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Research

Within-stem variation of respiration in *Pseudotsuga menziesii* (Douglas-fir) trees

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Summary

- A technique for measuring *in vitro* respiration was investigated to understand why rates were higher than those reported *in vivo* and to elucidate trends within mature *Pseudotsuga menziesii* (Douglas-fir) trees.
- Extracted increment cores were divided into 3–4 radial depths and a gas chromatograph was used to compare respiration rates radially and vertically within stems.
- Respiration of inner bark was 2–3 times greater than sapwood, and 50–70% higher in outer than inner sapwood. Inner bark and outer sapwood released > 40% more CO_2 at treetops than at bases. Trends were robust for CO_2 production on a core dry-mass, volume, or total carbon basis. By contrast, CO_2 production on a nitrogen basis showed almost no significant variation.
- This *in vitro* technique provided an effective index for relative differences in respiration within tree stems. Discrepancies between *in vitro* and *in vivo* measurements might be related to the gaseous environment in stems. The estimated within-stem gradients in respiration were possibly determined by enzyme quantity and availability and could be useful in scaling to whole-trees.

Key words: stem respiration, stem aeration, inner bark, sapwood, *Pseudotsuga menziesii* (Douglas-fir).

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Introduction

In most studies, estimates of whole tree and stand level respiration rates are obtained from scaling up small sample measurements acquired using infra red gas analysis (IRGA) chambers at one or more position(s) on tree stems (Kinerson, 1975; Ryan *et al.*, 1995; Edwards & Hanson, 1996). Stem surface area beneath the chamber has often been used as an index of the amount of living tissue to associate with the measured respiration rate (Linder & Troeng, 1981; Matyssek & Schulze, 1988). This rate can then be extrapolated to the entire surface area of the stem and from there to the ecosystem level. However, sapwood volume has proved to be a better index for scaling maintenance respiration to whole trees or ecosystems (Sprugel & Benecke, 1991; Ryan & Waring, 1992), probably because sapwood volume is proportional to the amount of living parenchyma cells therein (Ryan, 1990; Larson, 1994; Stockfors & Linder, 1998).

Scaling respiration to the whole-tree level by sapwood volume from measurements at only one location assumes uniform respiration rates among all the stem parenchyma cells. This assumption is unlikely to be valid because sapwood is not uniform in age (number of years since the cells developed) or maturity (cambial age at which cells were produced). Tissue age and maturity depend on radial and vertical position in the stem, both of which may impact respiration. Radial trends in sapwood respiration have been recorded from inner bark to the heartwood/sapwood boundary for tissues extracted just above ground level in stems of *Pinus radiata* (Shain & Mackay, 1973), *Fraxinus nigra* and *Acer rubra* (Goodwin & Goddard, 1940), and in *Picea abies* and other species (Møller & Müller, 1938). Radial and vertical trends in sapwood respiration were recorded for *Pinus ponderosa* (Pruyn *et al.*, 2002).

The impact of such within-stem variations on whole-tree level respiration rates was addressed in *P. abies* (Stockfors, 2000). Assuming a constant temperature-respiration relationship,

stem temperature was measured at multiple heights and radial depths to predict respiration. Within-stem temperature variations were observed that resulted in scaling errors between 2 and 72% for a single sunny day and 2 and 58% for a whole year. Within-stem variation in respiration was also accounted for by averaging respiration rates (measured via IRGA) from various heights on stems (Lavigne, 1987; Ryan *et al.*, 1996), or from different compass directions (Edwards & Hansen, 1996). In a third approach, the distribution of live cell volume in sapwood samples was estimated by using vital staining techniques and image analysis tools, which provided a percentage of living tissue for scaling respiration rates to the whole stem-level (Ryan, 1990; Stockfors & Linder, 1998).

Although these three methods are improvements for representing variation of respiration within sapwood, multiple sources for inaccuracy remain. First, the use of temperature to predict respiration rates may be problematic because of temperature-independent variation in CO_2 efflux from stems (Martin *et al.*, 1994). Second, the use of IRGA systems may sometimes underestimate respiration because all sapwood CO_2 production may not reach the stem surface. Some respired CO_2 could be dissolved in the transpiration stream (Negisi, 1975; Sprugel, 1990; Levy *et al.*, 1999), refixed by bark photosynthesis in some species (Nilsen, 1995; Cernusak & Marshall, 2000), or stored within sapwood parenchyma cells (Lev-Yadun & Aloni, 1995). Third, vital staining techniques only indicate whether cells are alive or dead, they do not directly convey the degree of respiratory activity among the living cells.

In a previous study, we measured rates of CO_2 production under controlled laboratory conditions of cores extracted from mature *Pinus ponderosa* (ponderosa pine) stems. A primary objective was to quantify within-stem variation of respiration, and then scale to the whole-tree level (Pruyn *et al.*, 2002). We learned that respiration in sapwood of those pine trees was not homogenous and that scaling to whole-trees resulted in rates substantially higher than those reported *in situ* from the literature. In the current study, we first examined the basis for higher *in vitro* than *in vivo* respiration rates of excised cores of *Pseudotsuga menziesii* (Douglas-fir) by exploring potential artifacts of the method. Second, we determined whether bark-to-pith and treetop-to-base trends in respiratory potential also existed within Douglas-fir stems. Third, we compared core respiratory potential on four different indices: dry mass, volume, moles carbon and moles nitrogen. Finally, we discussed the likely physiological mechanisms responsible for variations in respiration within trees, across sites and between seasons.

Materials and Methods

Species and site characteristics

We collected samples from mature (60–112 yr-old) Douglas-fir trees, *Pseudotsuga menziesii* (Mirb.), from a site just east of the Coast Range in southern Oregon, near Riddle (N42°57′-W123°22′, elevation 215 m) and in McDonald-Dunn Research Forest in the Williamette Valley, near Corvallis, OR, USA (N44°38′-W123°17′, elevation 305 m). Unless indicated otherwise, samples were collected either before the growing season for this region, in early March of 1998 and 1999, or afterwards in October of 1999, 2000, and 2001. The rationale behind selecting these sampling dates was to capture maintenance respiration, and thus avoid the complications of growth respiration in estimating core respiration.

Tree sampling

All trees sampled were free of broken tops, stem deformities, or visible disease. Twenty to 30 trees were selected randomly from each site to be used for one or more of the following experiments. Stem diameter 1 m from the ground ranged from 40 to 57 cm (Corvallis) or 55–70 cm (Riddle). Tree age at breast height was 64–109 yr (Corvallis, from cores) and 110–112 yr (Riddle, from cross-sectional disks).

Respiration measurements

Respiratory potential was estimated from 12 mm increment cores extracted from either standing stems (1 m from ground), or felled trees (1 m from ground and nodes 35 and 15 from treetop). When extracting multiple cores from a specific stem height, we took them evenly from about the stem's circumference. All cores were analyzed within 1 wk of sampling. Twenty-four hours before measurement, cores were cut into four segments: inner bark (phloem and cambium) and outer, middle and inner sapwood. Sapwood was defined as the woody tissue extending from the first growth ring interior to the inner bark to the last growth ring interior to the transition zone (one or two lighter colored rings at the sapwood/heartwood boundary). Outer, middle, and inner sapwood samples were obtained by dividing sapwood into three equal radial lengths. For this study, respiration of heartwood was not measured because preliminary data revealed almost no CO_2 evolution from heartwood samples (< 0.01 nmoles CO_2 g (d. wt)$^{-1}$ s^{-1}, M. L. Pruyn, unpublished data). Number of rings per segment was recorded, so that a mean age could be determined for each segment. These segments were weighed, wrapped tightly in plastic, then stored at 25°C overnight to allow metabolic activity in core segments to stabilize (Goodwin & Goddard, 1940; Hari *et al.*, 1991; Levy *et al.*, 1999).

Immediately before 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, carbon dioxide concentration within vials was measured with a Hewlett-Packard (5700 A) gas chromatograph (GC) (Hewlett-Packard, Avondale, PA, USA) immediately after closing the vials and again after an incubation period. Core segments were incubated at 25°C (unless indicated otherwise)

between GC measurements. Incubation period was held constant at either 6 or 20 h, with the longer incubation necessary to accommodate the processing time of large numbers of samples. Details of GC analysis and calculation of respiratory potential (nmoles CO_2 g (d. wt)$^{-1}$ s^{-1}) are in Pruyn *et al.* (2002). From this point onward, we refer to the reported values as respiratory potential, rather than respiration rate because the conditions of our measurements on these excised samples are probably different from those within the tree.

Immediately following the GC analysis, core segments were weighed a third time. The three successive wet masses verified that water loss was low (between 1 and 3%) between sampling and the end of the measurement period. Fresh volume of core segments was estimated as displaced water by submerged samples (D2395, ASTM, 2001). Dry masses were determined after oven drying at 60°C for 48 h. The OSU Central Analytical Laboratory determined total core carbon and nitrogen content using a LECO CNS-2000 Micro Analyzer (LECO, Corp., St. Joseph, MN, USA).

Potential artifacts of technique

To address the question of why *in vitro* respiration rates from the current technique differ from those reported *in vivo*, we conducted six tests to examine potential artifacts from extraction, handling and storage before analysis.

Effects of time in cold-storage at 4°C To determine whether rate of core segment CO_2 production was affected by storage time before respiration measurement, we extracted four 12 mm diameter cores at breast height from six trees from the Corvallis site in late August of 2000. For each tree, each core was assigned to one of four treatments: 1, 2, 4 or 9 d of storage at 4°C before GC measurement. The night before GC analysis, cores were segmented according to radial position as described above, with the exception that only inner bark, outer and inner sapwood tissues were sampled to capture the extremes of activity. Core segments were then analyzed for CO_2 production over a 6-h incubation period. We tested published results that a storage time of < 15 d would have no effect on core segment respiratory potential (Goodwin & Goddard, 1940; Shain & MacKay, 1973). The presence of a trend between core segment CO_2 production rate and storage time would indicate that the response was not stable and thus not likely indicative of respiration. Decreased CO_2 production rate with increased storage time may suggest a decreasing wound response, decreasing diffusion of stored CO_2 from within the core as it equilibrates to atmospheric concentrations, or increasing parenchyma cell death; whereas increased CO_2 production with storage time suggests the increased contribution of microbial respiration.

Microbial presence and respiration rate To determine the extent to which microbial respiration contributed to the observed CO_2 production of core segments, we compared the respiratory potential of samples 2 d and 1 month after coring. One core each was extracted from three of the six trees in the storage time experiment, segmented according to radial position and measured for core respiratory potential as described above. Core segments were then placed separately into sterile Petri dishes containing a malt extract agar (1.5% malt extract, 1% agar), sealed with Para-film 'M'® laboratory film (American Can Company, Greenwich, CT, USA) and then stored at 20°C. After 2 and 4 wk, the type of microbe (fungal or bacterial) visible to the naked eye on each core was recorded. We predicted that microbial growth would be visible on core surfaces. After the fourth week, we again measured core respiratory potential. To verify that CO_2 produced by these core segments was exclusively from microbes and not sapwood parenchyma, we exposed the cores to vital stain. Immediately following the GC analysis, we submerged them in a 1% aqueous triphenyl tetrazoilium chloride solution (TTC), which is reduced to a deep-red color in the presence of living cells (Feist *et al.*, 1971; Ryan, 1990). Red-stained tissues were considered alive and nonstained tissues dead.

Using differential scanning calorimetry to measure core respiratory potential To verify that the CO_2 released by core segments was a product of respiration, we compared our GC measurements with core segment metabolic heat rate, that is the rate at which heat is produced by respiration (Criddle *et al.*, 1991), using a differential scanning calorimeter (DSC, Hart Scientific Model 7707) (Calorimetry Sciences Corp. (CSC), American Fork, UT, USA) in the isothermal mode (25°C). Because the DSC ampules were only 1 cm^3, we reduced the size of each sample to 0.5 cm diameter × 0.8 cm length. Two replicates for each radial position (inner bark, outer sapwood, and inner sapwood) from 12 trees from the Corvallis site were used for the experiment because of high variability of preliminary results (M. L. Pruyn, unpublished). Extracted cores were stored at 4°C, until the night before analysis, when they were segmented as described above and stored at 25°C. The DSC analysis was implemented according to Criddle *et al.* (1991) and Anekonda *et al.* (1994), with the exception that each phase of the reaction required 75 instead of 45 min because respiration in stem tissues is considerably less than in leaves. We analyzed one tree per day, and each replicate set (inner bark, outer sapwood, and inner sapwood) separately, which enabled us to keep cores intact until the night before analysis, thereby reducing risk of desiccation.

Effects of temperature on respiration rate To determine if response of core segment respiratory potential to temperature was consistent with literature values from intact Douglas-fir trees, four 12 mm diameter cores were extracted from breast height from each of five trees at each site (Corvallis and Riddle) in early March 2000 for a total of 20 cores per site. For

each tree, each core was assigned to one of four temperatures (5, 10, 15, 25°C). Core response to temperature was measured, and the Q_{10}s (coefficient for changes in respiration with respect to temperature) for the 5–15°C and 15–25°C temperature ranges were calculated as described in Pruyn *et al.* (2002).

Seasonal variation of within-stem CO_2 and O_2 concentration We examined the seasonal pattern of within-stem O_2 and CO_2 concentrations *in vivo* to gain insight as to how different these conditions were from the environment of cores *in vitro*. To determine the CO_2 and O_2 concentration within stems, four holes of different depths were drilled into 12 trees at breast height (1 m from the ground) in early March of 2000 from the Corvallis site. This experiment was modeled after Eklund (1990). Depths were as follows: outer bark (1–2 cm), inner bark (1.5–3 cm), middle sapwood (3–7 cm) and sapwood/heartwood (5–13 cm). Depths were assigned to alternating directional faces by tree to distribute the depths evenly among the four aspects (N, S, E, W). There were a few exceptions to this design due to overshooting the drilling depths, which introduced a confounding variable of direction on depth. To determine the four drilling depths for each tree, we extracted one 5 mm increment core from each of the NE and SE aspects. Before drilling into each tree face, we extracted a 5-mm core to the assigned depth. This sample core was compared with the neighboring diagonal core to verify that the depth was correct. For example, this comparison would indicate if the bark thickness had changed, or whether we had overshot the sapwood/heartwood boundary. We marked the confirmed depth on a 12-mm auger drill-bit and then drilled into the 5 mm increment-core hole. Stainless steel tubes were inserted all the way into the holes so that only 1 cm^2 of wood (tangential face) was exposed to the air in the tube and the other end of the tube emerged from the tree. The area around the tube at the point of insertion was sealed with silicone to avoid leakage, and a gas-tight rubber stopper was inserted into the end of the tube.

For trees in which resin initially threatened to block some of these stainless steel tubes, we installed drainage apparatuses, which consisted of a Y-connecting glass tube inserted into the septum on the tree. Each exposed end of the Y-tube was connected to either a 2-ml glass vial for gas collection, or to a 10-ml vial via a septum for resin collection (pointed towards the ground to facilitate flow of resin). The gas collection vial was sealed with a silicone septum and open-top screw cap and connected to the Y-tube via a double-ended needle that penetrated the seal of each tube. All septa connecting Y-tubes to steel tubes or glass vials were tightened with plastic cinch-straps. Additionally, all exposed septa surfaces were sealed with Parafilm 'M'® and covered with duct tape to impede environmental degradation of the rubber and thus minimize gas leakage. All septum-sealed, steel tubes without drainage apparatuses were connected directly to gas-collection vials via double-ended needles. The connections to the gas-collection vials were initiated on April 1st and maintained for 3 wk. The sealed glass vials were then removed and the inside gas composition determined using the GC. Immediately after removing the sealed glass tube, a replacement was connected to the steel tube for the next 3-wk period. Vials were collected during the morning (800–1000 h), once every 3 wk from April through October Drainage apparatuses were replaced as resin-collection vials filled-up, as were any rubber septa that showed excessive degradation. To compare the measured fluxes of stem O_2 and CO_2 to seasonal climate trends, daily mean maximum and minimum temperature and precipitation for the site were obtained from the Oregon State University (OSU) Integrated Pest Management Weather Data web site (http://www.orst.edu/Department/IPPC/wea/).

Responses in core respiratory potential to variations in ambient CO_2/O_2 concentrations To understand the effect of CO_2/O_2 concentration on core segment respiratory potential, we extracted four 12 mm diameter cores from five trees at the Corvallis site in October 1999. Each core was assigned to one of four treatments: ambient in the laboratory (control, 0.04% CO_2/21% O_2), O_H (2% CO_2/5% O_2), O_L (10% CO_2/2% O_2), and nitrogen-flushed (0% CO_2/0% CO_2). Cores were cut into the three radial segments and stored at 25°C overnight. The morning of the experiment, segments were placed in test tubes and septum-sealed. Test tubes were flushed with nitrogen using a two-way syringe system. Control segments were not flushed. For the O_H and O_L treatments, a volume of gas equal to that which would be added was removed, then the appropriate volume of each gas was added to each test tube. Appropriate volumes were calculated by an application of the Ideal Gas Law ($\text{concentration}_1\ \text{volume}_1 = \text{concentration}_2\ \text{volume}_2$). Gas concentrations ($CO_2$ and O_2) in the test tubes were analyzed using the GC initially, as well as at 5, 10, and 32 h.

Application of the method – stem radial and vertical trends in core respiratory potential

We selected and felled three trees from the Riddle site. After felling, we sawed 20 cm tall stem disks from stems at the 100th (mean ± SE, 0.3 ± 0.02 m from ground), and just above (to avoid branch whorls within the crown) the 35th (35 ± 1 m from ground) and 15th (39 ± 1 m from ground) nodes (years) from the treetop. Total stem height averaged at 44 ± 2 m and base of the live crown (first stem position above ground level with three live branches) at 28 ± 2 m. A second, short disk (< 5 cm tall) was taken from each vertical position for calculations of inner bark, sapwood and heartwood thickness and for determining the age of the tissues. The tall disks for respiration measurements were wrapped in 4 mm-thick black plastic bags with moist paper toweling inside to reduce desiccation before experimentation. Tall disks were

stored at 4°C. The short disks were kiln dried, and then radial distances and ring counts from pith to the distal edge of each tissue (outer and inner bark, sapwood and heartwood) were recorded. Within 1 wk after harvesting, five 8 mm diameter increment cores were extracted from each height position (tall disk), wrapped separately in plastic bags and immediately returned to cold storage. The evening before GC analysis, cores were cut into four radial positions (inner bark, outer sapwood, middle sapwood, and inner sapwood), rewrapped in plastic, and stored overnight at 15°C. The following morning the samples were placed in vials, septum-sealed, and analyzed for CO_2 production as described above. The incubation period was 22 h at 15°C.

Statistical analysis

All data were analyzed 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 natural log) 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 intervals for transformed variables. Within a specific table or figure, if confidence intervals were required for one variable, they were presented for all.

A one-way ANOVA in PROC GLM was used to make comparisons among cold-storage time treatments. Specific pair-wise comparisons among treatments were conducted for each radial position separately using Fisher's Protected Least Significant Difference (FPLSD) procedure (Fisher, 1966). Paired *t*-tests were used to compare respiratory potential of control cores before and after 30 days on agar medium. Comparisons among radial positions for the DSC analysis of core respiratory potential were made using PROC MIXED, with randomized block design and strip-plot (split-block) treatments (Little & Hills, 1978; Milliken & Johnson, 1984). Trees were blocks and the effect of radial position was tested. Pair-wise comparisons among tissue radial positions were conducted using FPLSD procedure. Comparisons among Q_{10} values at different temperature ranges were made using a strip-plot analysis in PROC MIXED. Trees were blocks, and the effects of tissue radial position, temperature range, site and all possible interactions were tested. Pair-wise comparisons among tissue radial positions and temperature ranges were conducted using FPLSD.

Repeated measures analysis in PROC MIXED was used to test the effects of sampling date, radial position and their interaction on respiratory potential (Little *et al.*, 1996). We initially included aspect (N, S, E, W) and the interaction of aspect and sampling date in the model, but dropped these effects when they proved to be nonsignificant. Trees were treated as blocks, and the tree by treatment interaction was also blocked using the SUBJECT option in the repeated statement with a TYPE = UN covariance structure. A repeated measures analysis in PROC MIXED was also used to test the effects of incubation time, atmospheric (CO_2/O_2) treatment, and their interaction on core respiratory potential. This repeated measures model was identical to the previous model, except that tree was included as a random effect and each radial position was analyzed separately.

A strip-plot analysis was used to make comparisons (using FPLSD) among tissues at various radial and vertical positions within trees. We tested the effects of tissue radial position, vertical position and their interaction. This analysis was also used to test the effects of radial position, season (Corvallis site) or vertical position (Riddle site), and their interaction on respiratory potential on four different indices (i.e. core dry mass, volume, carbon or nitrogen).

Results

Effects of cold-storage time and microbial respiration

There were no significant differences in respiratory potential (nmoles CO_2 g (d. wt)$^{-1}$ s^{-1}) of inner sapwood from Douglas-fir trees on the Corvallis site, stored for 1, 2, 4, or 9 d at 4°C before GC analysis ($P \geq 0.05$, Table 1). Outer sapwood respiratory potential was also fairly constant, regardless of storage length, with the exception of tissue stored for 4 d, which respired significantly higher than tissues stored for 1, 2, or 9 d ($P < 0.04$, Table 1). By contrast, inner bark showed a trend of increasing activity with storage time that was significant ($P = 0.05$) after 9 d of storage, when respiratory potential was 45% higher than after the first day. After 30 d on agar medium, microbial growth (fungal and/or bacterial) was visible on all core segments (except one inner bark sample). We concluded that microbial respiration probably did not contribute significantly to the respiratory potential of freshly sampled cores because respiratory potential of older cores with visible microbe growth was either > 50% less than (inner bark and outer sapwood) or equal to (inner sapwood) that of fresh cores with no microbes visible (Table 1). Further, we verified that CO_2 produced by cores with visible microbe growth was exclusively from microbes because the TTC only stained red where microbes were visible.

Differential scanning calorimetry (DSC)

Respiratory potential measured using DSC was significantly higher in inner bark than in sapwood, but not higher in outer than inner sapwood (Table 1). Inner bark and sapwood respiratory potentials measured with the DSC were comparable with GC-measured respiratory potentials from core segments in the storage time experiment (Table 1). However, DSC respiratory potentials for inner sapwood were generally higher than when measured with the GC.

Table 1 Respiratory potential at 25°C of core segments from three radial positions, testing three different variables or methods: storage period, microbial presence after 1-month incubation, and differential scanning calorimetry

Test	Respiratory potential (nmoles CO_2 g (d. wt)$^{-1}$ s^{-1}) by radial position		
	Inner bark LSMEAN	Outer sapwood LSMEAN	Inner sapwood LSMEAN
Storage period (days at 4°C) (*n* = 6)			
1	3.3 (2.6, 4.0)^{a}	0.63 (0.53, 0.74)^{a}	0.27 (0.22, 0.32)^{a}
2	4.1 (3.4, 4.9)^{a}	0.60 (0.49, 0.70)^{a}	0.27 (0.21, 0.33)^{a}
4	3.9 (3.1, 4.6)^{a}	0.79 (0.69, 0.90)^{b}	0.32 (0.27, 0.37)^{a}
9	4.8 (4.1, 5.5)^{b}	0.61 (0.50, 0.71)^{a}	0.30 (0.24, 0.35)^{a}
Microbial presence (*n* = 3) (days after coring, microbe status)	Mean	Mean	Mean
2 d, no growth visible	5.1 (5.0, 5.2)^{a}	0.63 (0.57, 0.69)^{a}	0.27 (0.20, 0.34)^{a}
30 d, microbe growth visible	0.69 (0.62, 0.76)^{b}	0.30 (0.20, 0.39)^{b}	0.19 (0.18, 0.20)^{a}
	LSMEAN	LSMEAN	LSMEAN
Differential scanning calorimeter (*n* = 12)	3.9 (3.4, 4.5)$^{A.}$	0.7 (0.5, 1.0)$^{B.}$	0.6 (0.4, 0.8)$^{B.}$

All cores extracted from breast height of (n) trees at the Corvallis site. Least Squares Mean (LSMEAN, ANOVA or Strip-plot analysis in PROC MIXED) or Mean (*t*-test) ±95% confidence intervals. For each test, different lower-case letters indicate significant differences within columns and different capital letters indicate significant differences within rows (LSMEANS by FPLSD, Means by *t*-tests, $P < 0.05$).

Table 2 Mean Q_{10} for three radial positions in stems at both the Corvallis and Riddle sites

Q_{10} (Temperature Range °C)	Radial position		
	Inner bark	Outer sapwood	Inner sapwood
Corvallis			
Q_{10} (5–15)	4.5 (3.3, 5.7)^{a}	4.8 (3.6, 6.0)^{a}	5.3 (4.1, 6.5)^{a}
Q_{10} (15–25)	2.2 (1.0, 3.4)^{b}	1.9 (0.7, 3.1)^{b}	2.0 (0.9, 3.2)^{b}
Riddle			
Q_{10} (5–15)	4.5 (3.3, 5.7)^{a}	7.6 (6.4, 8.8)^{c}	7.6 (6.4, 8.8)^{c}
Q_{10} (15–25)	2.4 (1.2, 3.6)^{b}	1.8 (0.6, 2.9)^{b}	1.9 (0.8, 3.1)^{b}

All cores extracted from breast height of (n = 5) trees at each site. Least Squares Mean (Strip-plot analysis in PROC MIXED) ±95% confidence intervals are given for each temperature range (i.e. 5–15 and 15–25°C). Different letters represent significant differences among all means (FPLSD, $P < 0.05$).

Temperature effects

For all radial positions in trees from both sites, the Q_{10} for the 5–15°C temperature range, averaging between 4.5 and 7.6, was significantly higher than for the 15–25°C range, averaging 2.0 (Table 2). The effect of temperature on Q_{10} was thus significant ($P < 0.0001$). The large variability of the Q_{10} average at the lower temperature range resulted from outer and inner sapwood samples from the Riddle site. Thus, these two positions were exceptions to the trend of no significant variation among radial positions for each temperature range and the effect of radial position on Q_{10} was not significant ($P > 0.2$). However, the interaction of temperature by radial position and the interaction of temperature by site were significant ($P = 0.01$), likely a result of high outer and inner sapwood Q_{10}s at the Riddle site.

Seasonal variation of within stem CO_2 and O_2 concentration

The effects of aspect (N, S, E, and W) and the interaction of aspect and sampling date were not significant to the repeated measures model for response variables, O_2 and CO_2 concentration ($P > 0.1$). Thus, we found no evidence in these data of a confounding variable of aspect on gas concentration by radial position and dropped these effects from the model. However, we could not completely rule out the possibility of confounding because of the experimental design, which had an unequal number of radial positions assigned to each compass direction. Within stem O_2 and CO_2 varied significantly by sampling date ($P < 0.0001$) and radial position ($P < 0.0001$). The interaction of sampling date by radial position was significant for stem O_2 ($P = 0.003$),

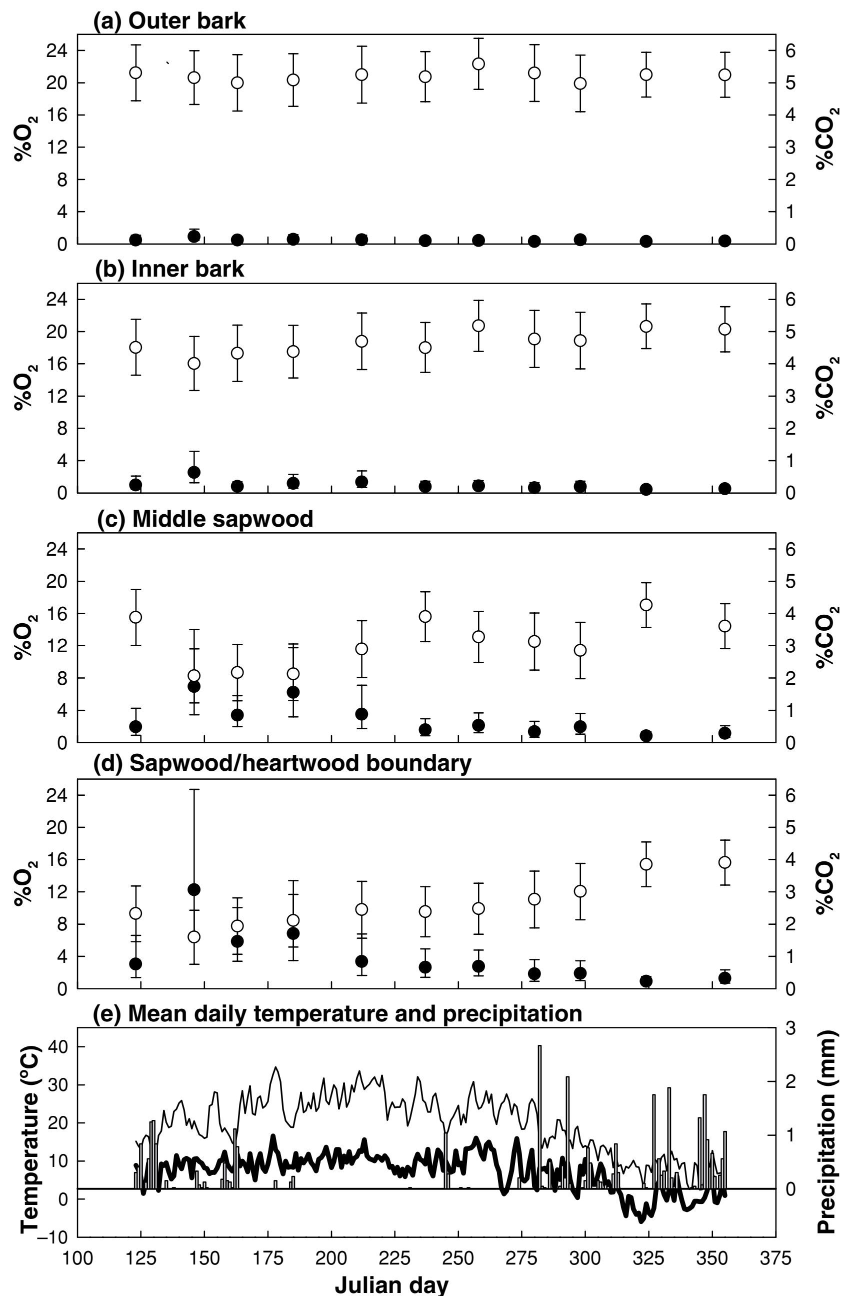

Fig. 1 Seasonal flux of within-stem gas concentrations of trees at the Corvallis site. Concentration of carbon dioxide (% CO_2, closed circles) and oxygen (% O_2, open circles) in the gas phase in equilibrium with (a) outer bark (b) inner bark (c) middle sapwood, and (d) heartwood. (e) Daily mean maximum temperature (lines) and minimum temperature (bold lines) and precipitation (bars). Least Squares Means ±95% confidence intervals (n = 12 trees) in PROC MIXED.

suggesting that the relationship between O_2 and sampling date varied among the four radial positions. This result was in contrast to the nonsignificant interaction of sampling date by radial position for stem CO_2 ($P = 0.2$).

The trend in O_2 concentration by sampling date and radial position were essentially inversely related to the corresponding trend in CO_2: when O_2 was low, CO_2 was high. Lowest within stem O_2 (mean = 6.4%) and highest CO_2 (mean = 3.0%) occurred at the sapwood/heartwood boundary during the growing season on Julian day 146 (Fig. 1a–d). This hypoxic environment within stems corresponded with the onset of the growing season, higher temperatures and less precipitation (Fig. 1e). As the growing season ended in late September–early October (Julian day 280), within stem O_2 gradually returned to the atmospheric levels recorded on the first sampling date in early May (Julian day 123). Within stem CO_2 followed a similar pattern, yet returned to atmospheric levels more rapidly. Middle sapwood and the heartwood/sapwood boundary were nearly always more hypoxic than the inner and outer-bark, yet significant differences between these two outer, or two inner radial positions were rare.

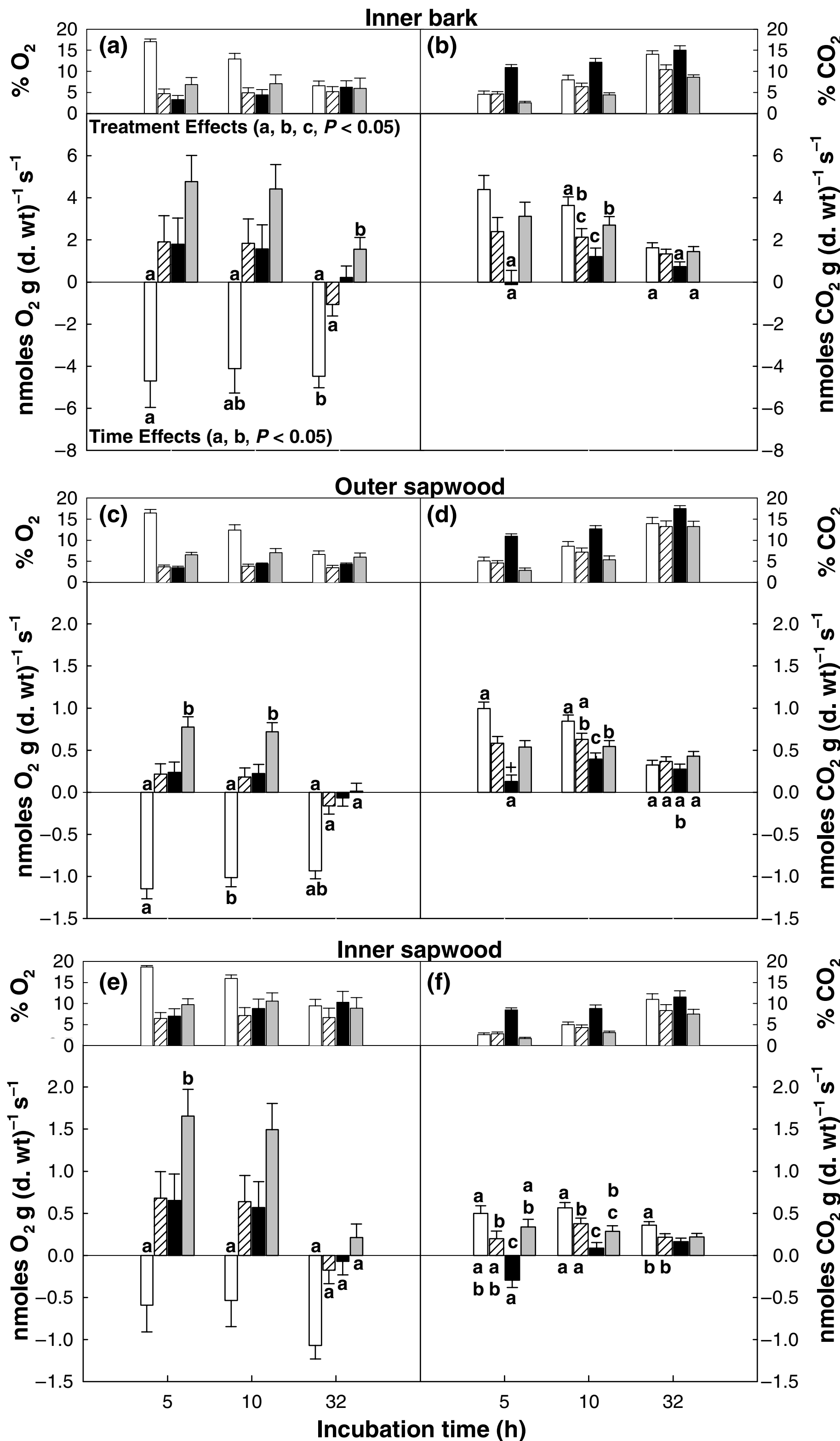

Fig. 2 Effects of four different gaseous environments on respiratory potential at 25°C, O_2 uptake (nmoles O_2 g (d. wt)$^{-1}$ s^{-1}) and CO_2 production (nmoles CO_2 g (d. wt)$^{-1}$ s^{-1}), of cores extracted at breast height from trees at the Corvallis site. Positive values for O_2 rates and negative values for CO_2 rates are the result of the respective diffusion of O_2 out of, or CO_2 into the core. Each lettered pair of graphs shows gas concentration (% CO_2 or percentage O_2) within test tubes, and respiratory potential (O_2 uptake or CO_2 production) of tissues from each radial position at three incubation times. Least Squares Means ± SE (n = 5 trees) from repeated measures analysis in PROC MIXED. Above the x-axis, different letters indicate significantly different means among treatments at each incubation time (treatment effects), and below the x-axis, different letters indicate significantly different means among incubation times for each treatment (time effects) (FPLSD, $P < 0.05$). For each effect, data without letters are not significantly different from one another. Ambient, open columns; 5% O_2 2% CO_2, hatched columns; 2% O_2 10% CO_2, black columns; N_2 flushed, gray columns.

Responses in core respiratory potential to variation in atmospheric CO_2/O_2 concentration

The initial gas concentration treatments (ambient, O_H – 5% O_2, 2% CO_2; O_L – 2% O_2, 10% CO_2; and N_2 flushed) changed over the course of the experiment because of core segment respiration (oxygen uptake or carbon dioxide production) and gas diffusion (stored gas from within the cores into the head-space of the vials or visa versa, Fig. 2). As a result, gas con-centrations were nearly equal among the four treatments as the experiment reached completion, which changed the effect of treatment on core respiratory potential over time.

Respiratory potential, carbon dioxide production (nmoles CO_2 g (d. wt)$^{-1}$ s^{-1}) and oxygen uptake (nmoles O_2 g (d. wt)$^{-1}$ s^{-1}) rates varied significantly by incubation time ($P < 0.0005$) and treatment ($P < 0.005$) for all three radial positions (Fig. 2). The interaction between time and treatment was significant for O_2 uptake in inner bark and outer sapwood ($P < 0.05$), but not for inner sapwood ($P = 0.07$). By contrast, the interaction for CO_2 production was significant for all three radial positions ($P < 0.01$). This result indicated that the trend in core respiratory potential over time varied by treatment. All radial positions of core segments under ambient conditions (control) showed the highest rates of O_2 uptake and CO_2 production among the four treatments tested (Fig. 2). As O_2 concentration in the vials decreased and CO_2 increased over time, O_2 uptake and CO_2 production of the inner bark and outer sapwood controls decreased significantly (Fig. 2a–d). Inner sapwood O_2 uptake and CO_2 production for the controls did not show any significant trends over time (Fig. 2e–f).

For the O_H, O_L, and N_2 flushed treatments O_2 uptake was not evident after 5 or 10 h of incubation in samples from any of the radial positions (Fig. 2a,c,e). The positive values for O_2 rates were likely the result of O_2 diffusion from within the core segments into vial headspace. After 32 h, it was evident that all core segments (from the O_H, O_L, and N_2 flushed treatments) were consuming O_2 because their O_2 uptake rates had decreased or become negative (indicating O_2 uptake). Inhibition of core segment respiratory potential was evident by the trend of decreased CO_2 production in the treated core segments compared with the controls (Fig. 2b,d,f). The most notable inhibition of CO_2 production rates occurred in the O_L treated core segments. The most extreme cases of this trend were in the inner sapwood and to a lesser extent inner bark, where negative CO_2 rates were recorded, indicating the likely diffusion of CO_2 from the vial headspace into the core segment.

Stem radial and vertical trends in core respiratory potential

When considering only stem sapwood respiratory potential, the effects of stem radial position ($P = 0.0003$) and the interaction of radial by vertical position ($P = 0.04$) were significant, whereas the effect of stem vertical position was not significant ($P = 0.15$). At all three vertical positions, outer sapwood respiratory potential was > 60% higher than middle or inner, and there was no significant difference between middle and inner sapwood (Fig. 3a). The significant interaction term indicated that the relationship between respiratory potential and radial position (ring number) varied by stem vertical positions. When both inner bark and sapwood respiratory potential were compared, effects of stem radial position, vertical position and their interaction were all significant ($P < 0.03$, Fig. 3b). At node 15 from the treetop, inner bark respiratory potential was > 3 times higher than all sapwood positions (Fig. 3b). However, at node 100 inner bark respiratory potential was equal to that of all sapwood positions, except inner sapwood. Outer and middle sapwood positions at node 15 from the treetop were at least 50% higher than their corresponding radial positions at node 100 (Fig. 3b).

Respiratory potential using moles carbon or moles nitrogen as a basis

At both the Corvallis and Riddle sites, core segment respiratory potential per moles carbon (μmoles CO_2 moles C^{-1} s^{-1} at 25°C) followed trends similar to the mass and volume based indices (Table 3). The effect of radial position was significant at both sites for all three indices ($P < 0.0001$). For the carbon, mass and volume indices, effects of season and the interaction of radial position by season were not significant ($P > 0.2$), whereas effects of vertical position and the interaction of radial by vertical position were ($P < 0.01$). For these indices respiratory potential at treetops was at least 1.5 times greater than at the bases. The within-stem trends for respiratory potential per moles nitrogen (μmoles CO_2 moles N^{-1} s^{-1} at 25°C) were not as obvious as when indices of carbon, mass or volume were used (Table 3). For example, although the effect of radial position on respiratory potential per unit nitrogen was significant (Riddle site, $P = 0.01$), the inner bark value was only 1.2 times greater than that of sapwood compared with > 5 times greater for any of the other indices. Moreover, there were no significant differences among stem radial and vertical positions for respiratory potential per moles nitrogen at the Corvallis site.

Discussion

The methods tests indicated that respiration rates in core segments were stable and not likely from wounding or microbial respiration (Table 1). Previous studies have also shown stable respiration rates in stored samples of tree stem tissue, for storage lengths < 15 d, in two hardwoods (Goodwin & Goddard, 1940) and in *Pinus radiata* (Shain & Mackay, 1973). In the current study, inner bark respiratory potential was less stable than the sapwood tissues and thus

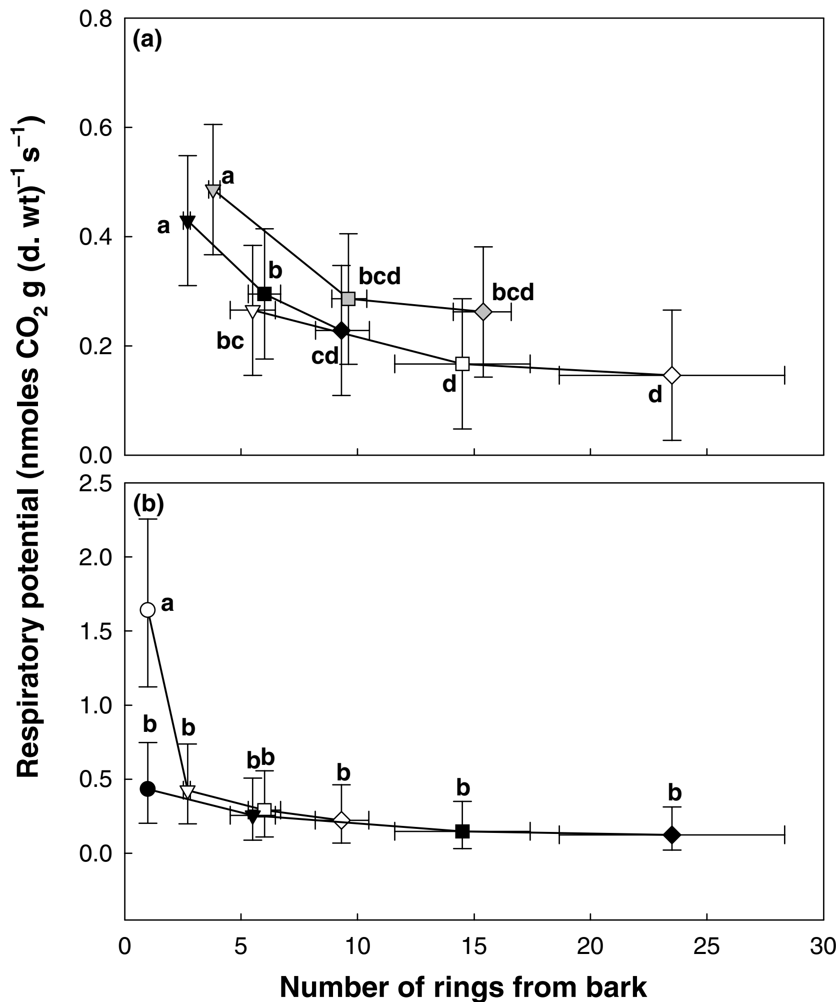

Fig. 3 Relationship between tissue age and respiratory potential at 15°C (nmoles CO_2 g (d. wt)$^{-1}$ s^{-1}) of cores extracted from two to three vertical positions within (4a) sapwood and (4b) inner bark and sapwood of trees from the Riddle site. Different shapes represent each stem radial position, and different shades represent each vertical position. Least Squares Means ±95% confidence intervals from strip-plot analysis in PROC MIXED (*n* = three trees). In each panel, different letters indicate significant differences (FPLSD, $P < 0.05$). For clarity, not all significant differences were indicated in (4b), namely: node 100, inner bark vs inner sapwood; outer sapwood, node 15 vs node 100; and middle sapwood, node 15 vs node 100. Inner bark, circles; outer sapwood, triangles; middle sapwood, squares; inner sapwood, diamonds. Black, node 15; gray, node 35; white, node 100.

should be analyzed within the first few days of sampling. In a previous study, we concluded that the CO_2 production of extracted ponderosa pine cores was from the respiration of living parenchyma cells and not from degassing of stored CO_2 by demonstrating that there was no respiration from cores exposed to a toxic fumigant compared with controls (Pruyn *et al.*, 2002). Results from differential scanning calorimetry in the current study further supported this conclusion.

In the current study, the mean Q_{10} of 2.0 for all tissues at both sites for the 15–25°C range is consistent with literature values for intact Douglas-fir stems (L. A. Cernusak, N. McDowell, N. Balster, & J. D. Marshall, unpublished data). The higher sapwood Q_{10}s in the current study for the 5–15°C temperature range are unusual compared with Larcher (1983), who reported that plant Q_{10}s approach 2.0 at 5–25°C, but have been recorded for decomposing root tissues (Chen *et al.*, 2000). These data suggest that at lower temperatures (5–15°C), Douglas-fir sapwood may have been more reactive to temperature increases than would be expected from literature values. Further research is necessary to ascertain whether a Q_{10} lower than 2.0 should be used when normalizing respiration measurements of Douglas-fir sapwood to temperatures in the 5–15°C range. The relative uniformity of sapwood Q_{10} within a given temperature range (excluding the 5–15°C range at the Riddle site) was notable because sapwood age ranged from 2 to 30 yr. Thus, respiratory enzymes in live stem wood of these Douglas-fir trees responded to temperature similarly, regardless of tissue age.

The O_2 and CO_2 concentrations within Douglas-fir stems *in vivo* differed from the ambient concentrations of the standard *in vitro* set-up used in the current study, which likely impacted the respiration rates of extracted cores. Low O_2 and high CO_2 within stems was also recorded in *Pinus strobes* and *Picea abies* during the growing season (Chase, 1934; Eklund, 1990, respectively). Mean O_2 from the Douglas-fir trees in the current study did not reach levels as low, nor did mean CO_2 reach levels as high as in these previous studies. However, individual Douglas -fir trees did reach such levels of O_2 (2%) and CO_2 (9%), but trees were not synchronized by sampling date, resulting in the lower means reported here. The inverse relationship between O_2 and CO_2 suggests that respiration of the living cells in the sapwood regulates their concentrations

Table 3 Respiratory potential on a carbon, nitrogen, mass, or volume basis of cores extracted from 1 to 2 stem vertical positions from trees at the Corvallis (March and October) and Riddle (March) sites

Carbon and Nitrogen measurements by location, season, and stem position	Radial position		
	Inner bark	Outer sapwood	Inner sapwood
Corvallis, OR – March and October (1 m from ground)			
Respiratory potential per moles Carbon at 25°C (μmoles CO_2 moles C^{-1} s^{-1})			
March	0.13 (0.10, 0.17)[a]	0.024 (0.018, 0.033)[b]	0.016 (0.012, 0.022)[bc]
October	0.09 (0.07, 0.13)[a]	0.024 (0.018, 0.033)[b]	0.012 (0.009, 0.016)[c]
Respiratory potential per moles Nitrogen a 25°C (μmoles CO_2 moles N^{-1} s^{-1})			
March	35 (20, 49)[abc]	29 (15, 43)[bc]	22 (8, 36)[c]
October	49 (35, 63)[a]	41 (27, 55)[ab]	33 (19, 48)[bc]
Respiratory potential per grams dry mass at 25°C (10^{-3} μmoles CO_2 g (d. wt)$^{-1}$ s^{-1})			
March	5.5 (4.1, 7.5)[a]	1.03 (0.77, 1.39)[b]	0.68 (0.50, 0.92)[cd]
October	3.9 (2.9, 5.2)[a]	0.97 (0.72, 1.31)[bc]	0.48 (0.35, 0.64)
Respiratory potential per core volume[d] at 25°C (10^{-3} μmoles CO_2 cm^{-3} s^{-1})			
March	2.7 (2.0, 3.6)[a]	0.5 (0.4, 0.7)[b]	0.33 (0.24, 0.44)[c]
October	1.8 (1.3, 2.4)[a]	0.5 (0.4, 0.7)[b]	0.26 (0.19, 0.34)[c]
Riddle, OR – March (Node 15 and Node 100 from treetop)			
Respiratory potential per moles Carbon at 15°C (μmoles CO_2 moles C^{-1} s^{-1})			
Node 15	0.05 (0.04, 0.06)[a]	0.01 (0.003, 0.02)[b]	0.006 (0.0, 0.01)[b]
Node 100	0.01 (0.003, 0.02)[b]	0.006 (0.0, 0.01)[b]	0.003 (0.0, 0.01)[b]
Respiratory potential per moles Nitrogen at 15°C (μmoles CO_2 moles N^{-1} s^{-1})			
Node 15	6.3 (0.2, 11.7)[a]	12.8 (2.9, 21.8)[a]	9.1 (0.6, 13.8)[a]
Node 100	3.8 (0.9, 16.5)[a]	10.2 (4.4, 25.5)[a]	5.0 (2.4, 20.2)[a]
Respiratory potential per grams dry mass at 15°C (10^{-3} μmoles CO_2 g (d. wt)$^{-1}$ s^{-1})			
Node 15	2.2 (2.0, 2.4)[a]	0.4 (0.2, 0.6)[b]	0.2 (0.1, 0.4)[bc]
Node 100	0.4 (0.2, 0.6)[b]	0.2 (0.1, 0.4)[bc]	0.1 (0.0, 0.3)[c]
Respiratory potential per core volume at 15°C (10^{-3} μmoles CO_2 cm^{-3} s^{-1})			
Node 15	1.04 (0.94, 1.14)[a]	0.23 (0.14, 0.31)[bc]	0.13 (0.04, 0.22)[d]
Node 100	0.28 (0.20, 0.37)[b]	0.16 (0.07, 0.24)[cd]	0.08 (0.0, 0.17)[d]

Note: mass and volume indices are in units of 10^{-3} μmoles CO_2, whereas molar indices are in μmoles CO_2. For each measurement, Least Squares Means (Strip-plot analysis in PROC MIXED) ±95% confidence intervals for Corvallis (n = 5) and Riddle (n = 3) trees. For each group of two rows, different letters indicate significant differences among means for all radial positions (FPLSD, $P < 0.05$).

(Eklund, 1990). Further, the seasonal hypoxia within tree stems has been attributed to the formation of heartwood extractives in *Acacia mearnsii* (Carrodus, 1971) and latewood formation in *Picea abies* (Eklund, 1990).

Oxygen supply to sapwood is hypothesized to be a function of both radial influx into the trunk through intercellular gas spaces and transport of dissolved oxygen via transpiration in the xylem (Gansert *et al.*, 2001). Considerable soil water deficiency during dry periods may reduce transpiration and prevent the entry (O_2) and exit (CO_2) of dissolved gases via the transpiration stream. The resulting CO_2 build-up is augmented by high respiration rates, characteristic of the growing season and associated with cambial divisions and photosynthate mobilization and storage (Chase, 1934). Hypoxia within stems may create negative partial pressures, favoring the radial influx of oxygen through bark lenticels to intercellular spaces of the cortex and phloem. The neighboring cambium and xylem are relatively impermeable to gas because they lack a continuum of intercellular spaces (Hook *et al.*, 1972), which explains the CO_2 increase and O_2 decrease from outer bark to the sapwood/heartwood boundary in the current study, Chase (1934), and MacDougal & Working (1933). Evidence that

O_2 is also supplied to sapwood from the soil and then the transpiration stream was demonstrated in *Picea abies* stems, where oxygen levels decreased with stem height during the growing season (Eklund, 2000). Further evidence of the transpiration stream as an O_2 source within stems is reported in *Betula pedula*, where sap flow was found to be a major determinant for the diurnal flux of dissolved O_2 concentrations (Gansert *et al.*, 2001).

When vial CO_2 concentrations were increased in the current study to mimic within-stem conditions *in vivo*, core respiratory potential was decreased compared with controls. This result is consistent with reports of a direct, immediate and reversible effect of reduced respiration in isolated tissues (Amthor, 1991) or intact plants (Griffin *et al.*, 1996) under conditions of elevated CO_2. The effect of respiratory potential inhibition was not as evident from patterns of O_2 uptake in treatments of low O_2 because of O_2 diffusion from within core segments into the vial headspace. This diffusion of dissolved O_2 from within cores into the vial headspace was likely the result of a positive partial pressure inside cores, created by the low O_2 treatments. The living cells within core segments were thus able to access and use gaseous and dissolved O_2 in respiration and produce CO_2, even when vial O_2 concentration was low or near zero. This ability of parenchyma cells to respire under low gaseous O_2 availability suggests a mechanism for how cells respire deep within stem sapwood. Accumulation of intracellular CO_2 from aerobic and/or anaerobic respiration (depending on the oxygen microenvironment of each cell) may create a gradient for O_2 diffusion into cells from nearby intercellular air spaces, or the transpiration stream within conducting elements of the xylem. Other mechanisms, such as the alternative pathway (cyanide-resistant) or anaerobic respiration may enable respiration to continue despite high ambient CO_2, simultaneously maintaining a gradient for O_2 influx while providing energy for stem tissue anabolism (Hook *et al.*, 1972; Amthor, 1991).

The trends of increasing respiratory potential from pith to bark and tree base to treetop that were identified within Douglas-fir stems at the Riddle site were similar to those in ponderosa pine (Pruyn *et al.*, 2002). Respiratory potential (normalized to 25°C) was similar in both species, with the exception of inner bark, which was 3–4 times higher in ponderosa pine than in Douglas-fir. Also, respiratory potential from breast height of the Riddle trees was nearly equal to values reported for O_2 uptake in stem tissue samples from *Fraxinus nigra* and *Acer rubrum* (Goodwin & Goddard, 1940), but twice that of *Pinus radiata* (Shain & Mackay, 1973). Possible explanations for the latter discrepancy were differences in sample dimensions (0.5 cm radial thickness vs our 2–5 cm long cores) and measurement techniques (volumetric respirometer vs our gas chromatograph) between studies. Further research is needed to ascertain whether similar within-stem trends exist in other species, and to incorporate diurnal or seasonal effects.

Potentially, the heightened respiratory activity of outer sapwood was related to its role in supporting growth and secondary wall formation in the cambial zone (Goodwin & Goddard, 1940), and/or other physiological activities associated with xylem maintenance (Lev-Yadun & Aloni, 1995). Enhanced activity may also be linked to a role in carbohydrate storage. Studies have shown increased amounts of soluble carbohydrates and starch in the outer sapwood and inner bark compared with the inner sapwood and heartwood of *Pinus sylvestris* (Saranapää & Höll, 1989). The reduced activity of middle and inner sapwood may be explained by age-related changes and/or dormancy of metabolic activity in sapwood parenchyma cells. These theories are supported by findings that ray cell nuclear morphology changed from outer to inner sapwood in various conifer species, thus indicating decreased ray vigor (Frey-Wyssling & Bossard, 1959; Yang, 1993; Gartner *et al.*, 2000). Also, heartwood formation or wound repair has been associated with enzymatic or chemical changes in rays of middle or inner sapwood, suggesting sapwood parenchyma cells may be genetically regulated to remain dormant until reactivation by signals from the cambial and/or apical meristem (Shain & Mackay, 1973; Bamber, 1976). The notably higher respiratory potential within the inner bark and outer sapwood at nodes 15 and 35 compared with the same positions at node 100 may also be explained by their location, which was within the crown, where physiological activities, such as growth, substrate supply, metabolism and transport are high.

Our results showed rates of CO_2 production that were nearly equal for the various radial positions when computed on a nitrogen basis vs on a core carbon content, dry mass, or volume basis, which is likely because tissue nitrogen content is an index of enzyme amounts in the tissue. Values for respiratory potential on a nitrogen basis for outer and inner sapwood in the current study were 1.5–2 times higher than oxygen consumption on a nitrogen basis of outer and inner sapwood extracts from two hardwoods (Goodwin & Goddard, 1940). Although carbon and nitrogen may be more representative of living cell material within stem tissue, it cannot be an absolute basis for measurement because some of the extracted carbon and nitrogen was likely obtained from dead cells (Goodwin & Goddard, 1940). Thus, other indices in addition to tissue nitrogen content should be explored for correlations to respiratory trends, such as total nonstructural carbohydrates (sugar, starch, and lipids), or percent live parenchyma cell volume.

Carbon dioxide production of extracted wood cores was generally 3–15 times higher than IRGA measurements of Douglas-fir stem respiration (L. A. Cernusak *et al.*, unpublished) and thus did not represent normal production within intact tree stems. The current results suggest that wounding, microbial respiration and leakage of stored CO_2 are not likely explanations for this discrepancy. We suggest three possible reasons why extracted cores respired at higher rates: inner bark and sapwood Q_{10} (for temperature ranges ≤ 15°C) may be

higher than has been indicated previously by IRGA measurements, which has implications for normalizing respiration to ≤ 15°C; within stem concentrations of high CO_2 and low O_2 may inhibit respiration *in situ*; and some respired CO_2 may exit the stem via the transpiration stream and not radial efflux, so that not all respired CO_2 is captured by the IRGA systems. Gradients of core respiratory potential within and among trees could be used as an index for scaling to the whole-tree level, in conjunction with IRGA measurements to provide a reference for *in situ* respiration rates. However, the IRGA rates will have to account for respired CO_2 that has left the stem undetected via the transpiration stream. Core based estimates for respiration could also be applied to such research areas as storage physiology, metabolism, wood development, ecosystem and individual tree modeling of carbon pools, and wood function in different life forms and growth habits.

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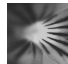

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