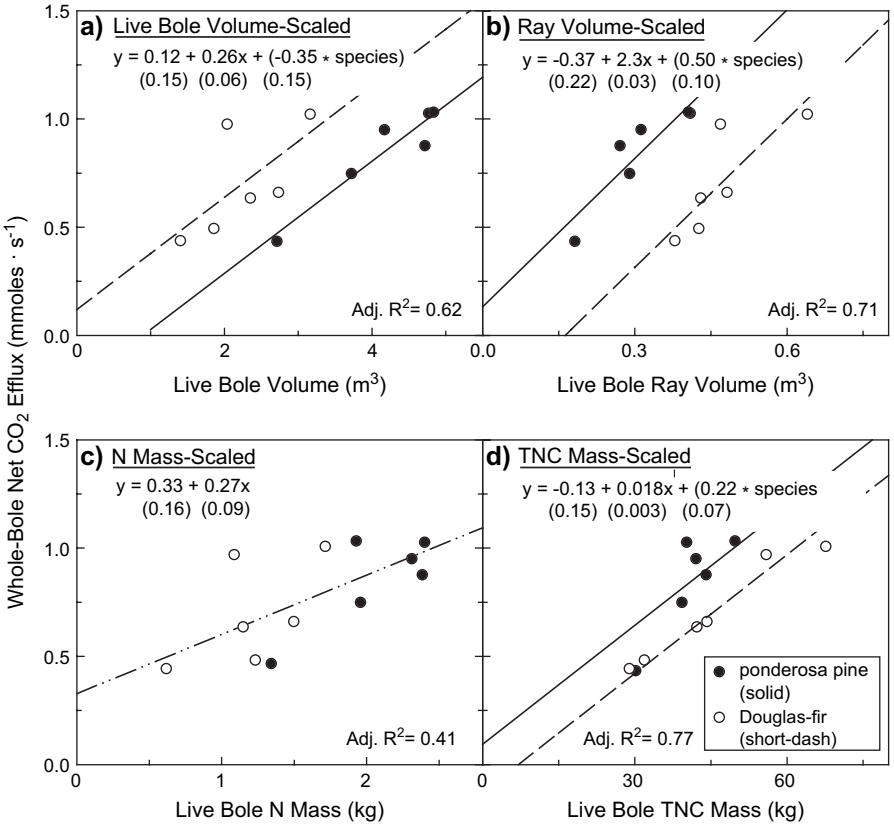

Fig. 4. Whole-bole net CO_2 efflux scaled from four indices [(a) core segment volume, (b) ray volume, (c) total nitrogen mass, and (d) total non-structural carbohydrate mass] versus the respective index on the whole-bole level for mature ponderosa pine and Douglas-fir trees sampled in September. Live bole, inner bark plus sapwood; N, nitrogen; TNC, total non-structural carbohydrates. Equation parameter estimates with (SE) (P <0.02, except y-intercepts P >0.1). Species effect: ponderosa pine=1 and Douglas-fir=0.

potential, which supported the original hypothesis that substrate was more important than parenchyma volume for tissue-level respiration in both of these species. The strongest example of this tendency was in the inner bark relative to the sapwood, where much higher respiration corresponded with much higher nitrogen and TNC levels, which may be explained by the cambium of the inner bark being involved in growth and thus more substrate-dependent than the storage-oriented sapwood (Dewar, 2000). Tree base to treetop trends in bole tissue chemical content in the current study also corresponded positively with core respiratory potential, for example, decreasing from tree base to treetop in ponderosa pine inner bark and the reverse for sapwood of both species (current data not shown: Pruyn *et al*., 2002*a*, *b*). Further, the often-higher levels of nitrogen and TNC in ponderosa pine boles corresponded with higher respiratory potentials on a mass or volume basis, as compared to Douglas-fir, yet when respiratory potential was represented on a nitrogen basis, the species no longer differed. These results indicated that enzyme (nitrogen), or sugar/starch (TNC) concentrations in woody tissue may limit respiration.

The current study's log–log relationship between core segment respiratory potential and core nitrogen content in ponderosa pine contrasted the linear relationship previously estimated for *Pinus* spp. with an infrared gas analysis system, in that a doubling of tissue N resulted in a 1.3–2-fold increase (as compared to the current, 4.2-fold increase) in bole respiration (for N contents 0.02–0.12 mol kg^{-1}, Vose and Ryan, 2002; Maier *et al*., 1998). However, the current study's regression for sapwood alone was more comparable: respiratory potential was 1.6 times higher with each doubling of core N, which suggested a different relationship between respiratory potential and nitrogen in the inner bark, possibly related to its growth and transport functions. Further, respiratory potential per unit N in the sapwood of both species (0.5–6 μmol mol^{-1} N s^{-1}, corrected to 15 °C, assuming a Q_{10} of 2.0) was higher than values reported for *Pinus taeda* bole sapwood and was closer to values of coarse roots (1.2 versus 3.4 μmol mol^{-1} N s^{-1} at 15 °C, respectively; Vose and Ryan, 2002).

Despite the positive correlation between nitrogen and TNC contents and respiratory potential in Douglas-fir and ponderosa pine boles, this relationship was not identifiable or consistent for all comparisons. Further, the radial constancy of nitrogen and TNC contents with respect to sapwood growth ring number in the current study did not reflect radial trends in respiratory potential (current data and

Pruyn *et al*., 2002*a*, *b*) and contrasted results from *Pinus sylvestris* L. (Saranpää and Höll, 1989; Fischer and Höll, 1992), *Quercus petraea* (Matt) Liebl. and *Fagus sylvatica* L. (Barbaroux and Bréda, 2002), where sugars and starches were more highly concentrated in the outer versus inner growth rings. One explanation for the current lack of a sugar/starch gradient across the sapwood is that the previous studies divided sapwood into five to six zones and analysed sugars and starches separately. By contrast, the current study analysed sugars and starches combined (TNC content) in only two zones, potentially causing subtle differences in specific rings to go unnoticed. For example, summing the sugar and starch values from Saranpää and Höll (1989) for each zone and then averaging the zones into outer and inner sapwood reduced the sugar/starch difference between outer and inner sapwood from 70–100% to 20–60%.

Interpreting the respiratory significance of tissue chemical content is problematic because sites of high respiration and of high carbohydrate content may be quite different (Farrar, 1985). For example, it has been shown that the concentration of the unloaded sucrose outside the phloem (i.e. in the differentiating xylem) of *P. sylvestris* was not related to annual growth ring width. This suggested that xylem production (a respiration-dependent process) along the stem was not limited by substrate unloading or local availability, but rather by greater specific mass transfer of sucrose (higher phloem and cambium activity) (Sundberg *et al*., 1993). Such observations have led researchers to conclude that only a better understanding of the sugar fluxes, between tissues and between subcellular compartments, will reveal the true nature of the relationship between carbohydrate status and respiration rate (Farrar, 1985; Cannell and Thornley, 2000; Dewar, 2000).

Whole-bole CO_2 efflux, parenchyma, N, and TNC

On the whole-bole level, there was a significant correlation between respiratory demand (whole-bole, potential net CO_2 efflux) and ray and substrate abundance. This correlation was shown by the positive, linear relationships between whole-bole net CO_2 efflux and live bole volume, the ray volume in the live bole, the live bole mass of N, and the live bole mass of TNC, all with an adjusted R^2 of 0.41–0.77. This relationship did not differ between species for live bole N mass, implying that it was a good estimator of CO_2 efflux. The relationship between net CO_2 efflux and the other scaling factors (live bole volume, ray volume in the live bole, and live bole mass of TNC) were similar between the two species in that they both shared the same slope for each factor. The difference was that ponderosa pine was either 20–50% higher (ray volume in the live bole and live bole mass of TNC) or 20–25% lower (live bole volume) than Douglas-fir. Thus, inconsistent, weak and/or complex correlations on the tissue-level scaled up to reveal important trends on the whole-bole level.

The differences in whole-bole net CO_2 efflux between the species were largely explained by the species' relative amounts of whole bole storage space or substrate mass. The higher ray proportions and TNC mass of the inner bark (relative to the sapwood) became important on the whole-bole level because ponderosa pine's inner bark volume was only 25% that of Douglas-fir. Scaling the high ray proportions and high TNC mass to ponderosa pine's smaller inner bark resulted in a smaller total ray volume and total TNC mass for ponderosa pine boles relative to Douglas-fir, which caused ponderosa pine's whole-bole net CO_2 efflux (per smaller ray volume or smaller TNC mass) to be higher than that of Douglas-fir. Likewise, the lower respiratory potential of the sapwood (relative to inner bark) strongly influenced ponderosa pine's whole-bole net CO_2 efflux/live bole volume because it was scaled to ponderosa pine's larger, live bole volume (twice that of Douglas-fir).

Because the influence of ray volume and substrate mass on whole-bole net CO_2 efflux was conveyed via the inner bark/sapwood volume, these two species likely had contrasting strategies for storage and metabolism in the live bole. Ponderosa pine's higher respiratory demand (whole-bole net CO_2 efflux) per unit storage space (ray volume) and sugar/starch concentration (TNC content) was possibly attributable to higher maintenance requirements of its proportionally larger sapwood compared with Douglas-fir. Further, for each level of whole-bole net CO_2 efflux, the smaller sapwood of Douglas-fir contained more storage space and more sugar/starch, which possibly resulted from its higher leaf area to sapwood area ratio (i.e. increased photosynthetic supply per unit sapwood, relative to ponderosa pine; Table 1). By contrast, nitrogen mass in the bole predicted whole-bole net CO_2 efflux independently of species, supporting the previously suggested hypothesis that maintenance respiration is directly or indirectly related to tissue protein content (Cannell and Thornley, 2000). The possibly high cost of sapwood maintenance for ponderosa pine was not evident for net CO_2 efflux per unit live bole volume, which was lower than that of Douglas-fir, resulting in the species with a proportionally larger live bole volume (ponderosa pine) having a lower live bole respiratory demand, a trend that has been shown for other conifer species (Pruyn *et al*., 2003). This result suggested that ponderosa pine's higher respiratory demand per unit storage space (living ray cells) and sugar/starch concentration was balanced out over its larger sapwood, possibly in support of non-living tracheids (e.g. ion uptake, transport, and gradient control) or other sapwood functions.

In the current study, the diverse tissues of the tree boles were analysed separately first to build an understanding toward the mechanistic controls of respiration and it was learnt that core-based respiratory potential may be limited by substrate concentration, but that the correlation was strongly driven by the high concentration of N and TNC in the inner bark relative to the sapwood. When comparing

the relationship between core-based respiratory potential and core N content to similar estimates from chamber-based, organ level measurements (via infrared gas analysis), similarities were found in the sapwood and also differences, possibly from the influence of the inner bark measurements. Further research is necessary to determine the relationship between core-based respiratory potential, live ray cells, and chamber-based, organ level measurements, which would clarify the proportion of the live bole that is metabolically active and help bridge the gap between tissue and whole-tree level respiration. Scaling from cores to whole boles indicated the importance of live bole geometry (inner bark and sapwood volume) to the outcome of net respiratory (CO_2) efflux, suggesting a need for more tissue-based measurements and scaling estimates to reveal/include the influence of inner bark and sapwood in branches, boles, and roots. However, before whole-tree respiration can be explained in terms of coarse, fine, and substrate-limited control of the respiratory pathway, analytical and integrated studies are required on carbon flux to and partitioning within respiratory metabolism and the related, energy-dependent processes (Farrar, 1985).

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