

AN ABSTRACT OF THE DISSERTATION OF

William T. Hicks for the degree of Doctor of Philosophy in Forest Science presented on June 2, 2000. Title: Modeling Nitrogen Fixation in Dead Wood

Abstract Approved: Signature redacted for privacy.

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A mechanistic simulation model of nitrogen fixation in dead wood was developed to help synthesize knowledge, develop hypotheses, and estimate rates of nitrogen fixation in the Pacific Northwest. In this model nitrogen fixation is directly controlled by log substrate, temperature, moisture, and oxygen content. Respiration and diffusion of oxygen indirectly affect nitrogen fixation and respiration by regulating log oxygen content.

The relationships of abiotic and biotic variables on nitrogen fixation and respiration and the relationships of wood moisture and density were determined in laboratory experiments to parameterize the model. Nitrogen fixation and respiration had similar responses to temperature, with nitrogen fixation being optimum near 30°C and respiration being optimum over a broader range from 30°C to 50°C. Nitrogen fixation and respiration responded similarly to wood moisture with little activity below 50%, and optimal activity above 175% to 100% moisture content for nitrogen fixation and respiration, respectively. Nitrogen fixation was optimized at 2% O₂. In contrast, respiration rates were optimal when O₂ exceeded 1%. Nitrogen fixation and respiration

in woody debris were significantly influenced by the degree of decay of the wood, and the woody tissue type, but not by the species of dead wood. In both the radial and longitudinal directions, the oxygen diffusion coefficient (D_{O_2}) in wood increased exponentially as the fraction of pore space in air (FPSA) increased and as density decreased. D_{O_2} in the longitudinal direction was 1.4 to 34 times greater than for the radial direction at zero and one FPSA, respectively.

In comparison to independent data, the model of nitrogen fixation reasonably estimated seasonal patterns of log temperature, moisture, oxygen content, and respiration rate. The model estimates an annual nitrogen fixation rate of $0.7 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ for an old-growth stand at the H. J. Andrews, which is reasonably close to an independent estimate of $1.0 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ made for the same stand.

Despite low annual rates of asymbiotic nitrogen fixation in wood, soil, and litter, this process can contribute 9% to 42% of a stands nitrogen inputs over succession when symbiotic fixers such as *Alnus rubra* and *Lobaria oregana* are present and absent, respectively. Managed stands with reduced levels of woody debris and litter may therefore be losing a significant nitrogen input.

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Modeling Nitrogen Fixation in Dead Wood

by

William T. Hicks

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This dissertation would not have been possible without the support and love of my family. I thank Jill for sticking with me through good and bad times, and Twinky, and HoHo for their incredible stress reducing qualities. Finally, I thank my parents without whom I would truly not be where I am today.

Contribution of Authors

Dr. Mark E. Harmon was involved in the design, analysis, and writing of each manuscript. Dr. Robert P. Griffiths provided laboratory space and equipment and valuable help in the design and writing of Chapter 2. Dr. David D. Myrold was involved in the design, analysis, and writing of the $^{15}\text{N}_2$ portion of Chapter 3, as well as serving as a valuable source of information on nitrogen cycling. Dr. Steve Garman was involved in the design and development of the model of nitrogen fixation in dead wood.

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Modeling Nitrogen Fixation in Dead Wood

Chapter 1

Introduction

In the highly productive forest ecosystems of the Pacific Northwest, both tree and fungal growth are limited by nitrogen (Cowling and Merrill, 1966; Gessel, 1973; Spano *et al.*, 1982). Nitrogen fixation is an important input of this key nutrient, but little attention has been given to this process in woody debris because of its relatively low annual input. However, a significant portion of a forest ecosystem's nitrogen can be provided by asymbiotic fixation in woody debris when inputs are summed over succession and/or when symbiotic nitrogen fixers are absent (Cromack *et al.*, 1979; Sollins *et al.*, 1987).

Past attempts at elucidating the controlling mechanisms and the magnitude of nitrogen fixation in dead wood were preliminary. Most studies isolated one or a few of the factors controlling fixation in woody debris (*e.g.*, Roskoski, 1980; Sollins *et al.*, 1987; Griffiths, 1993), but none attempted to synthesize these mechanisms. Moreover, estimates of the annual amount of nitrogen fixed in dead wood in the Pacific Northwest involved extrapolation from a few substrates at one point in time (Sylvester *et al.*, 1982) or at most a few substrates at two points during a year (Sollins *et al.*, 1987). A model of nitrogen fixation in woody debris incorporating the primary controlling variables integrated over a year would therefore greatly expand our understanding of this process.

To this end I developed a mechanistic simulation model of nitrogen fixed in woody debris that synthesized the current knowledge of this process. Nitrogen fixation is directly controlled in the model by log substrate, temperature, moisture, and oxygen content. Respiration and diffusion of oxygen indirectly affect nitrogen fixation by regulating log oxygen content. Respiration is directly controlled by log substrate, temperature, moisture, and oxygen content. In the model oxygen diffusion is influenced by log substrate, moisture, and oxygen content.

The overall objective of this study was to develop a mechanistic simulation model of nitrogen fixation in woody debris. To develop this model I needed to address several questions:

1. **How do abiotic variables influence nitrogen fixation and respiration?**
2. **How do biotic variables influence nitrogen fixation and respiration?**
3. **How do wood moisture and density influence oxygen diffusion?**

With the finished model we can address several additional questions about nitrogen fixation in woody debris including:

4. **How does nitrogen fixation in woody debris vary with climate and potential changes in climate?**
5. **How much nitrogen is fixed at the stand scale in woody debris?**
6. **How do nitrogen fixation rates in woody debris compare to other nitrogen inputs?**

In Chapter 2, I addressed question one by measuring the response of nitrogen fixation and respiration to wood temperature, moisture, and oxygen concentration using

acetylene reduction (AR). I developed equations to estimate the relationships for use in parameterizing the model.

In Chapter 3, I addressed question 2 by measuring the influence of wood species, tissue, and the degree of decay on respiration and nitrogen fixation using acetylene reduction. A table of mean values was developed for each relationship for input to the model. I also measured the $^{15}\text{N}_2$:AR conversion ratio for converting the amount of acetylene reduced to nitrogen fixed.

In Chapter 4, I addressed question three by measuring oxygen diffusion rates through wood cores of varied density and moisture. In addition, I measured seasonal changes in field log oxygen concentrations to compare model output to field data.

In Chapter 5, I describe the model of nitrogen fixation in woody debris, analyze parameters in the model for uncertainty, and compare the model output to independent field data. I answer questions 4 and 5 by simulating different scenarios with the model. Model output and literature-based estimates of nitrogen inputs over forest succession were used to answer question 6.

Chapter 2

Abiotic Controls on Nitrogen Fixation and Respiration in Woody Debris in the Pacific Northwest

William T. Hicks, Mark E. Harmon, and Robert P. Griffiths

Abstract

We estimated the effects of wood temperature, moisture, and oxygen concentration on nitrogen fixation and respiration rates in woody debris and used this information to model seasonal variation in these processes. We measured acetylene reduction and CO₂ evolution to test wood samples for nitrogen fixation and respiration activity at various levels of wood temperature, moisture, and oxygen. The interactions of log temperature, moisture, and oxygen content were examined in a model to test if temperature alone can be used as a predictor of seasonal changes in nitrogen fixation and respiration rates in woody debris. Nitrogen fixation and respiration had similar responses to temperature with nitrogen fixation being optimum near 30°C and respiration being optimum over a broader range from 30°C to 50°C. Nitrogen fixation and respiration responded similarly to wood moisture content with little to no measurable activity below 50%, and optimal activity above 175% to 100% for nitrogen fixation and respiration, respectively. Nitrogen fixation rates were optimized at 2% O₂ with rates much reduced above and below this concentration. Respiration rates were optimal when O₂ exceeded 1%. Past studies generally have used seasonal variations in temperature to predict the annual amounts of nitrogen fixed in woody debris, ignoring limitations of other abiotic factors. In our simulations, annual nitrogen fixation and respiration rates were 7.8 and 1.7 times greater, respectively, when only temperature limitations were included as compared to when all three abiotic controls were used. Therefore, seasonal interactions of abiotic factors need to be considered when estimating annual N₂ fixation and respiration rates.

Introduction

In the highly productive forest ecosystems of the Pacific Northwest, both tree fungal growth are limited by the abundance of nitrogen (Cowling and Merrill, 1966; Gessel, 1973; Spano *et al.*, 1982). Nitrogen fixation is an important input of this key nutrient, but little attention has been given to this process in woody debris in part because of its relatively low annual input when compared to symbiotic nitrogen fixation. However, a significant (14%) portion of a forest ecosystem's nitrogen over succession can be provided by asymbiotic fixation in woody debris even when symbiotic nitrogen fixers are present (Cromack *et al.*, 1979; Sollins *et al.*, 1987; Chapter 5).

Several abiotic factors are known to influence nitrogen fixation in woody debris including: temperature, wood moisture content, and oxygen concentration. However, few studies have developed mathematical relationships describing the effect of these variables on nitrogen fixation in woody debris, particularly for species found in the Pacific Northwest. Several studies have noted a relationship between nitrogen fixation rates in woody debris and temperature, moisture, or oxygen concentration (Sharp, 1975; Roskoski, 1981; Silvester *et al.*, 1982; Jurgensen *et al.*, 1984; Sollins *et al.*, 1987; Cushon and Feller, 1989; Wei and Kimmins, 1998). Sharp (1975) measured the effect of temperature from 15°C to 55°C on nitrogen fixation in decayed *Fagus* veneers, and found the highest rates at 35°C with lower activity above and below that optimum temperature. Wei and Kimmins (1998) found a linear correlation between acetylene reduction and *Pinus contorta* wood moisture at contents below 90%. In a study of nitrogen fixation in

Pseudotsuga menziesii woody debris from the Pacific Northwest, Silvester *et al.* (1982) found nitrogen fixation rates to be greatest at an oxygen concentration of 5%.

Nitrogen and molybdenum availability are also known to influence nitrogen fixation (Silvester, 1989). In the Pacific Northwest, nitrogen concentrations in woody debris never reach the relatively high levels needed to cause inhibition of fixation (Harmon *et al.*, 1986; Silvester, 1989). Molybdenum additions have been shown to significantly increase nitrogen fixation rates in wood and litter from areas of the Pacific Northwest, but information on regional patterns of molybdenum availability does not exist (Silvester, 1989). Except for molybdenum, Silvester (1989) found that no nutrients, of 13 tested, limit nitrogen fixation in wood and litter in the Pacific Northwest.

An understanding of the abiotic controls of respiration is also important in modeling nitrogen fixation because respiration indirectly affects nitrogen fixation by removing oxygen, an element that can inactivate nitrogenase. More work has focused on determining the effect of temperature, moisture, and oxygen concentrations on respiration than nitrogen fixation in woody debris; however, not for the substrates and range of environmental conditions found in woody debris of the Pacific Northwest (Jensen, 1967; Griffin, 1977; Boddy, 1983; Scheffer, 1985). Respiration rates generally increase with increasing moisture and oxygen concentrations; although, respiration decreases have been observed at high moisture contents under conditions where oxygen diffusion may be limiting (Boddy, 1983). Respiration responds to temperature in a similar manner as nitrogen fixation with a slightly higher optimum (Boddy, 1983; Chen *et al.*, 2000).

The objective of this study was to estimate the effect of temperature, moisture, and oxygen concentration on nitrogen fixation and respiration in woody debris from the

Pacific Northwest. We also examined the interactions of log temperature, moisture, and oxygen content in a model to test if temperature alone can be used as a predictor of seasonal changes in nitrogen fixation and respiration rates in woody debris.

Methods

Study area

Samples of woody debris were taken from the H.J. Andrews Experimental Forest and Cascade Head Experimental Forest. The H.J. Andrews is located on the west slope of the Central Oregon Cascades. Wet, cool winters and warm, dry summers characterize the climate. Mean annual temperature is 8.9°C and mean annual precipitation is 230 cm. Soils are deep, well-drained Typic Dystrochrepts (Griffiths, *et al.*, 1993). Forests are dominated from 1000-1500 m by *Pseudotsuga menziesii* and *Tsuga heterophylla* (Franklin and Dyrness, 1988). Cascade Head is in the Oregon Coast Range and borders the Pacific Ocean. Forests are dominated by *Picea sitchensis* and *Tsuga heterophylla* with Haplohumult soils predominating in the area (Franklin and Dyrness, 1988).

Laboratory procedures

In general, we followed the methods of Griffiths *et al.* (1993) when measuring acetylene reduction and respiration. We used their method because it used samples small

enough to avoid gas diffusion influences, which is critical when trying to measure the response of respiration and nitrogen fixation to moisture and oxygen concentration.

Cross sections of logs taken from H. J. Andrews and Cascade Head were wetted and stored in sealed plastic containers and incubated at 30°C for at least a week prior to measurements. Initial tests indicated this allowed the wood to reach ideal conditions for nitrogen fixation and respiration. Weighed, matchstick-sized pieces of the cross sections were removed, placed in screw-topped culture tubes, and stoppered with serum bottle caps.

Respiration was measured before acetylene reduction. We tested the effect of measuring respiration before and after acetylene reduction and no detectable effect was observed on either the respiration or acetylene reduction rate. When measuring respiration rates, the samples were pre-incubated for 30 minutes to allow the samples to adjust to the incubation environment. Samples for respiration tests were incubated in lab air at 30°C except when testing the effect of oxygen concentration or temperature. Initial CO₂ readings were taken with a Hewlett Packard model 5830 gas chromatograph fitted with a thermal conductivity detector. The gas chromatograph integrator was calibrated with external Scott® gas standards. After incubating for at least two hours a final reading was taken.

For acetylene reduction, tube headspace was purged with argon; then a portion of the headspace was removed and replaced with lab air and acetylene. The final acetylene concentration was 10% in all samples except the controls with wood that did not receive acetylene. Oxygen concentration was 4% in all tubes except when testing oxygen effects. Samples were incubated at 30°C for 24 h. Ethylene was measured on a Hewlett Packard

model 5830 gas chromatograph fitted with a flame ionization detector. In addition to having controls with wood and no acetylene, we had controls with only acetylene to measure the background ethylene present. Griffiths *et al.* (1989) previously tested this method to check for effects from sample preparation time, oxygen concentration, incubation time, and air exposure. From these tests, they concluded the method did not introduce significant experimental error.

After respiration and acetylene reduction were measured, the samples were weighed, dried at 80°C for 24 h, and reweighed. Moisture content was calculated by dividing the difference between sample weight before and after drying by the oven dry weight.

The effect of temperature and oxygen were measured by incubating at specified temperatures (e.g., 0, 5, 15, 25, 30, 45, and 65°C) or oxygen concentrations (0.3, 1, 2, 4, 8, and 20%). We did not create groups of samples at specific moisture concentrations (e.g., 0%, 50%, 100%, etc.), because thoroughly drying wood to set a known lower limit before rewetting with a defined amount of water can affect metabolic activity. Also, decayed wood often does not absorb all of the water added for rewetting (e.g., wood colonized by fungi with hydrophobic hyphae will repel water). Instead we created a range of moisture conditions by drying previously wetted wood in sealed containers over various amounts of drying agent during a period of one week. The effect of air and drying on wood respiration and acetylene reduction rates then were tested. Activity before and after exposure was not noticeably different once samples were rewetted.

Curve fitting

We developed equations to model the response of nitrogen fixation and respiration to temperature, moisture, and oxygen concentration. Parameter values for equations were estimated with nonlinear regression using SAS (1985). When examining the relationships of temperature and oxygen to nitrogen fixation and respiration, data points were the average of eight sub-samples. For the relationships of moisture with nitrogen fixation and respiration, each data point is an average of one to eight sub-samples. Each of the substrates tested had different maximum activity levels. To standardize the data for different substrates, we defined the reference level for a given abiotic factor to have a metabolic activity of one. All data values for other levels of the abiotic factors were then adjusted proportionally.

We used the Chapman-Richard's function to model the response of nitrogen fixation and respiration to the abiotic variables (Sit & Poulin-Costello, 1994). We also used a modified Q10 function to model the temperature response of respiration and nitrogen fixation. Goodness of fit, biological relevance, and simplicity were considered when deciding which type of equation to use when fitting data. The Chapman-Richard's function has the general form:

$$Y = a[1 - e^{-bX}]^c$$

where Y is the dependent variable, X is the independent variable, a is a parameter that adjusts the height of the curve, and b and c are parameters that influence the shape of the

curve. This equation was used to create a rising curve, while a complementary function was used to model a falling curve:

$$Y = a - (a[1 - e^{-bX}]^c).$$

To create a curve that rises and then falls (e.g., the oxygen response of nitrogen fixation) we multiplied the general and complementary forms of the equation.

For the rising portion of the temperature response, we also fitted a modified Q10 equation. Instead of a constant value for Q10, we used the following exponential function that allows Q10 to vary with temperature:

$$Q10 = a * e^{(-b * X)}$$

where X is the independent variable, in this case temperature, and b equals Q10 when temperature is zero and c is the rate Q10 decreases with temperature. The varying Q10 function is then used in the traditional Q10 equation:

$$Y = Q10^{(X - REFTEMP)/10}$$

For these analyses we used 15°C for the reference temperature (REFTEMP).

Seasonal interactions

We examined the importance of including the effect of temperature, moisture, and oxygen when estimating seasonal nitrogen fixation rates by using a model of nitrogen fixation in dead wood that incorporates the response curves from this study (Chapter 5). This model tracks daily nitrogen fixation and respiration rates in a log composed of five layers. Daily temperature and precipitation data are used to generate temperature and moisture profiles within the log. Respiration and oxygen diffusion rates are used to produce a profile of oxygen concentration within the log. Daily nitrogen fixation and respiration rates are modified by indices developed in this study relating fixation and respiration to temperature, moisture, and oxygen concentration. We used a 50 cm diameter, *Tsuga heterophylla*, decay class one log (least decayed) and meteorological data from the H.J. Andrews Experimental Forest for simulating seasonal dynamics of nitrogen fixation, temperature, moisture, and oxygen concentration.

Results

Temperature response

Nitrogen fixation and respiration had different responses to temperature (Figure 2.1a & b). Measured nitrogen fixation rates were highest at 30°C, with the Chapman-Richard's and Q10 functions peaking at 29°C and 27°C, respectively. Fixation rates

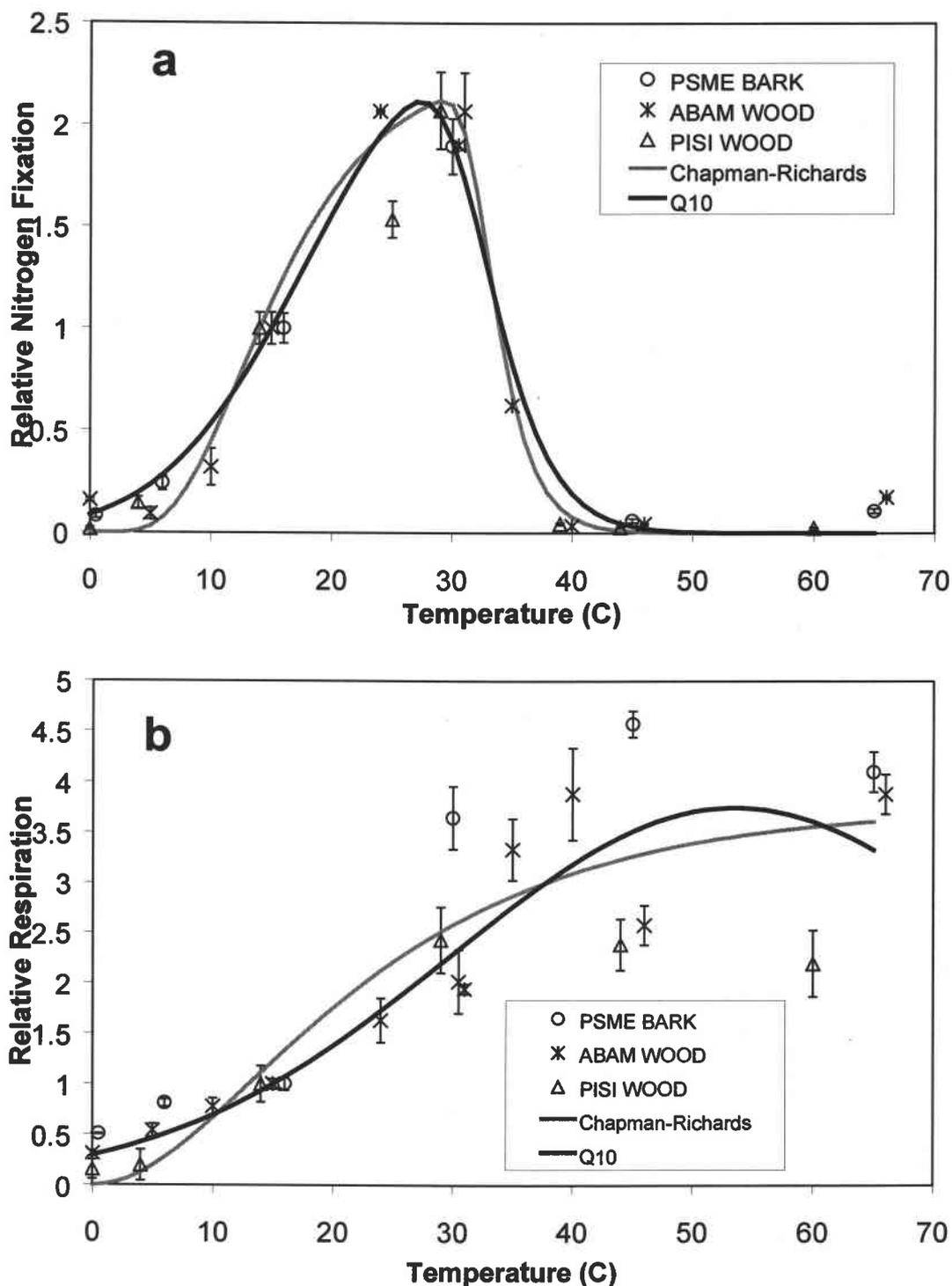


Figure 2.1. The effect of temperature on (a) nitrogen fixation and (b) respiration in *Pseudotsugamenziesii* (PSME) bark, *Abies amabilis* (ABAM) wood, and *Picea sitchensis* (PISI) wood. Error bars represent the standard error from the eight samples used at each temperature.

dropped more rapidly above 30°C than below. Both functions precisely fit the nitrogen fixation data (Adjusted $R^2 = 0.97$ and 0.96 ; Table 2.1). Respiration rates of *Pseudotsuga menziesii* bark and *Picea sitchensis* wood reached optimums at 40°C and 30°C respectively, while *Abies amabilis* wood respiration leveled off from 40°C to 65°C. The Chapman-Richard's equation provided a better fit, over the entire range of temperatures measured, to the respiration response data having an adjusted R^2 of 0.86 compared to 0.76 for the Q10 equation (Table 2.1). However, from 0°C to 30°C both equations fit the data equally well with an adjusted R^2 of 0.82.

Table 2.1. Parameter and adjusted R-squared values for the Chapman-Richard's (CR) and Q10 equations used to estimate the influence of the abiotic variables on nitrogen fixation and respiration.

Process	Variable/ Function	Rising Curve			Falling Curve			Adj. R^2
		a	b	c	a	b	c	
Nitrogen Fixation	Temperature CR	2.30	1.53 x 10^{-1}	6.78	1.00	4.66 x 10^{-1}	4.41 x 10^6	0.97
	Temperature Q10	5.10	3.70 x 10^{-2}		1.00	2.70 x 10^{-1}	7.00 x 10^3	0.96
	Moisture CR	5.48 x 10^{-1}	1.94 x 10^{-2}	2.89				0.68
	Oxygen CR	1.00	3.57	7.18	1.00	1.34 x 10^{-1}	2.42	0.72
Respiration	Temperature CR	3.75	6.26 x 10^{-2}	2.26				0.86
	Temperature Q10	2.27	8.91 x 10^{-3}					0.76
	Moisture CR	4.23 x 10^{-1}	4.54 x 10^{-2}	8.13				0.54
	Oxygen CR	8.49 x 10^{-1}	16.4	147				0.41

Moisture response

The response of nitrogen fixation and respiration rates to moisture was similar (Figure 2.2a & b). Nitrogen fixation rates ceased below approximately 50% moisture content. At log moisture contents greater than 50%, fixation rates rose to an optimum that was reached at 175% moisture content. The fitted Chapman-Richard's equation had an adjusted R^2 of 0.68 (Table 2.1). Respiration rates were slightly less sensitive to moisture content with activity increasing above 45% and leveling off after reaching a maximum at 100%. The fitted equation had an adjusted R^2 of 0.54 (Table 2.1).

Oxygen response

Nitrogen fixation and respiration rates responded differently to oxygen concentration (Figure 2.3a & b). Nitrogen fixation rates were optimum at 2% oxygen and decreased more steeply below this concentration than above. Respiration rates rose rapidly, reached a maximum at 0.5% oxygen, and then remained high. The curve describing the response of nitrogen fixation provided a better fit than the curve for respiration (Adjusted $R^2 = 0.72$ and 0.41 respectively; Table 2.1).

Seasonal interactions

Model simulations indicate that nitrogen fixation and respiration rates change greatly over a year and as abiotic factors are included in the model. Log moisture

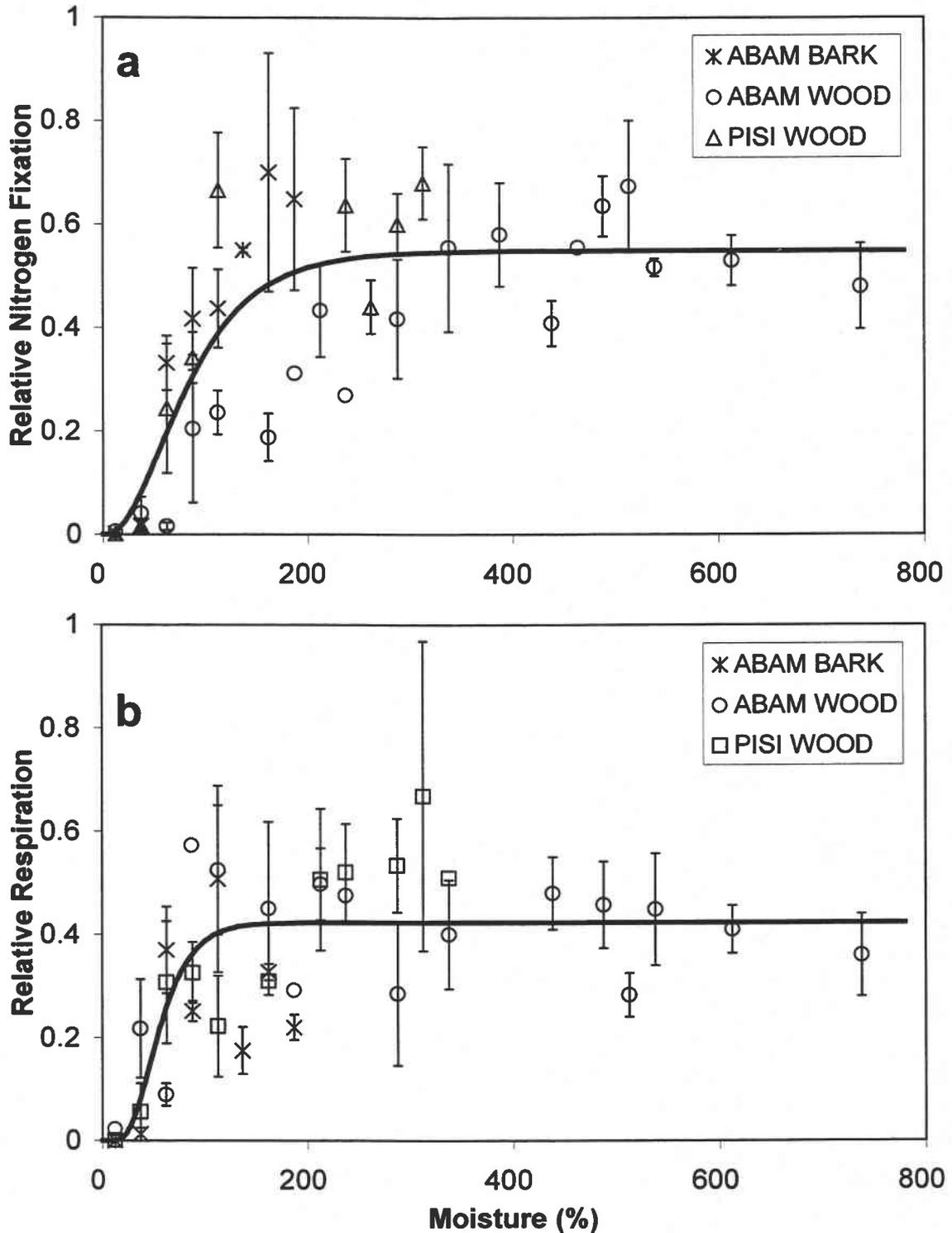


Figure 2.2. The effect of moisture on (a) nitrogen fixation and (b) respiration in *Abies amabilis* (ABAM) bark and wood, and *Picea sitchensis* (PISI) wood. Error bars represent the standard error from the one to eight samples used at each moisture content.

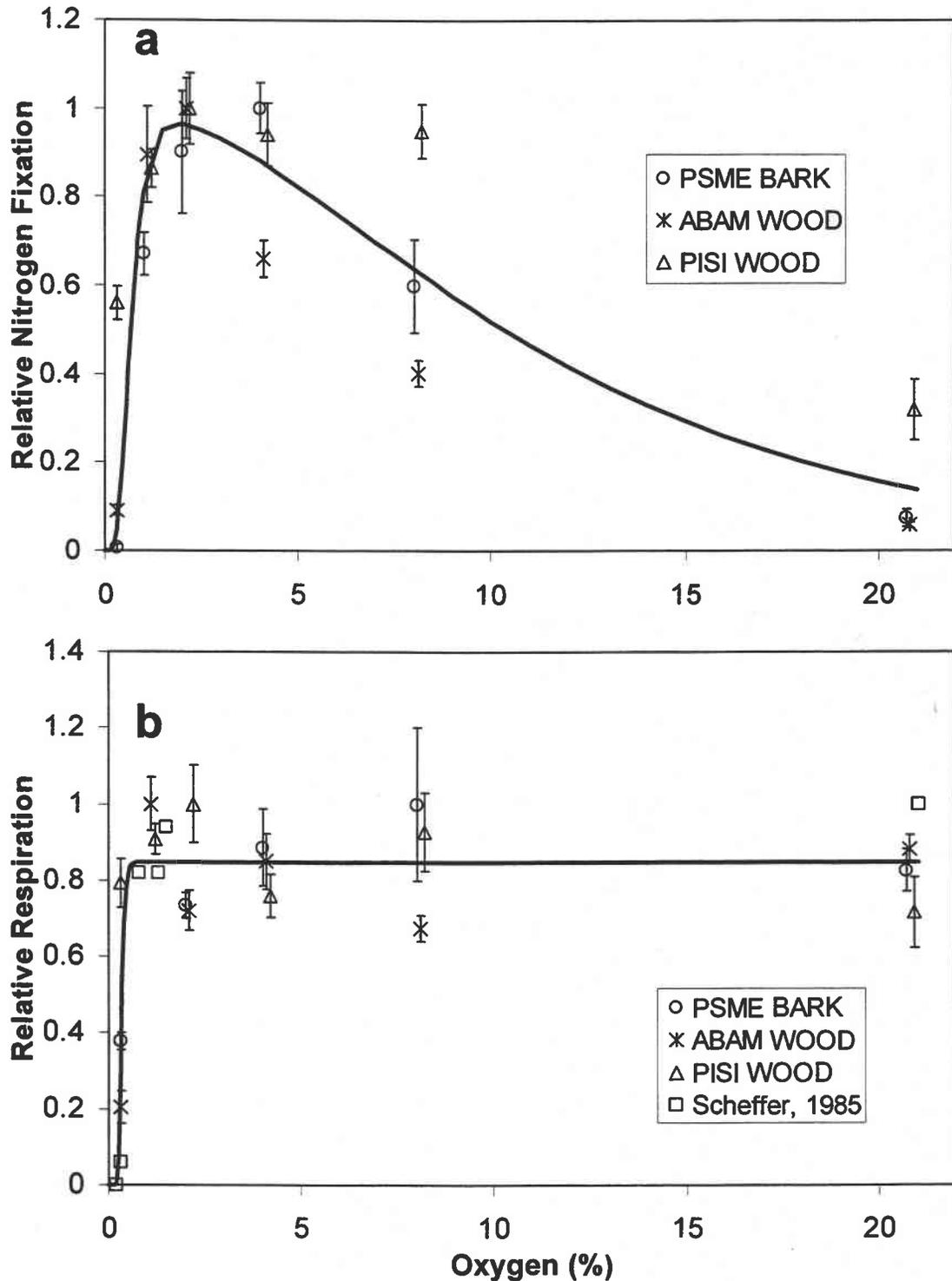


Figure 2.3. The effect of oxygen concentration on (a) nitrogen fixation and (b) respiration in *Pseudotsuga menziesii* (PSME) bark, *Abies amabilis* (ABAM) wood, and *Picea sitchensis* (PISI) wood. Error bars represent the standard error from the eight samples used at each oxygen concentration.

declines in the summer when temperatures are highest, while oxygen concentration is lowest when moisture is highest and vice versa (Figure 2.4a). When temperature is the only abiotic variable controlling nitrogen fixation in the model, daily rates closely track temperature changes (Figure 2.4b). If moisture and temperature limitations are both used in the model, daily nitrogen fixation rates closely track temperature until Julian Day 150 (May 30) when declining log moisture begins inhibiting nitrogen fixation. After Julian Day 275, fall rains rewet the log and fixation rates again track temperature changes. When the influence of temperature, moisture, and oxygen are included in the model, fixation rates decline further, especially from Julian Day 150 through 275 when dry conditions create high oxygen concentrations.

Annual estimates of nitrogen fixation and respiration rates drop greatly as abiotic factors are included in the model simulations (Table 2.2). When moisture and temperature are included in the simulation, annual nitrogen fixation rates are about one third the rates when only temperature is included and nearly eight fold lower when all abiotic controls are included as opposed to including only temperature. Annual respiration is less sensitive than nitrogen fixation to the inclusion of moisture and oxygen in the simulations, because it is optimum at lower moistures and oxygen concentrations.

Table 2.2. Annual nitrogen fixation and respiration rates from model runs that included various combinations of abiotic controls.

Abiotic Factors Included	Nitrogen Fixation (nmol·g⁻¹·yr⁻¹)	Respiration (μmol·g⁻¹·yr⁻¹)
Temperature	273.8	168.2
Temperature & Moisture	97.2	103.1
Temperature, Moisture, & Oxygen	35.3	102.9

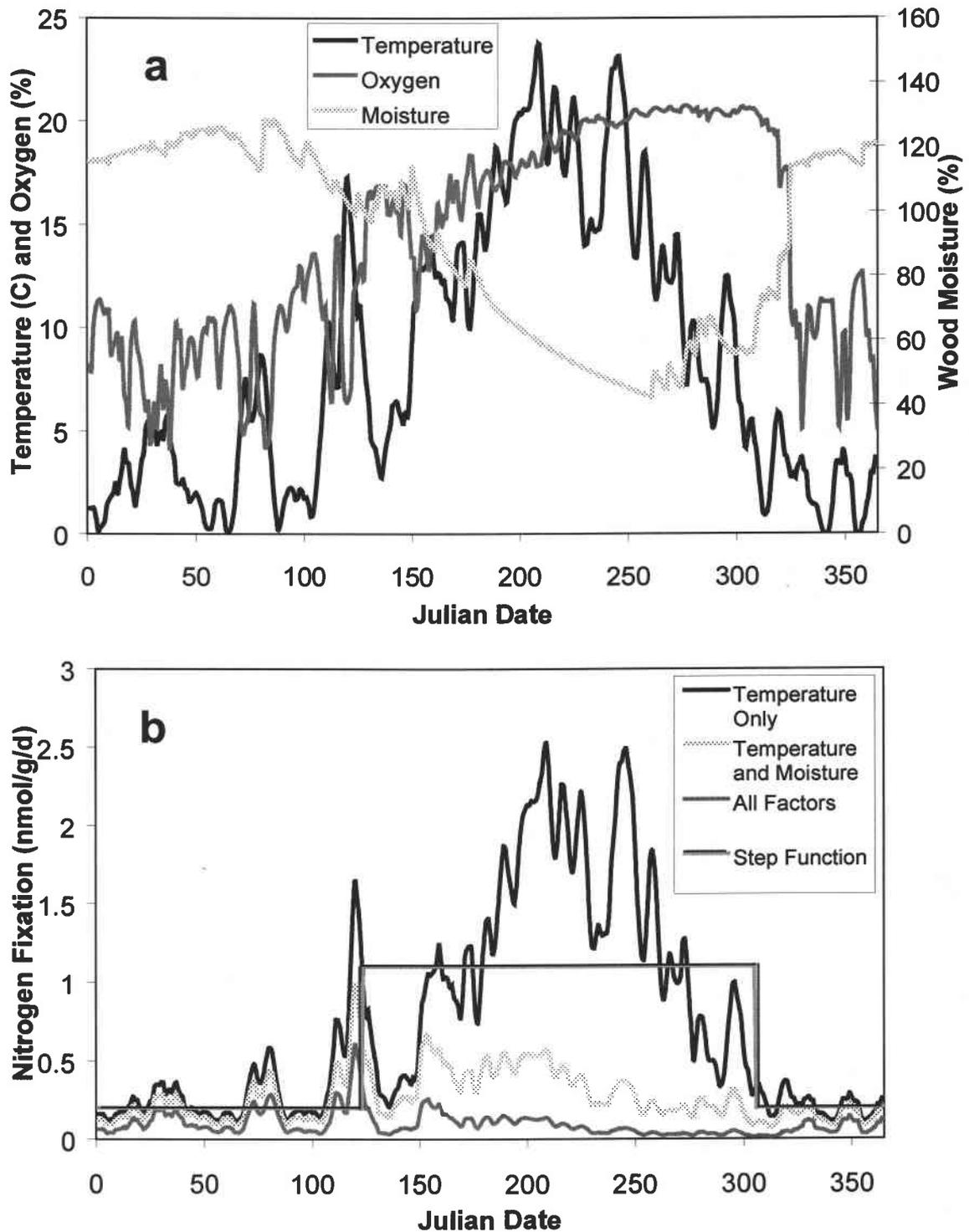


Figure 2.4. Seasonal changes in (a) average log temperature, moisture, and oxygen content and (b) nitrogen fixation rates from a simulation model using a 50 cm diameter, decay class one *Tsuga heterophylla* log and meteorological data from the H.J. Andrews Experimental Forest.

Discussion

Temperature response

Both the Chapman-Richard's and modified Q10 equations provided a precise fit to the nitrogen fixation data (Table 2.1); however, the Q10 function has been widely used and is slightly easier to interpret. While the Chapman-Richard's equation provides a better overall fit to the respiration data (Table 2.1), the modified Q10 equation does an equal or better job of modeling the temperature data from 0°C to 30°C. In addition, the modified Q10 equation declines above 50°C. Even though the data do not demonstrate an obvious decline, respiration rates must cease eventually as temperature increases and the modified Q10 function is more physiologically realistic in this sense. In the Pacific Northwest, wood temperatures rarely exceed 30°C except in settings such as clear-cuts where high radiation inputs occur. Thus, either function provides a reasonable model for the temperature response for forests with intact canopies.

Our nitrogen fixation temperature response curves are similar to those found in other studies. Sharp (1975) measured nitrogen fixation response to five temperatures (15, 25, 35, 45, and 55°C) in *Fagus veneers*, and found rates to be highest at 35°C. The response of nitrogen fixation in litter to temperature has also been studied. Nitrogen fixation in litter is similar to fixation in wood because both processes are carried out by free-living microorganisms as opposed to symbiotic nitrogen fixers such as those associated with plants and lichens. Heath *et al.* (1988) measured the response of nitrogen fixation in litter from the Pacific Northwest to temperature and found rates to peak at

22°C and a sharp drop above 27°C. O'Connell and Grove (1987) measured the response of nitrogen fixation in litter from south-western Australia to temperature and found rates to peak at 25°C and drop sharply above 28°C. The slightly lower optimum fixation temperature in litter as opposed to wood may be a result of the tendency for litter to dry more quickly than wood. Litter fixation often ceases in summer when temperatures are highest and litter is driest (Heath *et al.*, 1988; O'Connell and Grove, 1987). This creates a situation where the maximum temperature at which fixation occurs is lower than the highest litter temperatures. Microorganisms in litter would therefore have no selective pressure to evolve an optimum temperature as high as that found in wood.

Our results for the response of respiration to temperature are similar to those found in other studies where rates reach an optimum and decline above it (Flanagan & Veum, 1974; Moore, 1986; O'Connell, 1990). Boddy (1983) measured the response of wood respiration to temperature from 5°C to 25°C and found it to increase. Chen *et al.* (2000) found respiration rates in decomposing roots from the Pacific Northwest to be optimum from 30°C to 40°C. The optimum temperature for respiration by *Psuedotsuga menziesii* litter from the Pacific Northwest was 40°C (Moore, 1986). Similarly, O'Connell (1990) found litter from Australian eucalypt forests to respire optimally from 33°C to 34°C. Organic residue, including dead wood, from the Alaskan tundra respired at a lower optimum (25°C), possibly resulting from adaptation of the respiring organisms to lower temperatures (Flanagan & Veum, 1974).

The response of respiration in *Abies amabilis* wood in this study was somewhat different than other observed responses. Respiration did not exhibit much if any decline even at 65°C. We originally thought this might be due to experimental error because our

short pre-incubation period (30-60 min.) may not have allowed the wood to reach the incubation temperature or may have triggered an increase in activity in response to stress. To test this, we ran additional samples of *Abies amabilis* wood using a 24 hour pre-incubation period at 30, 40, 50, and 60°C. The respiration rate in this experiment was highest at 40°C ($1.8 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{hr}^{-1}$) and similar at 30, 50, and 60°C ($0.9\text{-}1.0 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{hr}^{-1}$). Thus, a Q10 response does not occur above 40°C and wood decomposers may be more tolerant of high temperatures than generally recognized.

Moisture response

The Chapman-Richard's function also provides a reasonable means for modeling the response of nitrogen fixation and respiration to moisture. The fitted equation modeled the response of nitrogen fixation to moisture well considering the variation in the data (Adjusted $R^2 = 0.68$; Table 2.1). The Chapman-Richard's function provides an adequate model for the respiration response to moisture despite the low adjusted R^2 of 0.54 (Table 2.1).

Our results for the response of nitrogen fixation to moisture are similar to those found in other studies. In general, nitrogen fixation activity ceases below a minimum moisture content where the remaining water is too tightly bound to the substrate for use by the fixing organism. Wei and Kimmins (1998) found a linear correlation between acetylene reduction and *Pinus contorta* wood moisture at contents below 90%. Silvester *et al.* (1982) measured nitrogen fixation rates in dead wood from the Pacific Northwest at moisture contents from 200% to 400% and found rates to be constant. Heath *et al.* (1988)

measured the response of nitrogen fixation to moisture in litter from the Pacific Northwest. They found that litter fixation did not occur below 35% moisture content. At moisture contents above 35%, Heath *et al.* (1988) found nitrogen fixation rates increase to an optimum at and above 170%. O'Connell and Grove (1987) found the same response of nitrogen fixation to moisture in litter from Australia with fixation ceasing below a minimum moisture content (~40%), and rising to an optimum at and above 100-200% moisture content.

The general response of respiration to moisture is the same as the response of nitrogen fixation except under conditions where oxygen diffusion may be limiting. Respiration rate can decline at high moisture contents, but presumably not because of the direct influence of moisture. Instead reduced oxygen levels, caused by slower diffusion at high moisture contents, inhibit respiration. We specifically avoided using samples large enough to have oxygen diffusion problems, because our intent was to directly measure the effect of oxygen and incorporate this in our model of nitrogen fixation in dead wood (Chapter 5).

Our results for the response of respiration to moisture are similar to those found in other studies. Boddy (1983) measured the response of respiration to moisture content in branch dead wood and found rates to cease below 30% moisture content, rise from 30% to an optimum, then level off. Flanagan and Veum (1974) measured respiration in organic residues from the Alaskan tundra and found a variable response to high moisture depending on the site, substrate, and temperature. At moisture contents above 300% and temperatures above 10°C inhibition occurred at one of the two sites tested. However, when substrates were incubated in 100% O₂, no inhibition of respiration was observed at

high moisture contents indicating reduced oxygen diffusion and availability was causing the respiration inhibition. Chen *et al.* (2000) found a slight inhibition of respiration in decaying roots from the Pacific Northwest at high moisture contents, but the inhibition was minor and not present in all species tested.

Oxygen response

The Chapman-Richard's function provides a reasonable means for modeling the response of nitrogen fixation and respiration to oxygen. This function provides a reasonably close fit; although, the adjusted R^2 for the respiration response is somewhat low (Table 2.1).

The response of nitrogen fixation to oxygen concentration we observed is similar to the results of others. Asymbiotic nitrogen fixers tend to fix optimally under microaerophilic conditions. Nitrogenase is inactivated by oxygen, but the fixing organisms require energy for fixation from aerobic respiration or from the byproducts of aerobic respiration by other organisms (Hendrickson, 1991). Silvester *et al.* (1982) found nitrogen fixation rates to be greatest at an oxygen concentration of 5% with fixation nearly absent at 0% oxygen and approximately half the optimum value at 20%.

The effects of oxygen on respiration that we observed are similar to those found in other studies. Highley *et al.* (1983) found wood decay as measured by mass loss to be lower at 1% than above 10% O_2 . Fungi are the principal organisms responsible for wood decay, and when wood-decomposing fungal isolates are exposed to varying oxygen concentrations, similar respiration responses are observed. Scheffer (1986) found nearly

the same pattern as we did in a thorough examination of the relationship between fungal growth and oxygen on a number of fungal isolates from the Pacific Northwest (Figure 2.3b). By precisely controlling oxygen content, he found growth rates to increase from no growth at and below 0.2% oxygen to nearly optimal levels at 0.8%. In general, other studies find the same pattern; although, the rate of increase above 0% oxygen is not always as steep (Jensen, 1967; Highley *et al.*, 1983). The curve used to model the response of respiration to oxygen captured Scheffer's (1986) and our data well (Figure 2.3b). Therefore, we feel this curve does an adequate job of capturing the average response of respiration by several substrates and fungal species from the Pacific Northwest.

Seasonal interactions

In the Pacific Northwest, wood temperature, moisture, and oxygen content fluctuate throughout the year (Chapter 4; Harmon and Sexton, 1995). The dry, warm summers and cool, wet winters create a pattern of wood temperature and moisture that make it difficult to predict nitrogen fixation rates in wood from temperature alone. Despite this, the best seasonal estimates we have of nitrogen fixation in wood for the Pacific Northwest rely on sampling at a few points in time and using a Q10 temperature response function to estimate rates for the rest of the year (Sollins *et al.*, 1987). Using a simulation model of nitrogen fixation and respiration developed in part from the functions in this study, we examined how past approaches might over- or underestimate annual estimates (Chapter 5, Figure 2.4).

Using temperature as the only variable to control nitrogen fixation rates could produce gross over- or underestimates. Sollins *et al.* (1987) used a step function to estimate annual nitrogen fixation rate where the average winter and summer temperatures were used to estimate rates throughout the year (Figure 2.4b). The samples used by Sollins *et al.* (1987) included moisture and oxygen limitations to some degree; however, without repeated sampling during the year or knowledge of annual changes in wood moisture and oxygen getting the correct annual fixation rate is fortuitous. Our model results indicate that annual fixation rates could be greatly miscalculated when measurements are made only a few times during a year. If samples are taken during times when samples are not limited or greatly limited by moisture and oxygen conditions, annual nitrogen fixation and respiration rates will be over- or underestimated, respectively.

Conclusions

Despite the regulatory importance of abiotic variables on metabolic processes in dead wood, there is little information on seasonal changes of these variables and the mechanisms that control them. Data and models similar to those gathered and developed in the soil sciences would greatly improve our understanding and ability to predict metabolic processes such as nitrogen fixation and respiration in woody debris.

Microbial population size may also influence the seasonal dynamics of a metabolic process. Lags in activity would result if microbial populations respond slowly

to changes in abiotic factors. Key areas for future research include measuring the effect of population size on nitrogen fixation and respiration and seasonal population dynamics of the microorganisms.

Acknowledgments

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Chapter 3

Biotic Controls on Nitrogen Fixation and Respiration in Woody Debris of the Pacific Northwest

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Abstract

We estimated the effect of wood species, tissue, and degree of decay on nitrogen fixation and respiration in woody debris from the Pacific Northwest. We also examined differences among sites and between actual and potential rates of nitrogen fixation and respiration where samples for potential measurements were amended with water and incubated for a week. We determined nitrogen fixation and respiration rates in several wood species, tissues, and decay classes, and at three sites using acetylene reduction and CO₂ evolution, respectively. We also directly measured nitrogen fixation with ¹⁵N₂ on a subset of samples to calculate the conversion ratio of acetylene reduced to ¹⁵N₂ fixed (AR: ¹⁵N₂ ratio). The average AR: ¹⁵N₂ ratio increased as acetylene reduction and ¹⁵N₂ fixation rates increased. For example, the average AR: ¹⁵N₂ ratio increased with temperature from 3.6 at 10°C to 4.9 at 30°C. Increased nitrogen fixation rates may result in increased rates of inhibitory processes, such as hydrogen evolution, that can inhibit nitrogen fixation but not acetylene reduction. Actual and potential nitrogen fixation and respiration rates peaked in moderately decayed wood. The relationship between nitrogen fixation and respiration and the degree of decay is probably due to changing patterns of moisture, microbial colonization, and resource quality. Actual nitrogen fixation and respiration rates were significantly higher at a warmer, wetter coastal site when compared to two interior sites, but potential rates were not significantly different. There were no significant differences among *Pseudotsuga menziesii*, *Tsuga heterophylla*, or *Picea sitchensis* for nitrogen fixation or respiration. Nitrogen fixation rates dropped from 0.72 $\eta\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ in outer bark, to 0.57 $\eta\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ in inner bark, to 0.24 $\eta\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ in sapwood, to 0.09 $\eta\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ in heartwood. Respiration rates were highest in inner bark

at $1.51 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$, followed by outer bark at $1.14 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$, then by sapwood at $0.88 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$, and finally heartwood at $0.17 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$. Potential nitrogen fixation and respiration rates averaged $0.62 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ and $0.08 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ higher than actual rates, respectively. Patterns of microbial colonization and abundance, resource quality, and climate probably explain most of the patterns observed in our study.

Introduction

In the highly productive forest ecosystems of the Pacific Northwest, both tree and fungal growth are limited by the abundance of nitrogen (Cowling and Merrill, 1966; Gessel, 1973; Spano *et al.*, 1982). Nitrogen fixation is an important input of this key nutrient, but little attention has been given to this process in woody debris in part because of its relatively low annual input when compared to symbiotic nitrogen fixation. However, a significant (14%) portion of a forest ecosystem's nitrogen over succession can be provided by asymbiotic fixation in woody debris even when symbiotic nitrogen fixers are present (Cromack *et al.*, 1979; Sollins *et al.*, 1987; Chapter 5).

Several biotic factors are known to influence nitrogen fixation in woody debris including: wood species, tissue, and degree of decay. Several studies have demonstrated that wood species significantly affects nitrogen fixation rate (Jurgensen *et al.*, 1989; Harvey, *et al.*, 1989; Griffiths *et al.*, 1993); whereas, Sollins *et al.* (1987) found no significant differences between the three species they examined. Griffiths *et al.* (1993) found nitrogen fixation rates varied with wood tissue during the first six years of

decomposition with the highest through lowest rates found in inner bark, outer bark, sapwood, and heartwood, respectively. Larsen *et al.* (1978) and Jurgensen *et al.* (1984) found nitrogen fixation rates increased as wood decay progressed, while Harvey *et al.* (1989) and Sollins *et al.* (1987) did not find a consistent pattern.

Decay type and the abundance of nitrogen fixing organisms are also known to influence nitrogen fixation in wood. The species of saprophyte influences nitrogen fixation rate (Larsen *et al.*, 1978; Jurgensen *et al.*, 1989; Harvey, *et al.*, 1989). Brown-rotted wood was shown to fix more nitrogen than white-rotted wood (Larsen *et al.*, 1978); however, Jurgensen *et al.* (1989) found the opposite pattern. Crawford *et al.* (1997) found the lowest numbers of nitrogen fixing organisms in wood with the lowest nitrogen fixation rates.

An understanding of biotic controls on respiration is also important in modeling nitrogen fixation because respiration indirectly affects nitrogen fixation by removing oxygen, an element that can inactivate nitrogenase. Wood species, tissue, and degree of decay affect respiration rate. Sollins *et al.* (1987) found no significant differences in respiration rate between *Pseudotsuga menziesii*, *Tsuga heterophylla*, and *Thuja plicata* when examining the entire range of wood decay; however, Carpenter *et al.* (1988) found *Pseudotsuga menziesii* to have higher respiration rates than *Tsuga heterophylla* early in decay. Yearly decomposition rates are known to differ between species (e.g., Harmon *et al.*, 1986; Yin, 1999). Wood tissues respire at different rates with the highest through lowest rates found in inner bark, outer bark, sapwood, and heartwood, respectively (Carpenter *et al.*, 1988; Griffiths *et al.*, 1993). Sollins *et al.* (1987) found no significant difference between respiration rates from various stages of decay.

The primary objective of this study was to estimate the effect of wood species, tissue, and degree of decay on nitrogen fixation and respiration in woody debris from the Pacific Northwest. We also examined differences between sites and between actual and potential rates of nitrogen fixation and respiration. Effects of other biotic factors such as decomposer species were not investigated because they are considered to be of secondary importance. Also, these factors are incorporated in the samples used for this study. The rates measured in this paper were used to parameterize a nitrogen fixation simulation model (Chapter 5).

Methods

Study area

Samples of woody debris were taken from the H.J. Andrews, Wind River, and Cascade Head Experimental Forests. The H.J. Andrews is located on the west slope of the central Oregon Cascades. Wet, cool winters and warm, dry summers characterize the climate. Mean annual temperature is 8.9°C and mean annual precipitation is 230 cm. Soils in the area we sampled are deep, well-drained Typic Dystrochrepts (Griffiths, *et al.*, 1993). Forests are dominated from 1000-1500 m by *Pseudotsuga menziesii* and *Tsuga heterophylla* (Franklin and Dyness, 1988). Wind River Experimental Forest is located on the west slope of the southern Washington Cascades. The climate and vegetation are similar to the H. J. Andrews. Mean annual temperature and precipitation are 8.8°C and

250 cm respectively. Forests are dominated by *Pseudotsuga menziesii* and *Tsuga heterophylla* with Haplorthod and Vitrandept soils predominating in the area (Franklin and Dyrness, 1988). Cascade Head is in the Oregon Coast Range and borders the Pacific Ocean. Mean annual temperature is 10°C and mean annual precipitation is 340 cm. Forests are dominated by *Picea sitchensis* and *Tsuga heterophylla* with Haplohumult soils predominating in the area (Franklin and Dyrness, 1988).

Field and laboratory procedures

We examined the effect of wood species and the amount of decay in samples from all three of the experimental forests, but tissue level effects were tested only with woody substrates from the H. J. Andrews. We selected *Pseudotsuga menziesii* and *Tsuga heterophylla* logs at the H J. Andrews and Wind River Experimental Forests; whereas, we sampled *Picea sitchensis* and *Tsuga heterophylla* logs at Cascade Head. Logs that could be identified to species were selected as randomly as possible. Logs were assigned to a decay class with decay class one being least decayed and five the most decayed (Harmon and Sexton, 1996). Wood samples for testing tissue level effects were taken from logs being used in a 200 year time series study of wood decay (Harmon, 1992). The effect of wood tissue on nitrogen fixation and respiration rates were obtained from published data and unpublished remeasurements (Griffiths *et al.*, 1993). Griffiths *et al.* (1993) measured nitrogen fixation and respiration rates in four wood tissues of four Pacific Northwest species during the first six years of wood decay at the H.J. Andrews

Experimental Forest. Subsequent unpublished resampling has extended this database to cover the first twelve years of wood decay.

Cross sections of logs taken from the experimental forests were wrapped in plastic then taken to the laboratory for sample preparation and measurement. Weighed, matchstick-sized pieces of the cross sections were removed, placed in screw-topped culture tubes, and stoppered with serum bottle caps. "Actual" acetylene reduction and respiration measurements were started within 24 hours of log sampling. In this study "actual" conditions indicate that fixation and respiration were measured as soon as possible and when wood moisture was not optimized. After these measurements were taken, samples were wetted and stored in their stoppered culture tubes and incubated at 15°C for at least a week prior to remeasurement. Initial tests indicated this allowed the wood to reach ideal conditions for nitrogen fixation and respiration. "Potential" nitrogen fixation and respiration rates refer to the measurements taken under these more ideal conditions.

In general, we followed the methods of Griffiths *et al.* (1993) when measuring acetylene reduction and respiration. Respiration was measured before acetylene reduction. We tested the effect of measuring respiration before or after acetylene reduction and no detectable effect was observed on either the respiration or acetylene reduction rate. When measuring respiration rates, the samples were pre-incubated for 30 minutes to allow the samples to adjust to the incubation environment. Samples for respiration tests were incubated in lab air at 15°C. Initial CO₂ readings were taken with a Hewlett Packard model 5830 gas chromatograph fitted with a thermal conductivity

detector. The gas chromatograph integrator was calibrated with external Scott® gas standards. After incubating for at least two hours a final reading was taken.

For acetylene reduction, the tube headspace was purged with argon; then a portion of the headspace was removed and replaced with lab air and acetylene. The final acetylene concentration was 10% in all samples except the controls with wood that did not receive acetylene. Headspace oxygen concentration was adjusted to an optimal 4% (Griffiths *et al.*, 1993). Samples were incubated at 15°C for 24 h. Ethylene was measured on a Hewlett Packard model 5830 gas chromatograph fitted with a flame ionization detector. In addition to having controls with wood and no acetylene, we had controls with only acetylene to measure the background ethylene present. Griffiths *et al.* (1989) previously tested this method to check for effects from sample preparation time, oxygen concentration, incubation time, and air exposure. From these tests, they concluded the method did not introduce significant experimental error.

After respiration and acetylene reduction were measured, the samples were weighed, dried at 80°C for 24 h, and reweighed. Moisture content was calculated by dividing the difference between sample weight before and after drying by the oven dry weight.

To convert acetylene reduction data to the actual amount of nitrogen fixed, we directly measured nitrogen fixation with $^{15}\text{N}_2$ on a subset of samples and calculate the ratio of acetylene reduced to $^{15}\text{N}_2$ fixed (AR: $^{15}\text{N}_2$ ratio). Acetylene reduction rates, using the above methods, were first measured on two different substrates (*Abies amabilis* and *Picea sitchensis* wood) at three temperatures (10, 20, and 30°C). After measuring acetylene reduction on the samples, the headspace was purged and oxygen was added to

produce a concentration of 4%. Finally, 100 atom percent $^{15}\text{N}_2$ was added to produce a headspace with 14 atom percent $^{15}\text{N}_2$ except in the control wood samples that received no $^{15}\text{N}_2$. Wood samples were incubated for two days then removed, ground, and analyzed with a mass spectrometer to get the absolute and relative amounts of the nitrogen isotopes in the samples. Initial and final headspace samples were also taken for nitrogen isotope ratio measurement to determine $^{15}\text{N}_2$ headspace concentrations and leakage rates. We used the average ratio for all samples when converting AR values to dinitrogen fixed.

Statistical analysis

All statistical analysis including Analysis of Variance (ANOVA), Analysis of Covariance (ANCOVA), Least Squares Means (LSMEAN), and 95% Confidence Limits were performed with SAS (1985). In general, we used ANOVA to determine if significant differences existed between the means of independent variables. Because wood moisture varied greatly among the samples used to measure the actual nitrogen fixation and respiration rates, we also performed ANCOVA with moisture included as a covariate to analyze the actual rates. We only used ANCOVA with moisture and sampling date as covariates to estimate differences in nitrogen fixation and respiration rate between wood tissues. Sampling date was included as a covariate, because the wood tissue data was collected periodically over the first twelve years of log decay. Only data from years nine through twelve were used to avoid periods early in decay when wood tissues were not fully colonized.

Nitrogen fixation and respiration rates had long-tailed distributions and required a natural log transformation prior to analysis. When reporting results in these cases, means of the log transformed values were backtransformed for ease of interpretation. Therefore, reported results are the medians of the untransformed data, because the backtransformed mean of the log transformed values equals the median (but not necessarily the mean) of the untransformed data. The ratios of acetylene reduced to dinitrogen fixed, and the differences of potential and actual nitrogen fixation and respiration rates did not require transformations as they were normally distributed.

For this study, we consider relationships to be statistically significant when the p-value is less than 0.05. The 95% confidence limits on figures provide a simple visual means to compare means. Using the terminology of Ramsey and Schafer (1995), we use the phrase “conclusive evidence” of a difference between two means to describe situations where confidence limits do not overlap at all and “strong evidence” to describe situations where confidence limits may overlap but not enough to include the mean being compared.

Results

AR: $^{15}\text{N}_2$ ratio

The AR: $^{15}\text{N}_2$ ratio significantly differed between the two wood species and among the three incubation temperatures (Table 3.1, Figure 3.1). *Picea sitchensis* had a

Table 3.1. P values and significance of the means for each of the independent variables for the different experiments from ANOVA and ANCOVA tests. The p-values for the covariate moisture were not included in the table.

Dependent Variable	Independent Variable	P Values	
		ANOVA	ANCOVA
AR: ¹⁵ N ₂ Ratio	Species	<0.001*	
	Temperature	0.024*	
Potential N ₂ Fixation	Decay Class	0.008*	
	Site	0.068	
	Species	0.722	
Actual N ₂ Fixation	Decay Class	0.046*	0.062
	Site	0.022*	0.023*
	Species	0.413	0.425
	Wood Tissue [†]		<0.001*
Potential Respiration	Decay Class	0.001*	
	Site	0.407	
	Species	0.354	
Actual Respiration	Decay Class	0.014*	0.003*
	Site	0.004*	0.004*
	Species	0.181	0.187
	Wood Tissue [†]		<0.001*
Difference of Potential and Actual N ₂ Fixation	Decay Class	0.027*	0.185
	Site	0.631	0.634
	Species	0.174	0.177
Difference of Potential and Actual Respiration	Decay Class	0.051	0.603
	Site	0.080	0.089
	Species	0.125	0.134

* Means for independent variables are considered to be significantly different from each other when p is less than 0.05.

[†] Data for estimating nitrogen fixation and respiration means for wood tissues are from Griffiths *et al.* (1993) and subsequent unreported resampling (see Methods section).

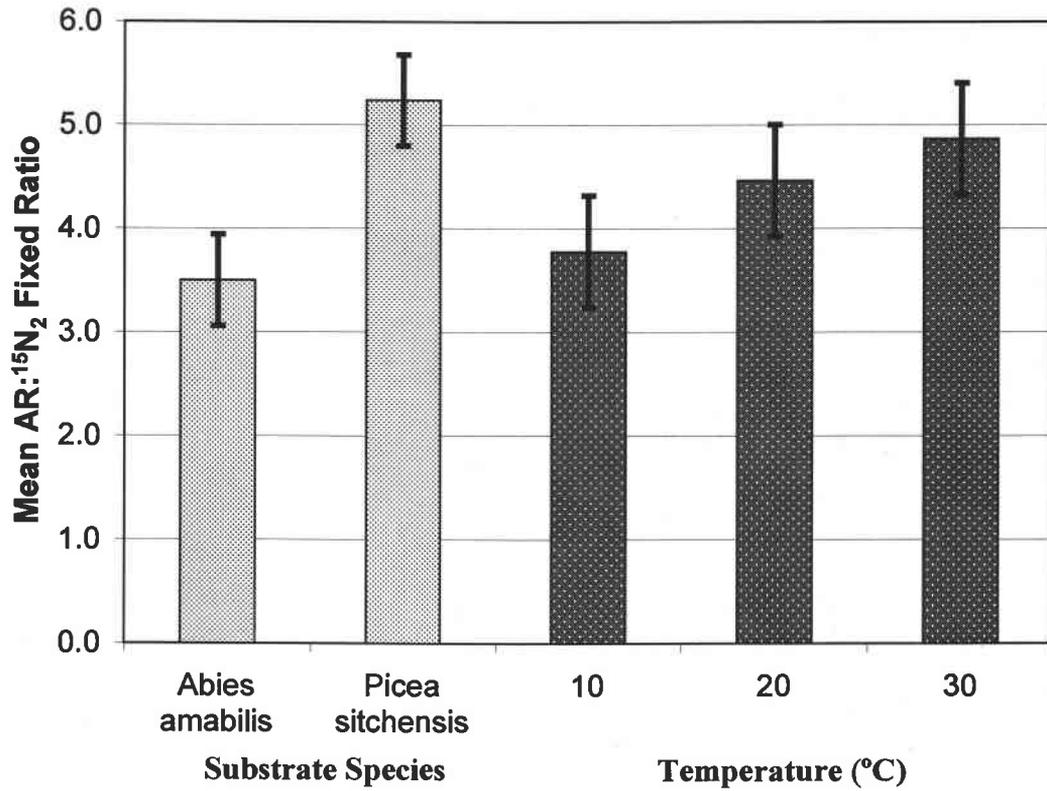


Figure 3.1. The least squares mean of the ratio of acetylene reduced to dinitrogen fixed and 95% confidence limits for two different species of wood and three incubation temperatures.

mean AR: $^{15}\text{N}_2$ ratio of 5.2 as compared to a ratio of 3.5 for *Abies amabilis*. The average AR: $^{15}\text{N}_2$ ratio increased with temperature from 3.6 at 10°C to 4.9 at 20°C. The average ratio for all samples was 4.4.

Degree of decay

Potential and actual nitrogen fixation rates peaked in moderately decayed wood and were lowest in the most decayed wood (Figure 3.2a). Potential nitrogen fixation rates differed significantly among the decay classes with the highest rates in decay class two and the lowest in decay class five (Table 3.1). There is conclusive evidence that actual nitrogen fixation rates differed among the decay classes, but only strong evidence when moisture is included as a covariate. Actual fixation rates were highest in decay class three and lowest in decay class one. When moisture was included as a covariate, actual nitrogen fixation rates were slightly higher in decay classes one and two and slightly lower in decay classes four and five (Figure 3.2a)

Respiration rates had a similar pattern as compared to nitrogen fixation with rates peaking for moderately decayed wood and lowest for the most decayed (Figure 3.3a). Potential respiration rates significantly differed among the decay classes with the highest rates in decay class two and the lowest in class five wood (Table 3.1). Actual respiration rates varied significantly among the decay classes even when moisture was included as a covariate. Actual respiration rates were highest in decay class three wood and lowest in decay class five. When moisture was included as a covariate, respiration rates were

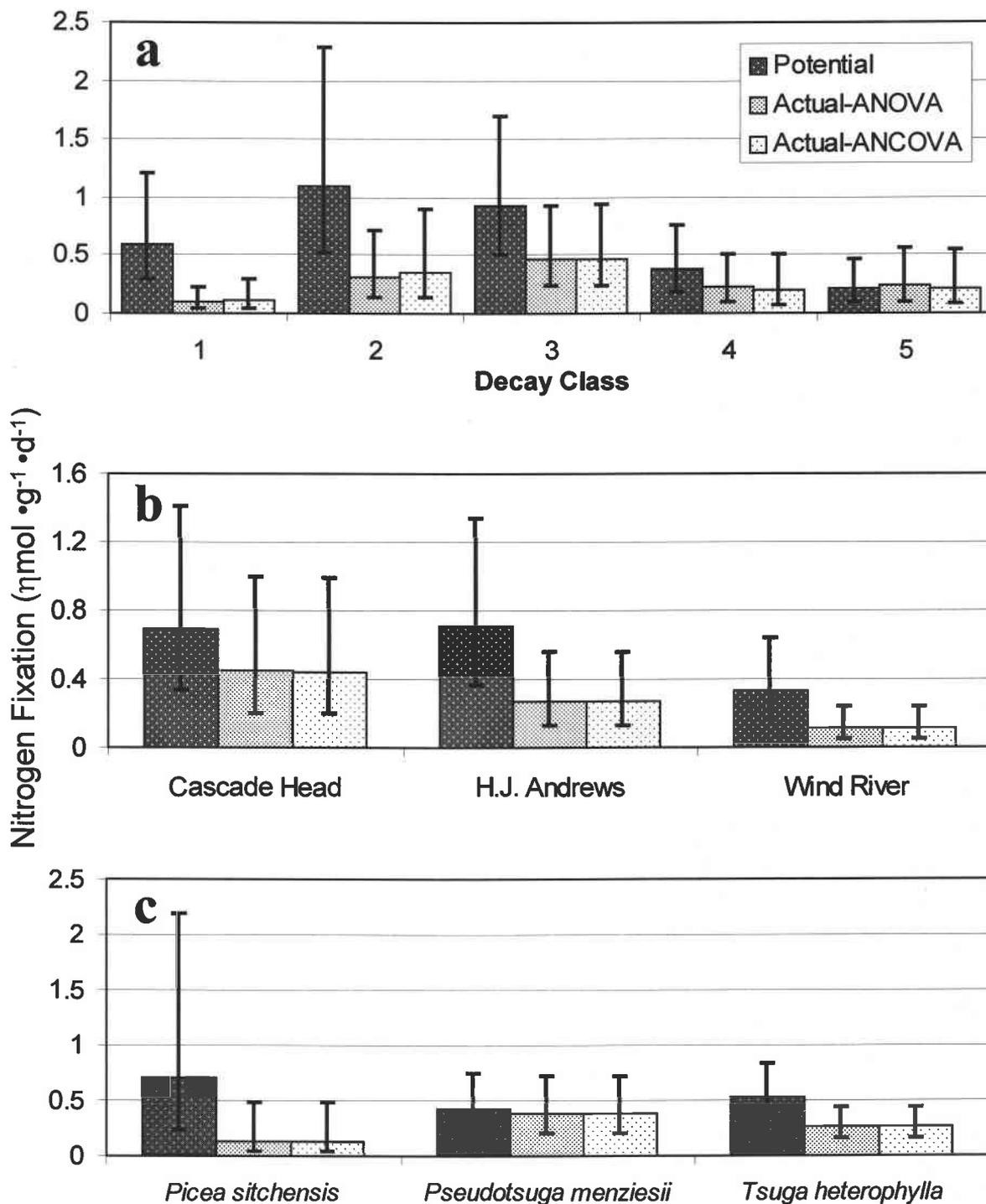


Figure 3.2. Medians and 95% confidence limits for potential and actual nitrogen fixation rates for (a) five decay classes of wood where one is least and five most decayed, (b) three sites in the Pacific Northwest, and (c) three species of wood. For actual nitrogen fixation rates, medians from both the ANOVA and ANCOVA were reported. The ANOVA did not, while the ANCOVA did include moisture as a covariate.

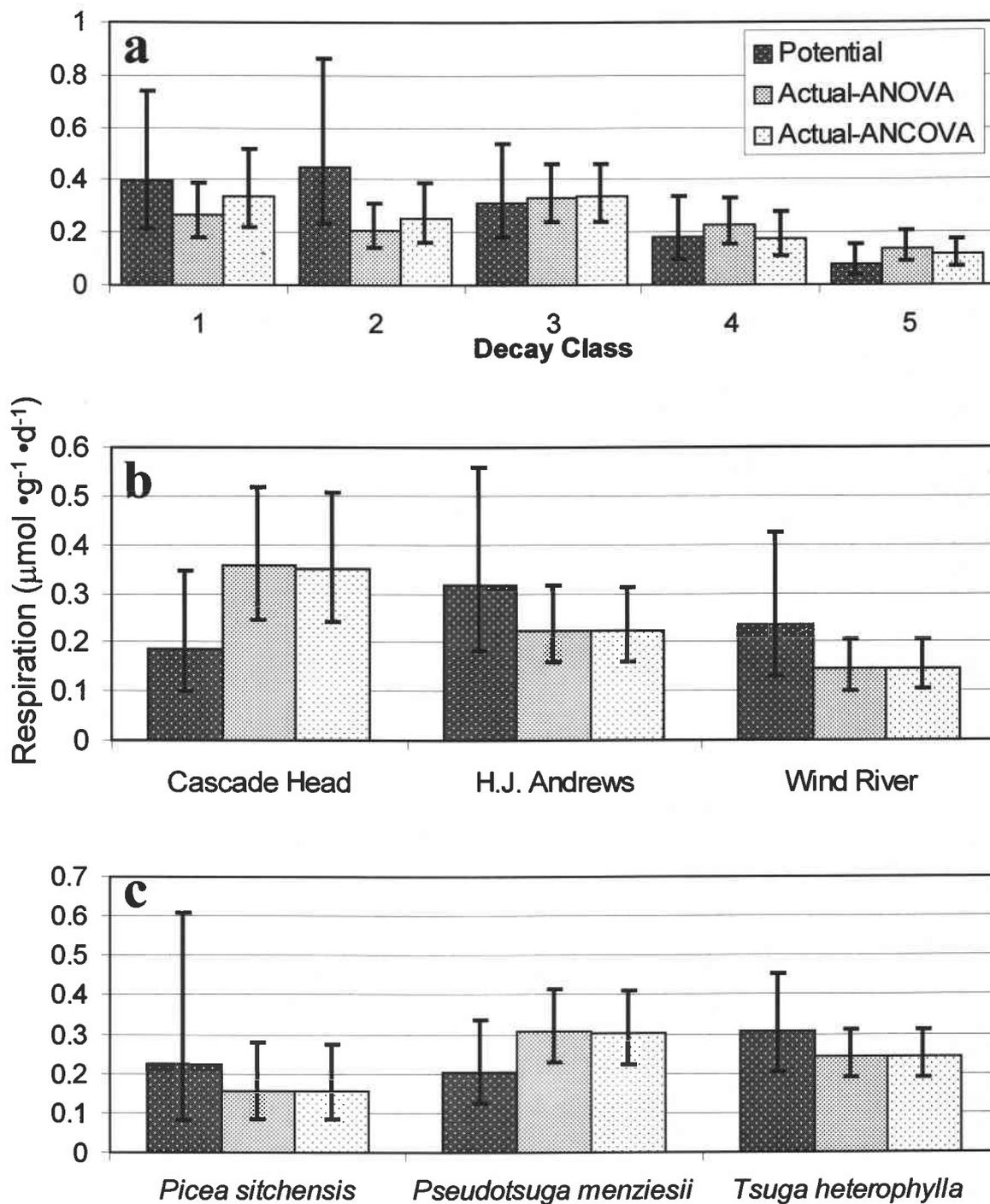


Figure 3.3. Medians and 95% confidence limits for potential and actual respiration rates for (a) five decay classes of wood where one is least decayed and five most, (b) three sites in the Pacific Northwest, and (c) three species of wood. For actual respiration rates, medians from both the ANOVA and ANCOVA were reported. The ANOVA did, while the ANCOVA did not include moisture as a covariate.

higher in decay classes one through three and lower in decay classes four and five (Figure 3.3a).

Site

Potential nitrogen fixation rates did not significantly vary among the three sites. In contrast, actual rates were significantly different (Table 3.1, Figure 3.2b). Average actual nitrogen fixation rates decreased from $0.45 \text{ } \eta\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ at Cascade Head, to $0.27 \text{ } \eta\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ at the H.J. Andrews, to $0.11 \text{ } \eta\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ at Wind River.

Potential respiration rates did not significantly vary among the three sites. Average actual rates were significantly different decreasing from $0.36 \text{ } \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ at Cascade Head, to $0.23 \text{ } \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ at the H.J. Andrews, to $0.14 \text{ } \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ at Wind River (Table 3.1, Figure 3.3b).

Wood species

No significant differences were found among the three species examined for potential and actual nitrogen fixation and respiration rates (Table 3.1). *Picea sitchensis* had the highest potential nitrogen fixation rates but the lowest actual rates (Figure 3.2c). *Tsuga heterophylla* had the highest potential respiration rates, while *Pseudotsuga menziesii* had the highest actual rates (Figure 3.3c).

Woody tissues

Nitrogen fixation and respiration rates were significantly different among the four woody tissues (Figure 3.4). Nitrogen fixation rates dropped from $0.72 \text{ } \eta\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ in outer bark, to $0.57 \text{ } \eta\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ in inner bark, to $0.24 \text{ } \eta\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ in sapwood, to $0.09 \text{ } \eta\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ in heartwood. Respiration rates were highest in inner bark at $1.51 \text{ } \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$, followed by outer bark at $1.14 \text{ } \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$, then by sapwood at $0.88 \text{ } \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$, and finally heartwood at $0.17 \text{ } \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$.

Differences between actual and potential rates

Potential nitrogen fixation rates averaged $0.62 \text{ } \eta\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ higher than actual rates. There was conclusive evidence that potential and actual fixation rates were different when moisture was not included as a covariate ($p = 0.044$) but only strong evidence when moisture was included ($p = 0.086$). Among the different decay classes, potential fixation rates were significantly different than actual rates particularly in decay class one, but not when moisture was included as a covariate (Table 3.1, Figure 3.2a). There were no significant differences between potential and actual nitrogen fixation rates among the different sites and species tested (Table 3.1, Figure 3.2b & c).

Potential respiration rates averaged $0.08 \text{ } \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ higher than actual rates. Potential rates were significantly different from actual rates whether moisture was included as a covariate or not ($p = 0.027$ and 0.021 , respectively). There was strong evidence to suggest that potential respiration rates were significantly higher than actual

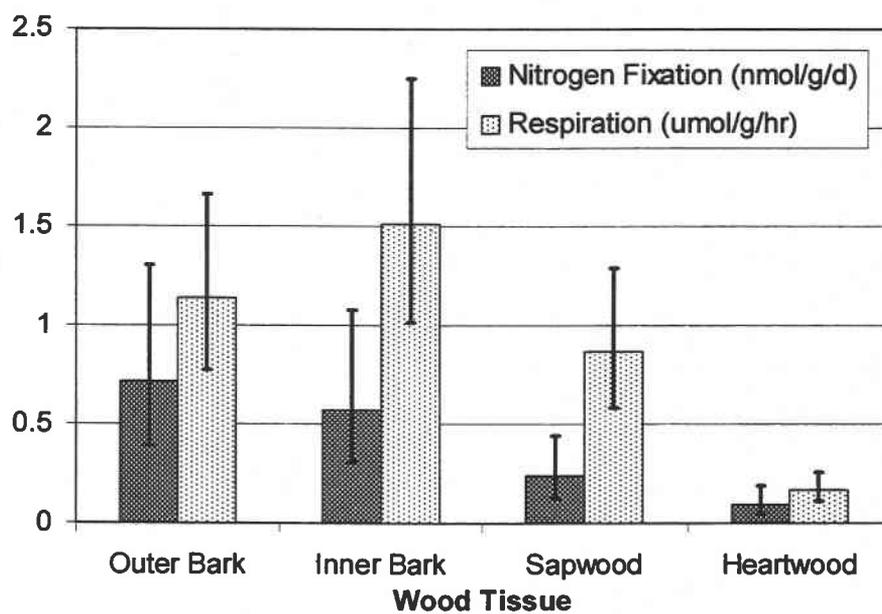


Figure 3.4. Medians and 95% confidence limits for nitrogen fixation and respiration rates for four wood tissues in years 9-12 of wood decay.

rates among the different decay classes, particularly in decay class two, but not when moisture was included as a covariate (Table 3.1, Figure 3.3a). There were no significant differences between potential and actual respiration rates among the different sites and species tested (Table 3.1, Figure 3.3b & c).

Discussion

AR: $^{15}\text{N}_2$ ratio

The differences in the AR: $^{15}\text{N}_2$ ratios between species and among the incubation temperatures share one striking similarity: the mean ratio increased as acetylene reduction and $^{15}\text{N}_2$ fixation rates increased. Average acetylene reduction and $^{15}\text{N}_2$ fixation rates were 2.4 and 1.7 times higher, respectively, for *Picea sitchensis* when compared to *Abies amabilis*. For incubation temperature, acetylene reduction and $^{15}\text{N}_2$ fixation rates were 6.2 and 4.6 times higher, respectively, for 30°C when compared to 10°C. Changes in the relative solubilities of N_2 and acetylene in water with increasing temperature might explain the increasing AR: $^{15}\text{N}_2$ ratio with temperature, but this does not seem to be the case since the relative solubility of acetylene compared to N_2 drops from being 70 times greater at 10°C to 62 times greater at 30°C (Wilhelm *et al.*, 1977). Another possible explanation is that higher rates of $^{15}\text{N}_2$ fixation, such as those in *Picea sitchensis* and at 30°C, could presumably lead to a greater degree of H_2 evolution and/or ammonia inhibition of nitrogen fixation but not acetylene reduction. H_2 evolution, which

is eliminated in the presence of acetylene, results in a decrease in the efficiency of nitrogen fixation (Burris, 1974; Sprent, 1979). In addition ammonia, which is not formed during acetylene reduction, can cause repression of nitrogenase synthesis and is involved in biosynthetic reactions that can affect nitrogenase (Hardy *et al.*, 1973; Sprent, 1979). Since AR rates would not be affected by these inhibitory processes, AR: $^{15}\text{N}_2$ ratios should generally increase as nitrogen fixation activity increases.

In general, AR: $^{15}\text{N}_2$ ratios from studies of wood and soil are similar to the theoretical ratio of four (Bergersen, 1991). These differences arise in part, because acetylene is more soluble in water than dinitrogen and the product of $^{15}\text{N}_2$ fixation, ammonia, can influence fixation rates (Hardy *et al.*, 1973; Wilhelm *et al.*, 1977). Hardy *et al.* (1973) found an average AR: $^{15}\text{N}_2$ ratio of 4.3 for several different studies of soils with higher values often being associated with water saturated conditions. Silvester *et al.* (1982) investigated nitrogen fixation in woody debris from the H.J. Andrews Experimental Forest and other sites in Oregon and found an average ratio of 3.5 to 4.5 for incubations lasting 6 and 42 hours, respectively. Roskoski (1981) found an unusually high AR: $^{15}\text{N}_2$ ratio of 8.5 for wood samples from the eastern deciduous forests of the United States. She measured AR and $^{15}\text{N}_2$ fixation on paired samples instead of the same sample and used a relatively long incubation period of five days. Using paired samples produced large variation in her data and long incubation periods are known to produce higher AR: $^{15}\text{N}_2$ ratios (Hardy *et al.*, 1973; Silvester *et al.*, 1982). In addition, the higher relative fixation activity and the moisture conditions and size of her samples may have contributed to the relatively high ratio.

Our overall average AR: $^{15}\text{N}_2$ ratio of 4.4 should produce conservative estimates of the amount of nitrogen fixed from our acetylene reduction data. The higher the ratio, the lower the amount of nitrogen that is fixed given the amount of acetylene reduced. Average yearly temperature at the three study sites ranges from 8.8 to 10°C, and the average AR: $^{15}\text{N}_2$ ratio for 10°C is 3.6. In addition, the substrates we used had relatively high nitrogen fixation rates. If our hypothesis is correct that higher fixation activity is associated with higher AR: $^{15}\text{N}_2$ ratios, then a value of 4.4 would overestimate the ratio for most of the wood substrates we surveyed and consequently nitrogen fixation rates are probably underestimated.

Degree of decay

The pattern of nitrogen fixation and respiration rates peaking in moderately decayed wood most likely reflects the changes in colonization extent, resource quality, and moisture conditions as a log decays. A fresh log in the Pacific Northwest generally takes many years to be completely colonized by wood-rotting and nitrogen fixing organisms (Harmon *et al.*, 1986). Portions of the heartwood normally remain sound even in decay class three logs. It has long been noted that the rate of decomposition of litter and resource quality declines with time (Heal *et al.*, 1997). The maximum and average moisture contents of logs increase with decay amount in the Pacific Northwest (Jurgensen *et al.*, 1984; Sollins *et al.*, 1987; Harmon and Sexton, 1995). In decay class one logs, resource quality is highest, but colonization and low moisture limit activity. Potential and actual nitrogen fixation and respiration rates are therefore low for this decay class. In

decay classes two and three, resource quality, colonization, and moisture content are probably not limiting, producing the highest rates of fixation and respiration. Activity is lower in advanced decay stages because resource quality becomes a limiting factor for nitrogen fixation and respiration.

In contrast to our results, Larsen *et al.* (1978) and Jurgensen *et al.* (1984) found nitrogen fixation rates in logs from Montana to increase with wood decay. However, both these studies examined the degree of decay within a log instead of between logs. The within log pattern of fixation rates and decay found by Larsen *et al.* (1978) and Jurgensen *et al.* (1984) is confounded by wood moisture, which covaries with the degree of decay. This same relationship may also hold in the logs we sampled, particularly in decay classes two and three. Sollins *et al.* (1987) measured nitrogen fixation and respiration from logs in the Pacific Northwest and did not find a significant pattern of nitrogen fixation or respiration with decay class. However, they did find that respiration rates peaked in decay classes two and three in *Pseudotsuga menziesii* and *Tsuga heterophylla* logs. The respiration rates they observed in *Thuja plicata*, which is not included in our study, increased with decay class. In addition, moisture was a confounding factor in the Sollins *et al.* (1987) study because they measured actual rates under field moisture conditions.

Site

Cascade Head Experimental Forest had the highest actual nitrogen fixation and respiration rates. The fact that Cascade Head, which is on the Oregon Coast, has a

milder, wetter climate than Wind River or the H.J. Andrews from the interior Cascade Range may explain this difference. The milder climate may allow larger microorganism populations to be maintained in woody debris. The relatively low respiration and nitrogen fixation rates at Wind River are somewhat surprising, since it is similar to the H.J. Andrews in terms of climate and species composition. Wood moisture content does not appear to be an explanation, because the medians and p-values were nearly identical when moisture was and was not included as a covariate. Also, wood samples were collected from Wind River in May when wood moisture is relatively high. Substrate differences are one possible explanation. However, chance alone may explain the differences between Wind River and the H.J. Andrews and is probably the best answer lacking any specific mechanism.

Wood species

While we did not find significant differences in nitrogen fixation and respiration rates among the species we examined, other studies have found differences between general taxonomic groups and among species. Jurgensen *et al.* (1989) found nitrogen fixation rates to be significantly higher in white-rotted hardwood litter when compared to brown-rotted conifer wood. Harvey, *et al.* (1989) demonstrated differences in nitrogen fixation among several decomposer-log associations in Idaho. Griffiths *et al.* (1993) found higher nitrogen fixation rates in *Pseudotsuga menziesii* and *Abies amabilis* than in *Thuja plicata* and *Tsuga heterophylla* at the H.J. Andrews during the first six years of log decay. Sollins *et al.* (1987) found no significant differences in nitrogen fixation or

respiration rate among *Pseudotsuga menziesii*, *Thuja plicata*, and *Tsuga heterophylla*. Species differences seem to be most pronounced when examining broad groups (e.g., angiosperms and gymnosperms) and species that are colonized by different decomposers (e.g., brown and white-rots).

Woody tissues

Tissue level patterns of nitrogen fixation during the ninth through twelfth years of log decay were generally similar to patterns during the first six years of decay with one exception: inner bark nitrogen fixation rates were higher than rates for outer bark (Griffiths *et al.*, 1993). During the first six years of decay nitrogen fixation rates were highest in inner bark, followed by outer bark, sapwood, and heartwood. Outer bark nitrogen fixation rates were higher than inner bark in years nine through twelve. Possible explanations include that outer bark may have become more completely colonized or inner bark substrate quality may be declining. Higher nitrogen fixation rates in outer and inner bark in comparison to wood may result from higher nutrient contents and carbon source availability in bark (Harmon *et al.*, 1986). Heartwood nitrogen fixation and respiration rates are still relatively low. Moisture was included as a covariate in these analyses, so low heartwood moisture can not explain all of this difference. Continued low rates in heartwood are probably due to incomplete colonization and extractives that inhibit decay (Harmon *et al.*, 1986).

Differences between actual and potential rates

Higher potential as compared to actual nitrogen fixation rates are probably a result of lower moisture and fixer abundance. After assaying for actual activity, samples were wetted and incubated for at least a week prior to testing potential activity. This allowed samples to thoroughly wet and for microorganism populations to adjust. The increase in activity is most pronounced in the difference between actual and potential fixation rates in decay class one logs (Figure 3.2a). Decay class one logs generally have the lowest moisture contents and are not completely colonized by decomposer microorganisms, so this decay class should have the greatest response to the treatment (Sollins *et al.*, 1987; Griffiths *et al.*, 1993; Harmon and Sexton, 1995).

Although we could detect significant differences between actual and potential respiration rates, the magnitude of the difference was not ecologically significant. Surprisingly, potential respiration rates were actually lower than actual rates for samples from Cascade Head, but this difference can adequately be explained by chance (Figure 3.3b). Potential respiration rates were higher in decay classes one and two and most of this difference is explained by moisture content as evidenced by the differences in the ANOVA and ANCOVA results (Table 3.1; Figure 3.3a).

Conclusions

The mechanisms that control the variability in the AR: $^{15}\text{N}_2$ conversion ratio in woody debris are poorly understood. Mechanisms such as hydrogen evolution and ammonia inhibition of nitrogen fixation may possibly explain the direct correlation between the AR: $^{15}\text{N}_2$ conversion ratio and nitrogen fixation activity. This may also explain the elevated AR: $^{15}\text{N}_2$ ratio observed by Roskoski (1981) for hardwoods, because nitrogen fixation rates are generally higher for hardwoods than softwoods (e.g., Todd *et al.*, 1975; Roskoski, 1980; Silvester *et al.*, 1982; Sollins *et al.*, 1987). Silvester *et al.* (1982) found the AR: $^{15}\text{N}_2$ ratio to increase with time and hypothesized that organisms exposed to acetylene, which inhibits nitrogen fixation, become nitrogen depleted causing stimulation of nitrogenase activity.

Until these ratios can be reliably predicted, it is advisable to determine the study specific conversion ratio when measuring nitrogen fixation rates with acetylene reduction (Roskoski, 1980; Silvester *et al.*, 1982). This is particularly important when the substrate of interest or AR methods differ from previous studies.

Patterns of microbial colonization and abundance, resource quality, and climate probably explain most of the patterns observed among the different decay classes, species, sites, and woody tissues examined in our study. Limitations of nitrogen fixation and respiration from incomplete microbial colonization and low microbial abundance probably decrease as decay proceeds, as wood resource quality increases, and the climate of a site becomes more favorable. Resource quality includes the chemical and physical properties of the dead wood that affect microbial colonization, abundance, and activity.

By our definition then, relative differences among different species and woody tissues are explained by resource quality. Climate is a major determinant of wood temperature and moisture, which in turn partially regulates metabolic activity and the colonization and abundance of microorganisms. Understanding how these factors vary and interact to determine metabolic activity is critical if we are to understand the current and future roles of woody debris in the carbon and nitrogen cycling of forest systems.

Acknowledgments

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Chapter 4

Diffusion and Seasonal Dynamics of O₂ in Woody Debris from the Pacific Northwest

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Abstract

We examined how O₂ diffusion rates in dead wood varied with moisture and density and examined its influence on the seasonal changes in oxygen concentration in logs in a Pacific Northwest old-growth *Pseudotsuga menziesii* forest. In the laboratory the oxygen diffusion coefficient (D_{O₂}) was determined in the longitudinal and radial directions on wood cores of varying moisture content and density. In the field O₂ was measured at three radial depths (2, 6, and 15 cm) within logs of two species (*Pseudotsuga menziesii* and *Tsuga heterophylla*) and five decay classes. In both the radial and longitudinal directions, D_{O₂} increased exponentially as the fraction of pore space in air (FPSA) increased and as density decreased. The regression results indicate that D_{O₂} increased from 1.88 x 10⁻⁶ cm²/s at zero FPSA to 1.94 x 10⁻⁴ cm²/s at a FPSA of one in the radial direction. Diffusion rates in the radial and longitudinal directions converge to 9 and 13%, respectively, of the D_{O₂} for water as FPSA in air approaches zero. D_{O₂} in the longitudinal direction was 1.4 and 34 times greater than for the radial direction at zero and one FPSA, respectively. In the field, mean O₂ concentrations in logs were not significantly different between species. In contrast, mean O₂ concentrations were significantly lower in decay class one and two logs as compared to decay class three through five logs. Higher density wood in decay class one and two logs and hence lower diffusion rates probably explains these differences. Mean O₂ concentrations only decreased with radial depth in decay class two logs. Seasonal O₂ levels did not consistently vary with log moisture, respiration, or air temperature. Low O₂ concentrations in observed in November 1998 may result from increased precipitation

following the summer drought. The comparison of the results from our model of oxygen diffusion in the radial direction and field data indicate that *in vivo* measurements of radial oxygen diffusion underestimate field oxygen concentrations. Cracks and passages in decay class five logs and longitudinal oxygen diffusion in decay class one logs may account for this discrepancy. In our logs, oxygen concentrations were rarely as low as 2%, indicating anaerobic conditions are not as common in logs as we previously thought. Oxygen limitations on decomposition may occur in relatively sound and/or water soaked wood, but probably not in decayed logs in a terrestrial setting.

Introduction

The importance of woody debris in terrestrial carbon and nutrient cycles is well recognized (e.g. Harmon *et al.*, 1986; Harmon and Sexton, 1996; Krankina *et al.*, 1999). Respiration and nitrogen fixation are key processes in the carbon and nitrogen cycles of dead wood; however, our understanding of the mechanisms that control these processes is still crude. For example, low levels of O₂ may be the reason respiration rates in woody debris are low in cool, wet forests such as those in the Pacific Northwest (Harmon and Sexton, 1996). However, O₂ levels in woody debris have rarely been monitored and O₂ diffusion processes in decayed wood have not been studied.

Oxygen regulates many physiologic processes in woody debris including respiration and nitrogen fixation, yet oxygen diffusion rates and levels within decayed wood are largely unknown (Scheffer, 1985; Silvester *et al.*, 1982). Huang *et al.* (1977)

measured the diffusion of dissolved O₂ in undecayed, chipped, liquid-saturated *Pseudotsuga menziesii* sapwood. Tarkow and Stamm (1960a & b) measured the diffusion of carbon dioxide and water vapor through undecayed veneers of *Picea sitchensis*. However, we do not know of any studies that have measured oxygen diffusion rates in decayed wood. Slightly more is known about oxygen levels in dead wood. Savely (1939) found O₂ concentrations as low as 9.4% in *Quercus* and *Pinus* logs from the deciduous forests of North Carolina. Paim and Beckel (1963) measured seasonal changes in O₂ partial pressure in decaying *Fagus grandifolia* logs from Ontario and found O₂ concentrations as low as 0.5% in partially submerged logs and as low as 3.4% in non-submerged logs.

The objectives of this study are to determine the effects of wood fiber orientation, density, and moisture on O₂ diffusion rates in decayed wood and to measure seasonal changes in O₂ concentration within logs. We also used a model of oxygen diffusion in woody debris to compare our diffusion data with the O₂ levels of the logs in the field.

Methods

O₂ diffusion

O₂ diffusion coefficients (D_{O_2}) in wood were determined using a core method developed for soils (Taylor, 1949). In this method an oxygen free diffusion chamber is

separated from the atmosphere by a cylindrical sample of wood (Figure 4.1). O₂ accumulation in the diffusion chamber is then measured over time. A plate within the diffusion chamber can be moved to start or stop diffusion. Pure N₂ was used to thoroughly flush the diffusion chamber prior to measurement. Modeling clay was used to cover the sides of the wood cores and seal the space between the cores and the diffusion chamber. The change in O₂ concentration with time inside the diffusion chamber was measured by periodically withdrawing 0.5 ml gas samples. O₂ concentrations were measured with a Hewlett Packard model 5830 gas chromatograph fitted with a thermal conductivity detector and Molecular Sieve 5A column. The effect of the diffusion apparatus and leakage were measured and corrected for (Taylor, 1949). D_{O₂} was calculated by fitting the concentration data to a steady-state diffusion equation (i.e. Fick's first law; Taylor, 1949).

Samples of *Pseudotsuga menziesii* wood in various stages of decay taken from the H. J. Andrews Experimental Forest in the central Oregon Cascades were cut into cylindrical cores with a diameter of 5.2 cm and a height of 3.6 cm. Cores were cut to measure diffusion in the longitudinal direction (along the fiber) or radial direction (perpendicular to the fiber along an axis from the bark to the pith). Cores were sterilized in a cobalt 60 gamma irradiator with 2.5 Mrad prior to diffusion measurements to inhibit biological respiration. Wood moisture was left at field conditions, decreased by drying, or increased by soaking in water. The fraction of pore space in air (FPSA) at various moisture contents was calculated for each core using the following equation:

$$\text{FPSA} = 1 - (\text{Wood Moisture} / \text{Wood Moisture}_{\text{max}}).$$

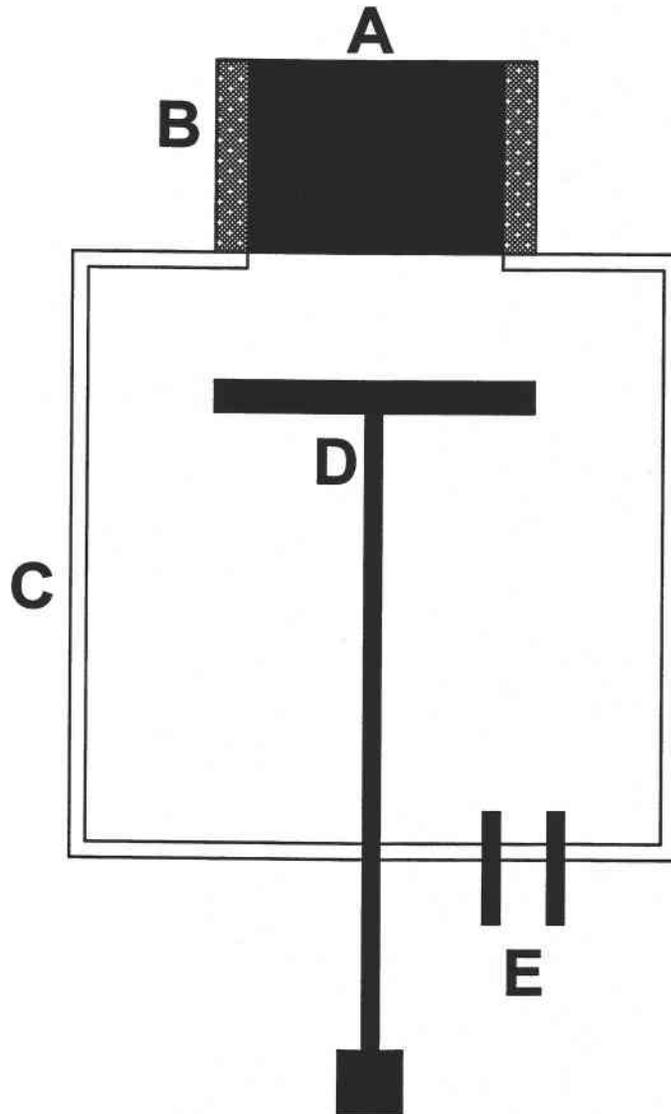


Figure 4.1. Diagram of a cross-section of the diffusion apparatus where A is the wood core, B is modeling clay, C is the diffusion chamber, D is the movable plate, and E designates ports used for obtaining gas samples and flushing the diffusion chamber with N_2 .

Wood Moisture content was calculated by dividing the difference between sample weight before and after drying by the oven dry weight then multiplying by 100. The maximum wood moisture (Wood Moisture_{max}) content of the wood cores was determined on cores submerged in water for at least one week. Density was determined for each core from the wet volume and dry weight.

O₂ concentrations within logs

To determine seasonal changes of O₂ in the field, we measured O₂ concentrations in logs in an old-growth stand in the Wind River Experimental Forest on the west slope of the southern Washington Cascades. Wet, cool winters and warm, dry summers characterize the climate of this site. Mean annual temperature and precipitation are 8.8°C and 250 cm respectively. Forests are dominated by *Pseudotsuga menziesii* and *Tsuga heterophylla* (Franklin and Dyrness, 1988).

Eighteen logs for oxygen measurement were selected as randomly as possible. Two *Pseudotsuga menziesii* and *Tsuga heterophylla* logs from each of five decay classes were selected except in decay class five where only one suitable log per species could be found. Decay class one logs are the least decayed and decay class five logs are the most (Harmon and Sexton, 1996).

Three PVC tubes with an inner diameter of 2.5 cm were tightly imbedded within each log to a depth of 2, 6, or 15 cm in order to monitor oxygen concentrations. Tube internal volume ranged from 60 ml in the 2 cm deep tubes to 125 ml in the 15 cm tubes. A bead of silicon caulk was applied along the edge of the tube in contact with the internal

wood surface to help ensure a tight seal. Tubes were attached to the logs with long screws to prevent movement and sealed to the log at the surface with caulk. In addition, silicon caulk was used to fill any spaces between the hole and tube. Each tube had a cap with a septum to allow gas sample removal.

The tubes within each log were sampled monthly from April 1998 to April 2000. Four ml gas samples were withdrawn with a syringe and transferred to evacuated 3 ml gas sample containers. The septa of the gas sample containers were further sealed with wax to help prevent contamination. The O₂ concentration of the gas samples was determined within 24 hours with a gas chromatograph (see O₂ diffusion section of the Methods). A gas mixing equation was used to correct for the average 0.02 ml of O₂ contamination that we found in the evacuated gas sample containers. In theory, the reduced pressure created by withdrawing 4 ml of gas from a 60 ml tube interior could increase oxygen concentration by up to 1.25% in the syringe barrel from the inrush of atmospheric air into the syringe. This could elevate the O₂ concentrations of samples taken from tubes with low O₂ concentrations. We tested for this effect by additionally measuring oxygen in tubes with histories of low oxygen levels using gas sample containers left for a month on double-ended needles that also penetrated the log tube septum. The O₂ concentrations determined with both methods never differed by more than 0.5% even at O₂ concentrations as low as 3%. This indicates that air flow into the syringe barrel did not greatly influence O₂ concentrations.

Log moisture, respiration, and meteorological data

Log moisture was monitored in the logs using time domain reflectometry (TDR; Gray and Spies, 1995). The TDR method determines average wood volumetric water content by measuring the elapsed time it takes an electromagnetic wave to travel the length of a pair of metal rods embedded in the wood. Two rods spaced 5 cm apart were inserted 30 cm into the logs resulting in a measured volume of approximately 47 cm³ (Gray and Spies, 1995). Readings from the rods were taken monthly or bimonthly from March 1998 to March 2000.

Log respiration was monitored using soda-lime (Edwards, 1982). Jars with soda lime were left for 24 hours once per month in lidded buckets sealed to the logs with silicone caulk and long screws. Control buckets with jars of soda-lime were used to correct for leakage of CO₂ into the buckets from the atmosphere.

Monthly temperature and precipitation data were from the National Oceanic and Atmospheric Administration's data archives for the nearby Carson Fish Hatchery weather station in Washington.

Statistical and modeling analysis

All statistical analysis including linear regression, Analysis of Covariance (ANCOVA), and calculation of means and 95% confidence limits were performed with SAS (1985). Linear regression was used to develop relationships between wood moisture, density and the log of the oxygen diffusion coefficients. ANCOVA was used

to test for significant differences among the means of the oxygen levels from all sampling dates for the two species, five decay classes, and three tube depths with the distance of the tubes from the nearest broken end of the log included as a covariate. This part of the study used a split-plot experimental design structure and the ANCOVA were performed using the appropriate procedures and random effects for this design. Means and confidence intervals of the oxygen concentrations at each sampling date were used to examine seasonal changes in oxygen concentrations.

For this study, we consider relationships to be statistically significant when the p -value was less than 0.05. The 95% confidence limits on figures provide a simple visual means to compare means. Using the terminology of Ramsey and Schafer (1995), we use the phrase “conclusive evidence” of a difference between two means to describe situations where confidence limits do not overlap at all and “strong evidence” to describe situations where confidence limits may overlap but not enough to include the mean being compared.

We used a model of nitrogen fixation in dead wood that generates log oxygen concentrations from diffusion and respiration rates to compare our laboratory O_2 diffusion results with the seasonal O_2 levels obtained from the field (Chapter 5). This model uses a modified form of Fick’s First Law to estimate oxygen diffusion rates in the radial direction after accounting for the effect of the log moisture content and density. Respiration rates are modified by log temperature, moisture, oxygen concentration, and substrate quality. Log temperature and moisture are estimated using meteorological data from the Carson Fish Hatchery. All logs used in model runs were 50 cm in diameter.

Model results consist of the average oxygen values in the outer 15 cm of the logs for *Pseudotsuga menziesii* and *Tsuga heterophylla* logs for each decay class examined.

Results

O₂ diffusion

In the radial and longitudinal directions, D_{O_2} exponentially increased with FPSA (Figure 4.2). The lines fitted to the log transformed radial and longitudinal data explain much of the variation in the data as indicated by adjusted r^2 values of 0.68 and 0.95, respectively (Table 4.1). The regression results indicate that D_{O_2} increased from $1.88 \times 10^{-6} \text{ cm}^2/\text{s}$ at zero FPSA to $1.94 \times 10^{-4} \text{ cm}^2/\text{s}$ at a FPSA of one in the radial direction, and increased from $2.64 \times 10^{-6} \text{ cm}^2/\text{s}$ at zero FPSA to $6.62 \times 10^{-3} \text{ cm}^2/\text{s}$ at a FPSA of one in the longitudinal direction. D_{O_2} was higher in the longitudinal direction when compared to the radial direction and this difference increased as FPSA increased. Thus, D_{O_2} in the longitudinal direction was 1.4 and 34 times greater than D_{O_2} in the radial direction at zero and one FPSA, respectively.

As wood density increased, D_{O_2} exponentially decreased (Figure 4.3, Table 4.1). The lines fitted to the log transformed radial and longitudinal data explain much of the variation in the data as indicated by adjusted r^2 values of 0.99 and 0.72, respectively (Table 4.1). The rate of decrease was less in the longitudinal direction than in the radial direction. At a wood density of zero, D_{O_2} in the longitudinal direction was 19 times

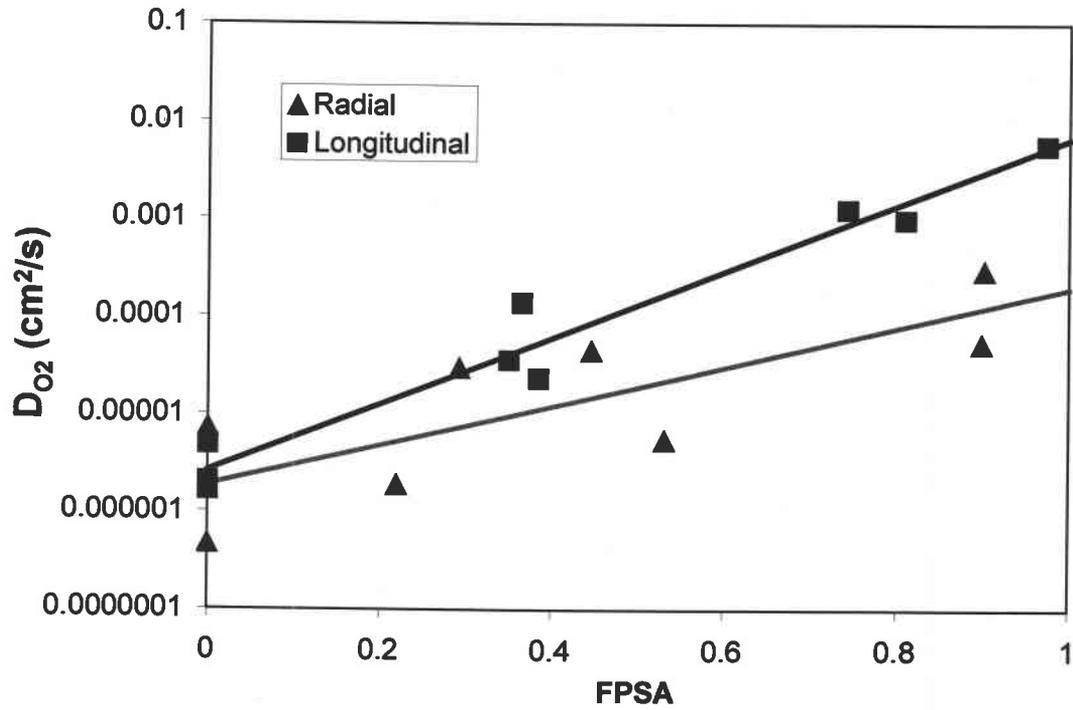


Figure 4.2. The relationship of the O₂ diffusion coefficient (D_{O_2}) and the fraction of pore space occupied by air (FPSA) in wood cores of various densities.

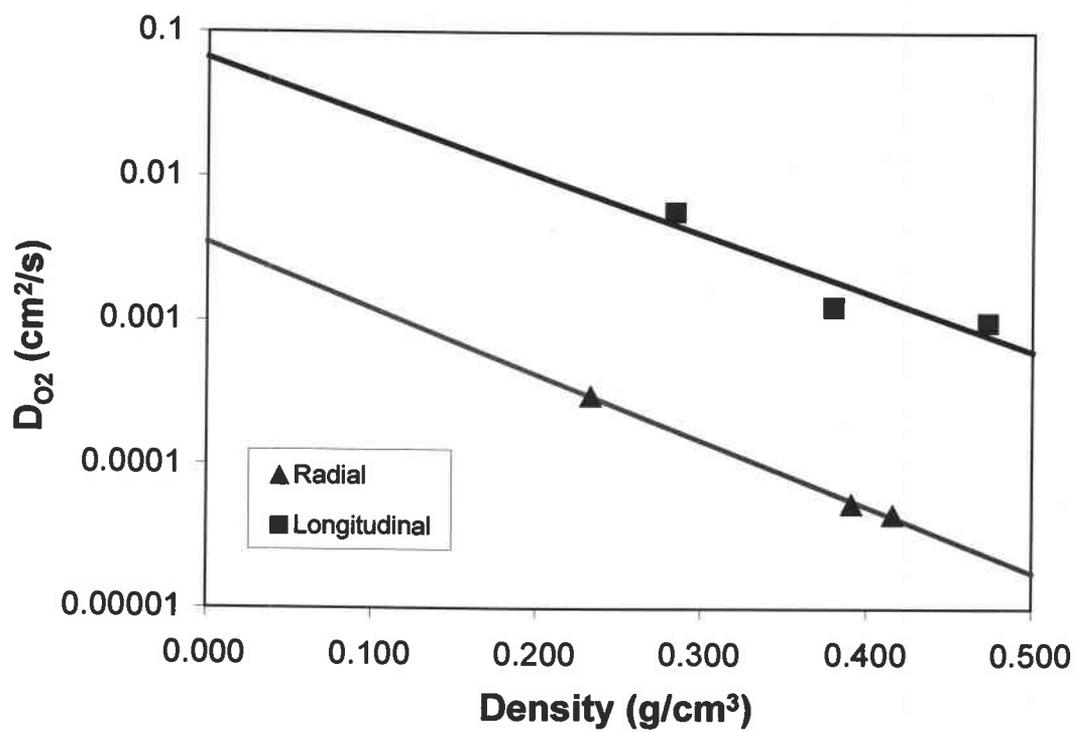


Figure 4.3. The relationship of the oxygen diffusion coefficient (D_{O_2}) and wood density.

greater than D_{O_2} in the radial direction (6.63×10^{-2} and $3.46 \times 10^{-3} \text{ cm}^2/\text{s}$, respectively).

At a wood density of 0.5, D_{O_2} in the longitudinal direction was 35 times greater than D_{O_2} in the radial direction (6.15×10^{-4} and $1.78 \times 10^{-5} \text{ cm}^2/\text{s}$, respectively).

Table 4.1: Coefficients for slopes and y-intercepts for equations fit to oxygen diffusion data relating the diffusion coefficient (D_{O_2}) to FPSA and wood density. Equations were of the form: $\log(D_{O_2}) = m \cdot x + b$.

Wood Variable (x)	Fiber Orientation	m	b	Adj. r^2	N
FPSA	Radial	2.01	-5.72	0.68	9
FPSA	Longitudinal	3.40	-5.58	0.95	9
Density	Radial	-4.58	-2.46	0.99	3
Density	Longitudinal	-4.06	-1.18	0.72	3

O_2 levels within logs

In the field mean O_2 concentrations for the two log species and for the three radial depths within the logs were not significantly different, whereas the means for the decay classes and combinations of decay class and depth were significantly different (Table 4.2; Figures 4.4 & 4.5). The covariate (distance of the tubes from the end of the logs) bordered on meeting the criteria for significance with a p-value of 0.0656 (Table 4.2). Average O_2 concentration rose from 15.1% in decay class one logs to approximately 20.5% in decay classes three through five (Figure 4.4c). Mean O_2 concentrations decreased significantly with radial depth in decay class two logs from 18.6% at a depth of 2 cm to 11.4% at a depth of 15 cm, but O_2 concentrations varied little with depth in the other decay classes (Figure 4.5).

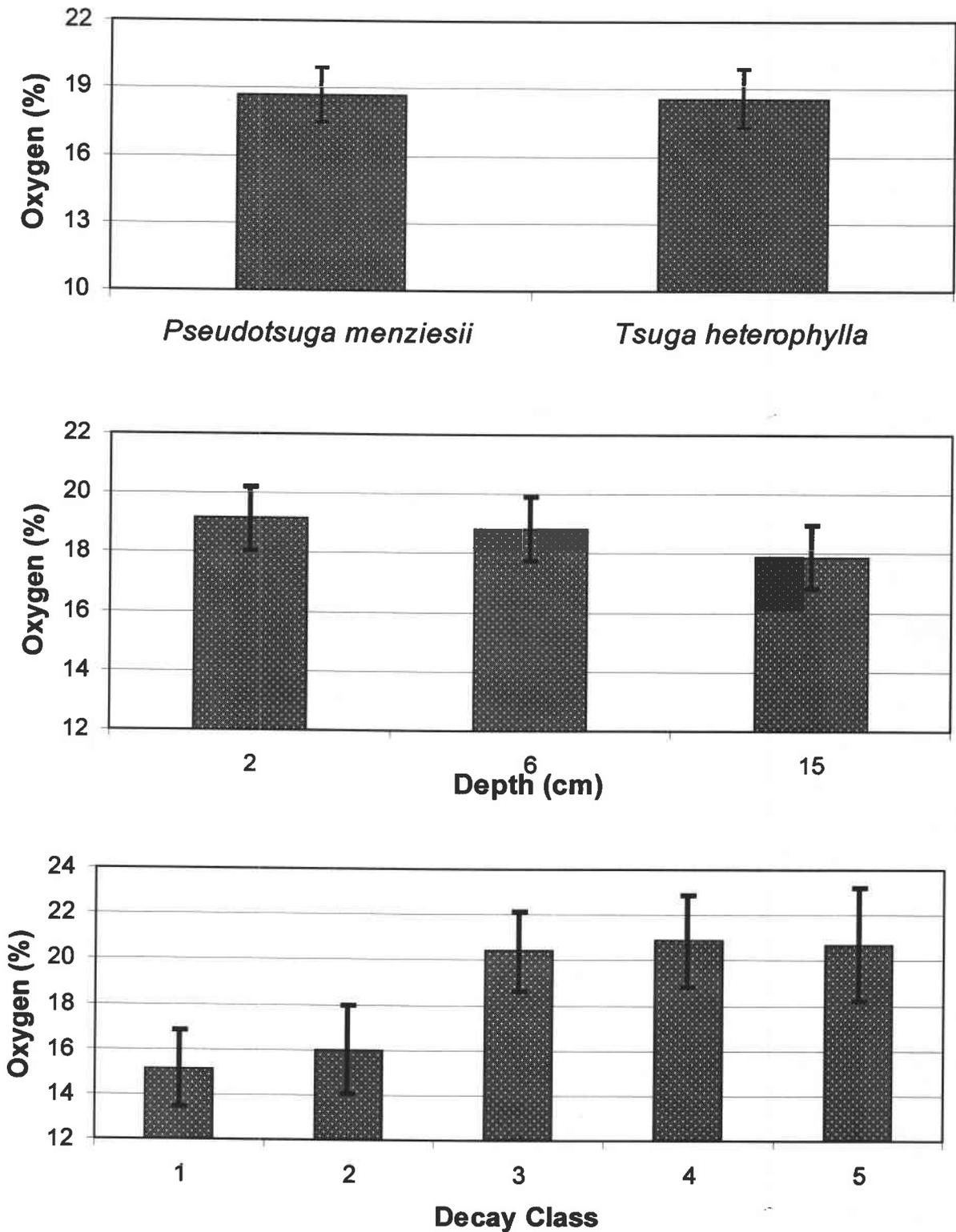


Figure 4.4. Mean oxygen concentrations and 95% confidence intervals in logs for different (a) species, (b) radial depths within the logs, and (c) decay classes.

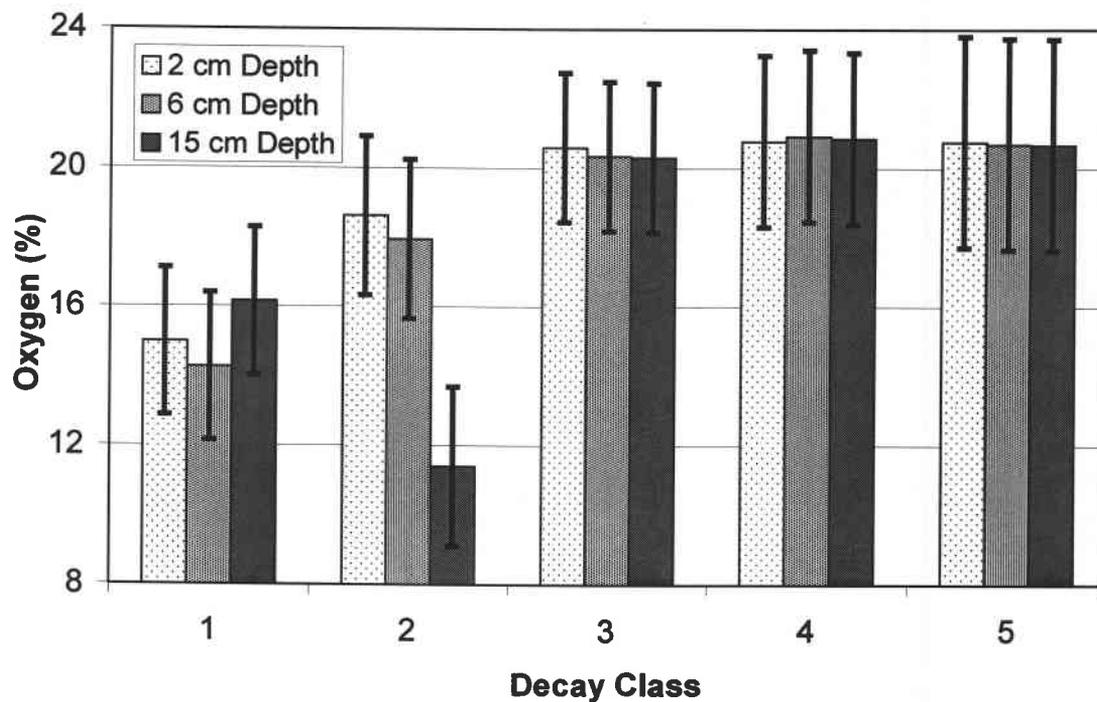


Figure 4.5. Mean oxygen concentrations and 95% confidence intervals for different combinations of radial depths within the logs and decay classes.

Table 4.2: P-values from ANCOVA results for testing if wood species, tube depth within the log, decay class or their interactions affect mean O₂ concentrations.

Effect	P-value
Species	0.9188
Depth	0.1225
Decay Class	0.0046*
Species x Depth	0.5715
Species x Decay Class	0.5618
Depth x Decay Class	0.0032*
Species x Depth x Decay Class	0.9651
Distance of tube from the log end	0.0656

*Indicates significance at p-values < 0.05

Log O₂ concentrations, moisture, and respiration varied seasonally (Figure 4.6). There was convincing evidence that oxygen levels were significantly lower and varied more in decay class one and two logs in comparison to logs in decay classes three through five (Figure 4.6a). In addition, O₂ concentrations dropped dramatically and significantly in November 1998 coinciding with an increase in precipitation and moderate temperatures in this month (Figure 4.6a & b). There was no indication of a consistent seasonal pattern in log O₂ concentrations associated with the patterns of log moisture, respiration, and air temperature. Log moisture levels were generally higher as decay class increased (Figure 4.6b). In decay class five logs, moisture levels peaked around 300% from November through May then declined to nearly 150% in August through October. Moisture levels varied much less in decay classes one through three. The respiration rates of the logs generally tracked average monthly temperatures, although there was more variation in the respiration data in comparison to the monthly temperatures (Figure 4.6b).

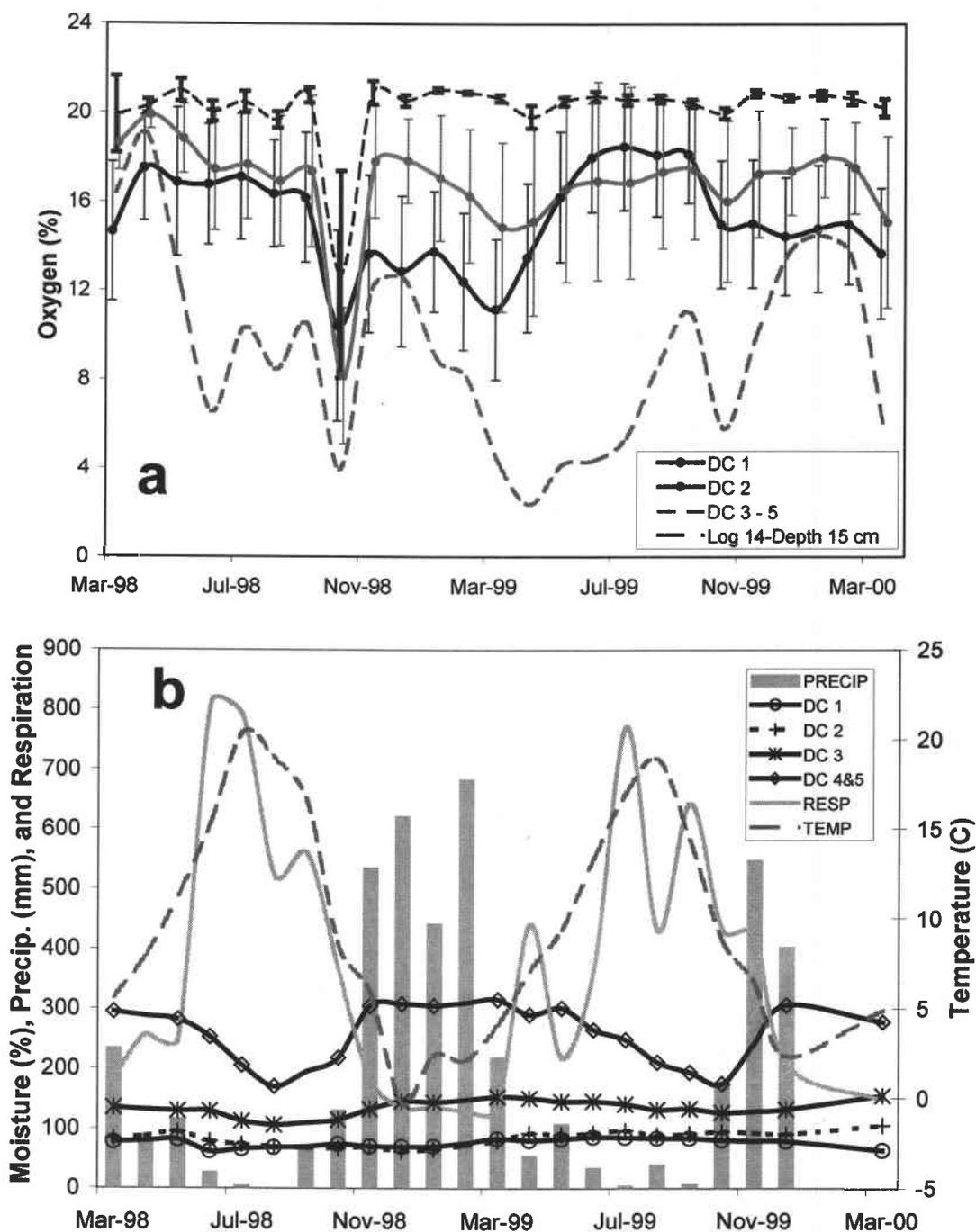


Figure 4.6. (a) Monthly mean O₂ concentrations and 95% confidence intervals for decay classes one, two, and three through five; and monthly O₂ levels at a radial depth of 15 cm in log 14 (*P. menziesii*, decay class two). (b) Average log moisture concentration in decay classes one, two, three, and four and five combined; mean monthly temperature, monthly precipitation, and respiration rate (mg CO₂·m⁻²·d⁻¹).

Modeled log oxygen concentrations generally underestimated or overestimated field oxygen concentrations when the model used parameters calculated from diffusion data from the radial or longitudinal directions, respectively; although, differences between model and field estimates were much greater for decay class one logs than for decay class five logs (Figure 4.7, Table 4.3). Modeled predictions closely tracked seasonal changes in field data in decay class five logs when model parameters were estimated from diffusion data from the longitudinal direction, but not when using parameters for the radial direction. Modeled predictions did not closely track seasonal changes in field O₂ in decay class one logs.

Table 4.3. Average oxygen concentration in decay class one and five logs from 1999 from a model and field data. Modeled results used diffusion parameters calculated from data for the radial or longitudinal directions.

Data Source	Parameter Source	Average O ₂ (%)	
		Decay Class 1	Decay Class 5
Model	Radial	8.6	18.1
Model	Longitudinal	18.9	20.9
Field		15.2	20.7

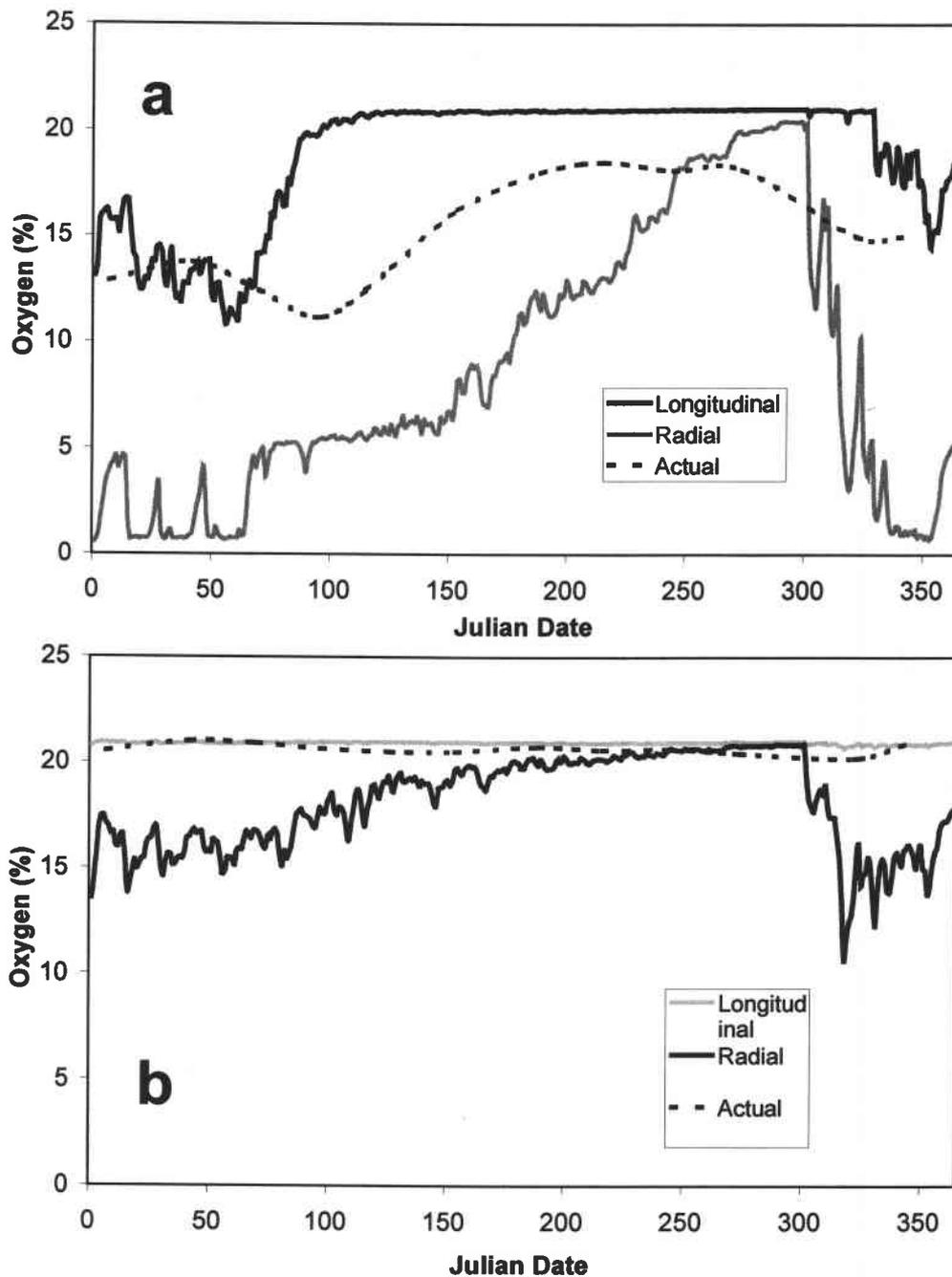


Figure 4.7. A comparison of log oxygen concentration from 1999 field data and a model of oxygen diffusion for (a) decay class one logs and (b) decay class five logs where actual indicates field data, longitudinal indicates model parameters were derived from data on oxygen diffusion in the longitudinal direction, and radial indicates model parameters were derived from data on oxygen diffusion in the longitudinal direction

Discussion

O₂ Diffusion

Our results for the relationships of D_{O_2} with FPSA in decayed wood agree with other studies of oxygen diffusion. Taylor (1949) measured oxygen diffusion in soil and found a similar exponential decrease of D_{O_2} with increasing moisture. At an FPSA of zero, D_{O_2} was 9 to 13% that of the D_{O_2} in water at 20°C (approximately 2.0×10^{-5} cm²/s; Lide, 1998) for diffusion in the radial and longitudinal directions, respectively. Huang *et al.* (1977) measured diffusion of dissolved oxygen in liquid-saturated *Pseudotsuga menziesii* sapwood and found D_{O_2} for water in the longitudinal and radial directions to be 1.4 and 7.6×10^{-6} cm²/s, which is 6 to 40% that of the D_{O_2} for water. Huang *et al.* (1977) used sapwood with wide annual rings, which may explain the higher D_{O_2} they found in the longitudinal direction.

Our results for the relationships of D_{O_2} with density in decayed wood generally agree with other studies of oxygen diffusion. Studies of oxygen diffusion in soil and wood found similar exponential decreases of D_{O_2} with increasing bulk density (Taylor, 1949; Huang *et al.*, 1977). In addition, the lines relating radial and longitudinal D_{O_2} values to wood density approach the D_{O_2} in air at 20°C (approximately 2.1×10^{-1} cm²/s; Lide, 1998). Theoretically, the lines should converge to the D_{O_2} in air at a wood density of zero. This indicates our rate of decrease for the relationship of D_{O_2} with density should be greater, or the rate of decrease is greater at lower wood densities than the ones we measured.

When comparing longitudinal and radial diffusion, Huang *et al.* (1977) and Tarkow and Stamm (1960a) also found higher D_{O_2} values for longitudinal diffusion. In addition, the convergence of radial and longitudinal D_{O_2} values as FPSA approaches zero agrees with theory and previous results (Tarkow and Stamm, 1960a; Huang *et al.*, 1977). Longitudinal O_2 diffusion is faster than radial because wood tracheids form paths that allow much faster diffusion along the path (longitudinal) as compared to perpendicular to the path (radial). As water fills wood fibers, radial and longitudinal O_2 diffusion rates should converge, because as pore space fills with water, O_2 diffuses much more slowly in water than through wood cell walls and air. This should reduce the relative differences between the longitudinal and radial directions. Tarkow and Stamm (1960a) found D_{CO_2} to be approximately 650 times greater in the longitudinal as compared to radial direction in dry, undecayed wood veneers. Huang *et al.* (1977) found longitudinal D_{O_2} to be 5.5 times greater than radial D_{O_2} in undecayed wood chips that were water saturated. The smaller relative differences between D_{O_2} for radial and longitudinal directions found in our study probably result from using decayed wood. As wood decays, cracks and passages form, and these reduce the importance of tracheid orientation.

O_2 levels within logs

The patterns of O_2 concentration among the different decay classes and between the two species can be explained by patterns of wood density and respiration. Wood density decreases with decay (Harmon *et al.*, 1986; Harmon and Sexton, 1996). Respiration and decomposition rates also tend to decrease with the degree of decay

(Chapter 3; Harmon *et al.*, 1986). Lower oxygen levels in decay class one and two logs relative to decay classes three through five result from relatively high respiratory consumption of oxygen and relatively low oxygen diffusion rates in the denser wood of decay classes one and two. *Pseudotsuga menziesii* and *Tsuga heterophylla* have similar wood densities and respiration rates during decomposition, thus they should have similar average oxygen levels (Chapter 3; Sollins *et al.*, 1987; Harmon and Sexton, 1996).

The interaction of decay class and depth is somewhat surprising. We expected O₂ levels to decrease with radial depth in each decay class because: 1) the distance for oxygen to diffuse increases, and 2) we assumed that respiration rates would be relatively constant along the length of the log, and 3) that spacing the tubes at least one meter from the ends of the logs would avoid the influence of longitudinal oxygen diffusion (Paim and Beckel, 1963). However, this only occurred in decay class two logs (Figure 4.5). The nearly constant, near atmospheric, values in decay classes three through five is probably a result of the relatively high O₂ diffusion rates in these lower density logs when compared to decay class one and two logs. The pattern in decay class one cannot be explained unless longitudinal O₂ diffusion and/or patchily distributed respiration activity is influencing oxygen levels. We measured the distance of the tubes from the nearest broken end of the log and included it as a covariate to test if longitudinal diffusion might be influencing oxygen levels in the tubes. The borderline significance of the covariate is suggestive that the distance of the tubes from the end of the logs was influencing oxygen levels in some of the logs. However, there is no way to tell if the mechanism producing this effect is longitudinal diffusion or something else such as patterns of respiration or moisture. The relatively dry and constant moisture content of heartwood early in the

decay process of logs (Harmon and Sexton, 1995) suggests that longitudinal diffusion rates might be higher in decay class one than in later decay classes. A patchy distribution of respiration may also explain the results for decay class one logs. It was not uncommon in decay class one logs for tubes at depths of 2 or 6 cm to have much lower O₂ levels (3-6%) when compared to levels at the 15 cm depth (10-20%). Logs are colonized in a patchy manner with many microbial decomposers introduced by channelising insects (Carpenter, 1988). This probably leads to a patchy distribution of respiration activity in the early decay stages of the log. This patchy distribution probably changes to a relatively uniform distribution in decay class two logs accounting for the decrease of O₂ with depth. Thus, both longitudinal O₂ diffusion and a patchy distribution of respiration activity may be influencing O₂ concentrations in the decay class one logs.

In an apparent contrast to our results, Paim and Beckel (1963) found O₂ levels to decrease with the radial depth within logs in a forest in Ontario, Canada. However, they sampled only *Fagus grandifolia* logs inhabited by *Orthosoma brunneum* beetles. These beetles do not inhabit logs until they have been on the ground several years, which is probably similar to decay class two logs in our study.

The seasonal pattern of moisture with decay class agrees with previous studies of wood moisture. Harmon and Sexton (1995) measured changes in seasonal fluctuations in the moisture content of decay class one *Pseudotsuga menziesii* and *Tsuga heterophylla* logs in the Pacific Northwest and found little seasonal variation in the average moisture content of logs. They also found the maximum moisture content of logs to increase with decreasing density. In addition, Harmon and Sexton (1995) noted that run off of throughfall precipitation decreases and drying rates increase as density decreases. Thus,

low density decay class four and five logs are more likely to wet up and dry out to a greater degree than higher density logs.

The dramatic decrease in O₂ concentrations in November 1998 was very interesting (Figure 4.6a). Gas-sampling procedures did not differ from other sampling dates and we double-checked the GC calibration, so we feel experimental error does not account for this event. Both in November 1998 and 1999, precipitation dramatically increased (Figure 4.6b). Log moisture as measured by TDR also increased in decay classes four and five at these times, but not in decay classes one through three. However, it is likely that the moisture content of the outer portions of logs (primarily bark and sapwood) in all decay classes also increased in November. This is supported by Harmon and Sexton (1995), who found that heartwood moisture content was relatively constant, while bark and sapwood moisture content decreased in the summer and were maximum in the fall and winter. It is likely that the outer portions of logs in all decay classes had greater seasonal moisture fluctuations than the inner portions. We hypothesize that a wetting event in November 1998 triggered reduced O₂ diffusion rates, and possibly high respiration rates. The high respiration rates could result from the scavenging of microorganisms in the outer portions of the logs that died from low moisture availability. In November 1999 there is strong evidence that oxygen levels dropped significantly in decay classes one and three through five; however, the decrease is not nearly as large as in November 1998 (Figure 4.6a). We may have missed this second rewetting event or a more gradual log rewetting may have spread the decrease out over time. We would have expected respiration rates to also increase in November 1998; however, respiration rates were measured two weeks after O₂ possibly missing the effect of this rewetting event

(Figure 4.6). These rewetting events are probably transient, lasting for days to weeks, so daily to weekly sampling is probably necessary to observe these hypothesized events.

Other than the response in November 1998, the lack of a consistent seasonal pattern of log oxygen concentrations is somewhat surprising. The relatively close relationship between temperature and respiration would presumably create a seasonal pattern of oxygen concentration. Paim and Beckel (1963) found average CO₂ concentrations in *Fagus grandifolia* logs to rise and fall with temperature from May to October in Ontario; however, their oxygen levels did not consistently relate to temperature or CO₂. In our case, the Mediterranean climate of the Pacific Northwest may contribute to the lack of a seasonal pattern of O₂ concentrations in logs (Figure 4.6b). The warm, dry summers and cool, wet winters create a pattern where low precipitation levels and log moisture in summer may increase D_{O2} when respiration is high, while high log moisture in the winter would decrease D_{O2} when respiration is low. This combination of high D_{O2} with high respiration and low D_{O2} with low respiration would obscure a seasonal pattern.

Individual logs often had unique responses that are difficult to discern from average values. For example, O₂ concentrations in log 14, a decay class two log, at a depth of 15 cm varied greatly from near atmospheric levels to 2.4% in April 1999 (Figure 4.6a). Oxygen concentrations below 5% occurred in 5 of the 18 logs, but primarily in decay classes one and two. In addition, O₂ levels in log 14 varied more than the average seasonal response. This suggests that logs, particularly in decay class one and two, probably have unique patterns of colonization and wetting that appear random at the scale we measured.

The comparison of the results from our model of oxygen diffusion in the radial direction and field data indicate that *in vivo* measurements of radial oxygen diffusion do not adequately explain field data (Figure 4.7, Table 4.3). Large cracks and passages in decay class five logs that were specifically avoided when cutting wood cores for diffusion measurements probably contribute to the generally higher field O₂ concentrations. In decay class five logs that do not have cracks and passages, oxygen diffusion in the longitudinal direction may be accounting for the underestimates of field O₂ levels when using model parameters calculated from radial O₂ diffusion data. Our model also tended to overestimate decay class one log moisture in spring and underestimate log moisture in summer in comparison to the TDR data. Model overestimates of log moisture will produce lower O₂ levels, whereas underestimates of log moisture will produce overestimates of log O₂ concentrations. The addition of longitudinal diffusion in the model, improvements in the moisture generation portion of the model, and better estimates of *in situ* oxygen diffusion rates may improve the seasonal daily estimates for decay class one logs.

Physiological processes in dead wood such as respiration and nitrogen fixation are influenced by O₂ concentration, but do the O₂ levels we observed limit either of these processes? Respiration does not seem to be inhibited much above 5% O₂ (Scheffer, 1985), while nitrogen fixation is optimum at concentrations from 2-5% O₂ (Chapter 2; Silvester *et al.*, 1982). Respiration is probably not greatly inhibited by the oxygen concentrations found in our logs. However, this does not take into account CO₂, which rises as oxygen declines and can also inhibit respiration. Nitrogen fixation rates are probably limited greatly by the generally high oxygen levels found in our logs. However,

our methods really examine large-scale patterns of O₂ relative to the size of microorganisms. Microscale patterns of depleted O₂ may be occurring in the vicinity of nitrogen fixing organisms in the wood. Nitrogen fixing organisms often have mechanisms for regulating the O₂ levels around them (Sprent, 1979). Still these mechanisms do not seem to compensate completely for high O₂ concentrations, because the organisms are inhibited in laboratory studies when incubated at O₂ concentrations above 5% (Chapter 2; Silvester *et al.*, 1982). Therefore, nitrogen fixation may be greatly inhibited by the O₂ levels we observed, while respiration is probably not.

Conclusions

The exponential increase and decrease of D_{O₂} with FPSA and wood density, respectively, that we found were reasonable and probably relatively accurate. *In situ* measurements of O₂ diffusion in wood similar to those made in soil (Jellick and Schnabel, 1986) would be useful for checking and improving the absolute accuracy of our results.

We were somewhat surprised that low oxygen levels in logs were not more common in the outer 15 cm of logs. Other investigations have demonstrated anaerobic conditions in wood or found oxygen levels below 1% in wood (Paim and Beckel, 1973; Huang *et al.*, 1977). These investigations dealt with wood that was partially or completely submerged in water, indicating that anaerobic conditions in terrestrial woody debris may not be as common as we previously thought. Oxygen limitations on

decomposition may occur in relatively sound and/or water soaked wood, but probably not in decayed logs in a terrestrial setting.

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Chapter 5

Modeling Nitrogen Fixation Rates in Dead Wood

William T. Hicks, Mark E. Harmon, and Steven L. Garman

Abstract

We developed a mechanistic simulation model of nitrogen fixation in dead wood to synthesize current knowledge, develop hypotheses, and estimate nitrogen fixation rates in the Pacific Northwest. Our model is a system of difference equations that estimate the annual amount of nitrogen fixed in a log of defined length and diameter and divided into five concentric layers. In our model nitrogen fixation is constrained by log substrate, temperature, moisture, and oxygen content. Respiration and diffusion of oxygen indirectly affect nitrogen fixation and respiration by regulating log oxygen content. Oxygen diffusion is influenced by log density, moisture, and oxygen content. Uncertainty analysis indicates that the focus of future research should be on improving estimates of the maximum nitrogen fixation rate, parameters involved in regulating log moisture content, and parameters involved in estimating oxygen diffusion rates. In comparison to independent data, our model reasonably estimated seasonal patterns of log temperature, moisture, oxygen content, and respiration rate. Our model estimates an annual nitrogen fixation rate of $0.7 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ for an old-growth stand at the H. J. Andrews, which is reasonably close to an independent estimate of $1.0 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ made for the same stand. Model output indicates that a decay class two, *Tsuga heterophylla* log fixes the most nitrogen in warm wet sites such as those near the coast, and the least in dry sites east of the Cascades and in the Klamaths. Raising the annual temperature by 2°C and decreasing precipitation by 10% caused nitrogen fixation rates to increase at all sites. Increases were greatest in warm wet sites and least in dry sites. Despite low annual rates of asymbiotic nitrogen fixation in wood, soil, and litter, asymbiotic nitrogen fixation

can contribute 9% to 42% of a stands nitrogen inputs over succession when symbiotic fixers such as *Alnus rubra* and *Lobaria oregana* are present and absent, respectively. Managed stands with reduced levels of woody debris and litter may be losing a significant nitrogen input.

Introduction

In the highly productive forest ecosystems of the Pacific Northwest, both tree growth and fungal wood decay are limited by nitrogen (Cowling and Merrill, 1966; Gessel, 1973; Spano *et al.*, 1982). Nitrogen fixation is an important input of this key nutrient, but little attention has been given to this process in woody debris because of its relatively low annual input. However, a significant portion of a forest ecosystem's nitrogen can be provided by asymbiotic fixation in woody debris when inputs are summed over succession and/or when symbiotic nitrogen fixers are absent (Cromack *et al.*, 1979; Sollins *et al.*, 1987).

Past attempts at elucidating the controlling mechanisms and the magnitude of nitrogen fixation in dead wood were preliminary. Most studies examined one to several of the factors controlling fixation in woody debris (*e.g.*, Roskoski, 1980; Sollins *et al.*, 1987; Griffiths, 1993), but none attempted to synthesize all major mechanisms. For example, past estimates of the annual amount of nitrogen fixed in dead wood in the Pacific Northwest involved extrapolation from a few substrates at one point in time (Sylvester *et al.*, 1982) or at most a few substrates at two points during a year (Sollins *et al.*, 1987). A model of nitrogen fixation in woody debris incorporating the primary

controlling variables integrated over a year would greatly expand our understanding of this process.

To this end we developed a mechanistic simulation model of nitrogen fixed in woody debris that synthesized our current knowledge of this process. Comparisons of model results with independent data were used to evaluate the accuracy of our model. The model was then used to examine situations that have not been or that would be difficult to assess experimentally (*e.g.*, annual and successional changes at log or stand scales; changing climate regimes). Finally, uncertainty analysis of model parameters was used to help direct future research towards areas that can be improved most.

Model Description

Our model is a system of difference equations that estimate the annual amount of nitrogen fixed in a log of defined length and diameter. A log is represented by five concentric layers of varying thickness with each layer corresponding to a wood tissue: bark, sapwood, and three layers of heartwood. All equations are solved on a daily time step. The model is programmed in BASIC. We scale up annual fixation rates from logs to stands by running the model for each decay class of each species present. Maximum nitrogen fixation and respiration rates, and densities for each species, tissue, and decay class are included in the model (Chapter 3; Griffiths *et al.*, 1993; Harmon and Sexton, 1996). These maximum rates are adjusted by daily air temperature and precipitation data provided by the user. The model also requires mean monthly temperature to calculate

evaporation potential. For stand level estimates, woody debris masses by species and decay class are required.

A conceptual diagram of modeled factors influencing nitrogen fixation in a log is shown in Figure 5.1. Nitrogen fixation is directly controlled by log substrate, temperature, moisture, and oxygen content. Respiration and diffusion of oxygen indirectly affect nitrogen fixation by regulating log oxygen content. Respiration is directly controlled by log substrate, temperature, moisture, and oxygen content. In our model oxygen diffusion is influenced by log substrate, moisture, and oxygen content.

Layer Surface Area, Volumes, and Mass

Several geometric quantities are necessary for calculating the temperature, moisture, and oxygen contents of the log layers. Log layers correspond to tissues: bark, sapwood, and heartwood. Radial tissue thicknesses are determined using equations relating log radius to tissue thickness (Lassen and Okkonen, 1969; Wilson *et al.*, 1987). The outer most log layer is composed of both outer and inner bark. Outer bark is ten percent of the log radius (Figure 5.2). Inner bark thickness (IBTHIK, cm) is determined from the following equation:

$$IBTHIK = IBTMAX * ((1 - \text{EXP}(-IB1 * \text{RADIUS}))^{IB2})$$

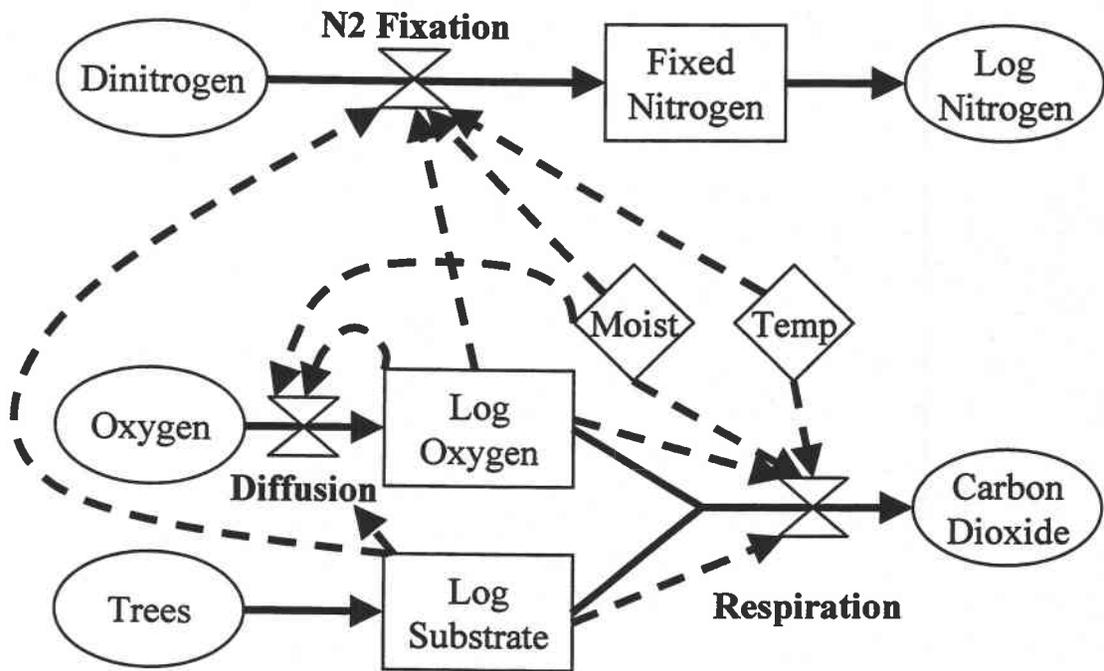


Figure 5.1. A diagram of the direct and indirect influences on nitrogen fixation included in our model.

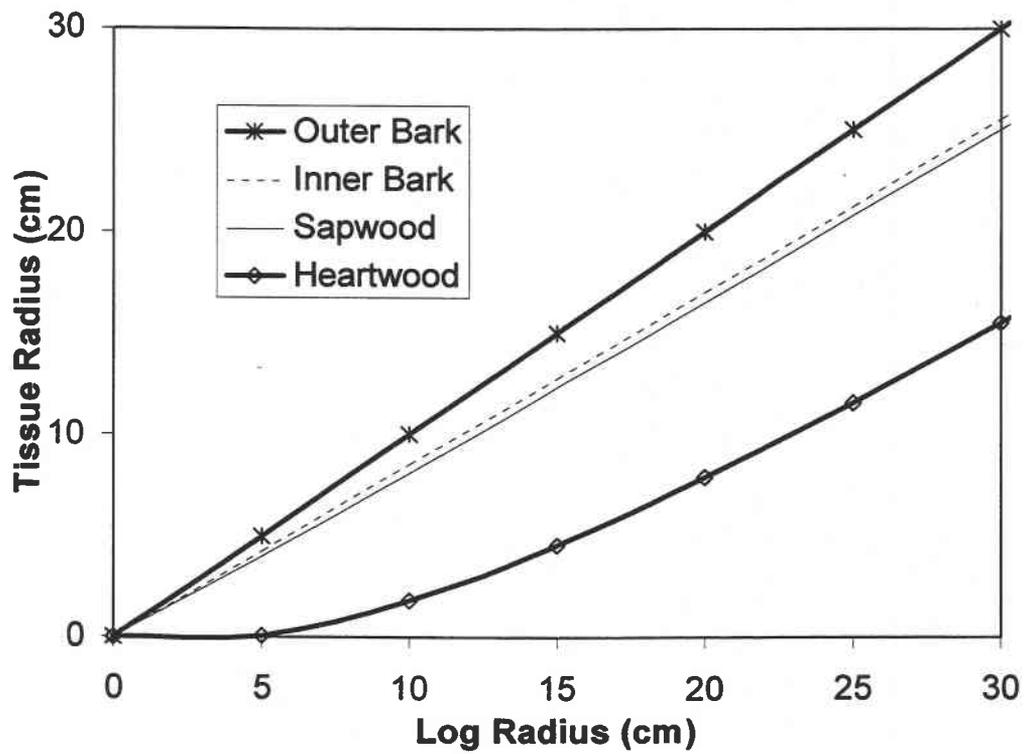


Figure 5.2. The relationship of tissue radii and log radius.

where IBTMAX is the maximum inner bark thickness, RADIUS is the log radius, and IB1 and IB2 are parameters that determine the shape of the curve (Table 5.1). Sapwood thickness (SWTHIK, cm) is determined from the following equation:

$$SWTHIK = SWTMAX * ((1 - \exp(-SW1 * RADIUS))^{SW2})$$

where SWTMAX is the maximum sapwood thickness, and SW1 and SW2 are parameters that determine the shape of the curve (Table 5.1). Heartwood thickness (HWTHIK, m) is calculated as any remaining thickness after subtracting the other tissue thicknesses from RADIUS. The surface area of a log layer (LOGSA, m²) is given by:

$$LOGSA = 2\pi * ROUT * LENGTH$$

where ROUT is the outer radius of the layer and LENGTH is the log length. The projected surface area (*i.e.* the effective area available to collect precipitation; PROSA; m²) of the log is equal to:

$$PROSA = 2 * ROUT * LENGTH.$$

Layer volume (LOGV, l) is defined as:

$$LOGV = LENGTH * 1000 * \pi * (ROUT^2 - RIN^2)$$

Table 5.1. List of parameters and values used in this model.

Parameter	Value	Description	Reference
IBTMAX	5.00×10^{-1}	maximum inner bark thickness (cm)	Wilson <i>et al.</i> , 1986
IB1	1.50×10^{-1}	coefficient for determining inner bark thickness	Wilson <i>et al.</i> , 1986
IB2	1.00	coefficient for determining inner bark thickness	Wilson <i>et al.</i> , 1986
SWTMAX	6.00	maximum sapwood thickness (cm)	Lass. & Okk. 1969
SW1	1.00×10^{-1}	coefficient for determining sapwood thickness	Lass. & Okk. 1969
SW2	1.00	coefficient for determining sapwood thickness	Lass. & Okk. 1969
RATEQN	5.10	Q10 response of nitrogen fixation and temperature	Chapter 2
SHPQN	3.70×10^{-2}	Q10 response of nitrogen fixation and temperature	Chapter 2
**REFTMP	15.0	reference temperature for Q10 (°C)	Chapter 2
SHPN2	2.70×10^{-1}	lethal response of n fixation and temperature	Chapter 2
LAGN2	7.00×10^3	lethal response of n fixation and temperature	Chapter 2
RATEQR	2.27	Q10 response of respiration and temperature	Chapter 2
SHPQR	8.91×10^{-3}	Q10 response of respiration and temperature	Chapter 2
SHPTR2	5.00×10^{-2}	lethal response of respiration and temperature	Chapter 2
LAGTR2	10.0	lethal response of respiration and temperature	Chapter 2
*MAXFAL	9.50×10^{-1}	maximum fraction of precip. that is throughfall	Rothacher, 1963
SHPINT	3.50	response of throughfall to precipitation amount	Rothacher, 1963
LAGINT	5.00	response of throughfall to precipitation amount	Rothacher, 1963
*SHPRUN	6.00	response of runoff to wood density	Harm. & Sext., 1995
LAGRUN	8.00	response of runoff to wood density	Harm. & Sext., 1995
MDMX	1.07	maximum moisture diffusion rate ($1 \cdot \text{m}^{-2} \cdot \text{d}^{-1}$)	Harmon [†]
MDDIXA	7.87×10^{-1}	density effect on moisture diffusion	Harmon [†]
MDTIXA	3.30×10^{-1}	temperature effect on moisture diffusion	Harmon [†]
MDTIXB	6.10×10^{-1}	temperature effect on moisture diffusion	Harmon [†]
*MMHITE	726	curve height for density effect on MAXMST	Harm. & Sext., 1995
**MAXCOM	4.08	rate constant for density effect on MAXMST	Harm. & Sext., 1995
SHPMN	1.94×10^{-2}	index of moisture effect on nitrogen fixation	Chapter 2
LAGMN	2.89	index of moisture effect on nitrogen fixation	Chapter 2
SHPMR	4.54×10^{-2}	index of moisture effect on respiration	Chapter 2
LAGMR	8.13	index of moisture effect on respiration	Chapter 2
SHPON1	3.57	index of O2 effect on N fixation-increasing portion	Chapter 2
LAGON1	7.18	index of O2 effect on N fixation-increasing portion	Chapter 2
*SHPON2	1.34×10^{-1}	index of O2 effect on N fixation-decreasing portion	Chapter 2
**LAGON2	2.42	index of O2 effect on N fixation-decreasing portion	Chapter 2
SHPOR	8.00×10^{-1}	index of O2 effect on respiration	Chapter 2
LAGOR	4.00	index of O2 effect on respiration	Chapter 2
*DIFMAX	2.65	maximum O2 diffusion rate ($\text{mol} \cdot \text{m}^{-1} \cdot \text{d}^{-1}$)	Chapter 4
**MIDDA	2.01	index of moisture effect on oxygen diffusion	Chapter 4
*MIDDB	2.01	index of moisture effect on oxygen diffusion	Chapter 4
**DIDDA	1.07	index of density effect on oxygen diffusion	Chapter 4
**DIDDB	4.58	index of density effect on oxygen diffusion	Chapter 4

*When these parameters were raised by 10%, the annual amount of nitrogen fixed changed by 5% to 10%.

**When these parameters were raised by 10%, the annual amount of nitrogen fixed changed by more than 10%.

[†]Unpublished data.

where RIN is the inner radius of the layer. The mass of a log layer (LMASS, kg) is equal to:

$$LMASS = LOGV * DENSE.$$

where DENSE ($\text{kg}\cdot\text{l}^{-1}$) is the density of the layer. The maximum gas volume (GASVM, l) and maximum water volume (H2OVM; l) for a layer are equivalent and given by:

$$GASVM = H2OVM = LOGV * DENSE * (MAXMST / 100)$$

where MAXMST is the maximum moisture content of the layer (the determination of MAXMST is described later in the *Log Moisture Content* section).

Difference Equations for Nitrogen Fixation and Respiration

Nitrogen fixation and respiration rates are directly controlled by log temperature, moisture and oxygen content:

$$\Delta NFIX = NFIXMAX * TMPIDN * MSTIDN * O2IDXN$$

$$\Delta RESP = RESPMAX * TMPIDR * MSTIDR * O2IDXR$$

where NFIXMAX ($\text{nmol N}_2 \cdot \text{g}^{-1} \cdot \text{d}^{-1}$) and RESPMAX ($\mu\text{mol CO}_2 \cdot \text{g}^{-1} \cdot \text{hr}^{-1}$) are the maximum nitrogen fixation and respiration rates for a given decay class, tissue, and species. Indices are included that describe the effect of log temperature (TMPIDN, TMPIDR), moisture (MSTIDN, MSTIDR) and oxygen content (O2IDXN, O2IDXR) on the nitrogen fixation and respiration rates, respectively. These indices are used to adjust NFIX and RESP from the maximum values to actual values for a given log temperature, moisture and oxygen content.

Log Temperature

Daily log temperatures (TEMP, °C) are estimated from the average daily air temperature and Fourier's Law of Heat Conduction. The temperature of the outer layer of the log is assumed to be the same as air temperature allowing us to ignore heat convection, radiation, and absorption. Heat conduction moves heat between layers within the log as described by Fourier's Law:

$$QCON = K \cdot LOGSA \cdot \Delta TEMPK \cdot TIME / THIK$$

where QCON is the amount of heat in Joules moved between two layers in a day, $\Delta TEMPK$ is the temperature difference in Kelvin between the two layers, TIME is the number of seconds in a day, and THIK is the radial thickness between the midpoints of the layers. The thermal conductivity coefficient ($K, \text{W} \cdot \text{m}^{-1} \cdot \text{K}^{-1}$) is affected by wood

density and moisture according to the following equation (U.S. Forest Products Laboratory, 1974):

$$K = 0.14*(DENSE*(1.39+0.028*MOIST)+0.165).$$

The total heat in the layer is calculated with:

$$HEAT = ((LMASS+H2OV)*C*TEMPK)+QCON$$

where HEAT is the amount of heat in Joules in the layer, and H2OV is the volume of water in liters in the layer (the determination of H2OV is described later in the *Log Moisture Content* section). The heat capacity (C; J·kg⁻¹·K⁻¹) is affected by wood moisture and temperature according to the following equation (U.S. Forest Products Laboratory; 1974):

$$C = (M + (0.27+0.0011*TEMP))/(1+M)+0.05$$

where M is the fractional wood moisture content. The temperature of the layer is then calculated from the amount of heat in the layer using:

$$TEMPK = HEAT/((LMASS+H2OV)*CTOT).$$

Temperature Effects

The response of nitrogen fixation and respiration to temperature has two components. The first component simulates the increase in activity that occurs as temperature rises from 0°C to the optimum temperature. We use a modified Q10 equation to describe the increase. Instead of a constant value for Q10, we used the following exponential equations that allow Q10 to vary with temperature:

$$Q10N = RATEQN * EXP(-SHPQN * TEMP)$$

$$Q10R = RATEQR * EXP(-SHPQR * TEMP)$$

where Q10N and Q10R are the Q10 values at a given temperature for nitrogen fixation and respiration respectively. RATEQN, RATEQR, SHPQN, and SHPQR are parameters that determine the height and steepness of the curve, and are generated from data collected on various substrates (Chapter 1; Table 5.1). The equations relating nitrogen fixation and respiration to temperature are given by:

$$TMPN1 = Q10N^{((TEMP-REFTMP)/10)}$$

$$TMPR1 = Q10R^{((TEMP-REFTMP)/10)}$$

where TMPN1 and TMPR1 are the first components of the temperature index for nitrogen fixation and respiration respectively. For these analyses we used our most common incubation temperature (15°C) for the reference temperature (REFTMP). The

second component of the temperature response describes the lethal effect of rising temperature on the fixing and respiring organisms. We used the following Chapman-Richards equations:

$$\text{TMPN2} = 1 - ((1 - \text{EXP}(-\text{SHPTN2} * \text{TEMP}))^{\text{LAGTN2}})$$

$$\text{TMPR2} = 1 - ((1 - \text{EXP}(-\text{SHPTR2} * \text{TEMP}))^{\text{LAGTR2}})$$

where TMPN2 and TMPR2 are the second components of the temperature index for nitrogen fixation and respiration respectively. SHPTN2, SHPTR2, LAGTN2, and LAGTR2 are parameters that determine the shape of the curve and are generated from data collected on various substrates (Chapter 2, Table 5.1). The overall effect of temperature on nitrogen fixation and respiration is given by combining the two components (Figure 5.3):

$$\text{TMPIDN} = \text{TMPN1} * \text{TMPN2}$$

$$\text{TMPIDR} = \text{TMPR1} * \text{TMPR2}.$$

Log Moisture Content

Daily log moisture content (MOIST) is estimated for each layer from precipitation data (PRECIP, mm), evaporation, and diffusion rates. The approach for each daily time-

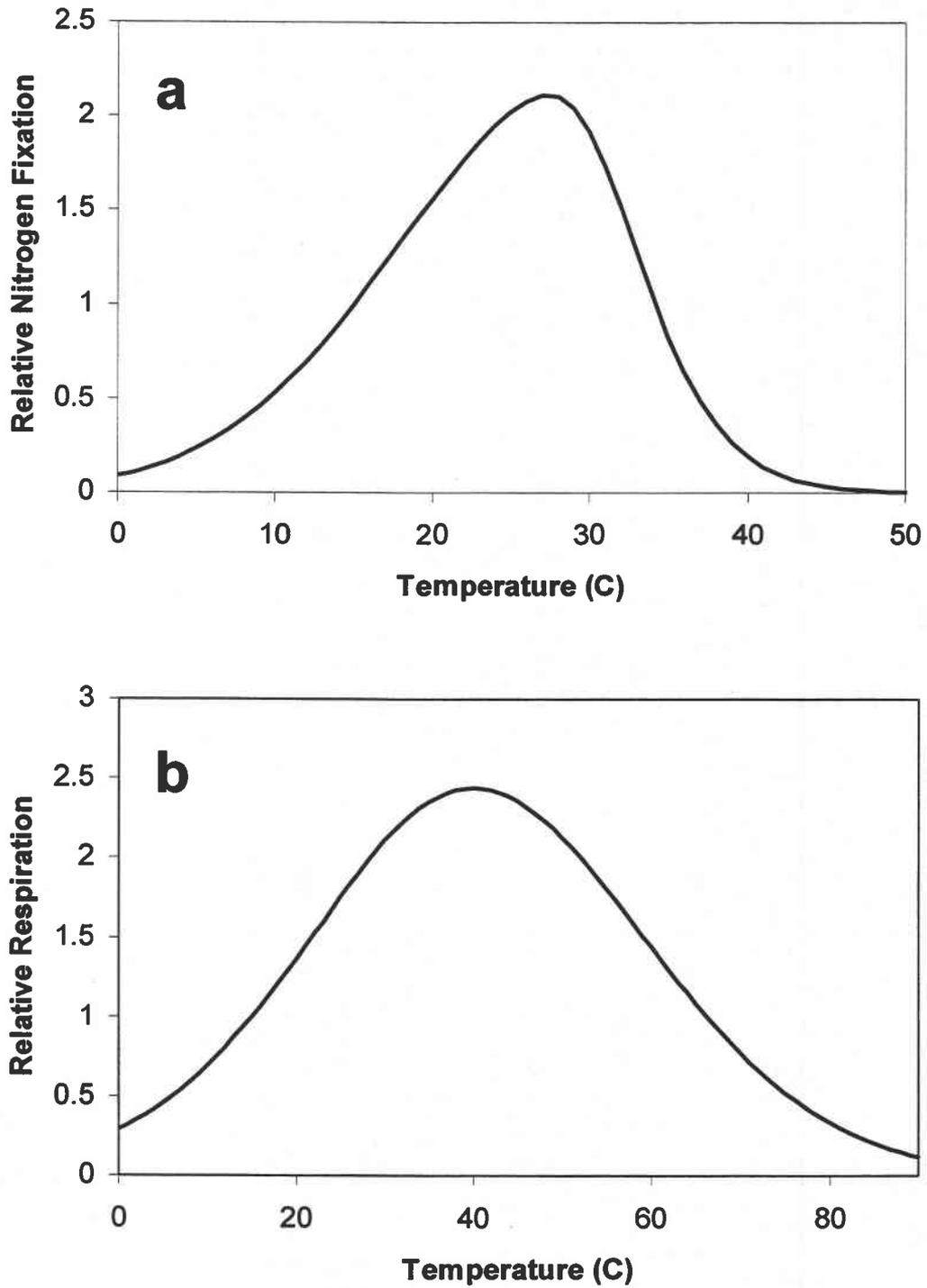


Figure 5.3. The effect of temperature on (a) nitrogen fixation and (b) respiration rate.

step is to wet the log with throughfall, calculate water loss from drying, and then estimate diffusion of water between layers.

First, we wet the log using precipitation data. Before precipitation can enter the log, canopy interception and runoff eliminate some of the water (Figure 5.4). The fraction of throughfall water (THRUFL) that is not intercepted by the canopy increases as the amount of precipitation increases (Rothacher, 1963; Figure 5.5a) as described by the following Chapman-Richards equation:

$$\text{THRUFL} = \text{MAXFAL} * ((1 - \text{EXP}(-\text{SHPINT} * \text{PRECIP}))^{\text{LAGINT}})$$

where MAXFAL is the maximum fraction for THRUFL. SHPINT and LAGINT are parameters that alter the shape of the curve. The fraction of throughfall that runs off the log surface (RUNOFF) is related to layer density according to the following equation:

$$\text{RUNOFF} = (1 - \text{EXP}(-\text{SHPRUN} * \text{DENSE}))^{\text{LAGRUN}}$$

where SHPRUN and LAGRUN are parameters which determine the shape of the curve and are generated from Harmon and Sexton's data (1995; Figure 5.5b). Thus, the water that enters the outermost log layer (H2OIN , $\text{l} \cdot \text{d}^{-1}$) is determined from the following equation:

$$\text{H2OIN} = \text{PRECIP} * \text{PROSA} * \text{THRUFL} * (1 - \text{RUNOFF})$$

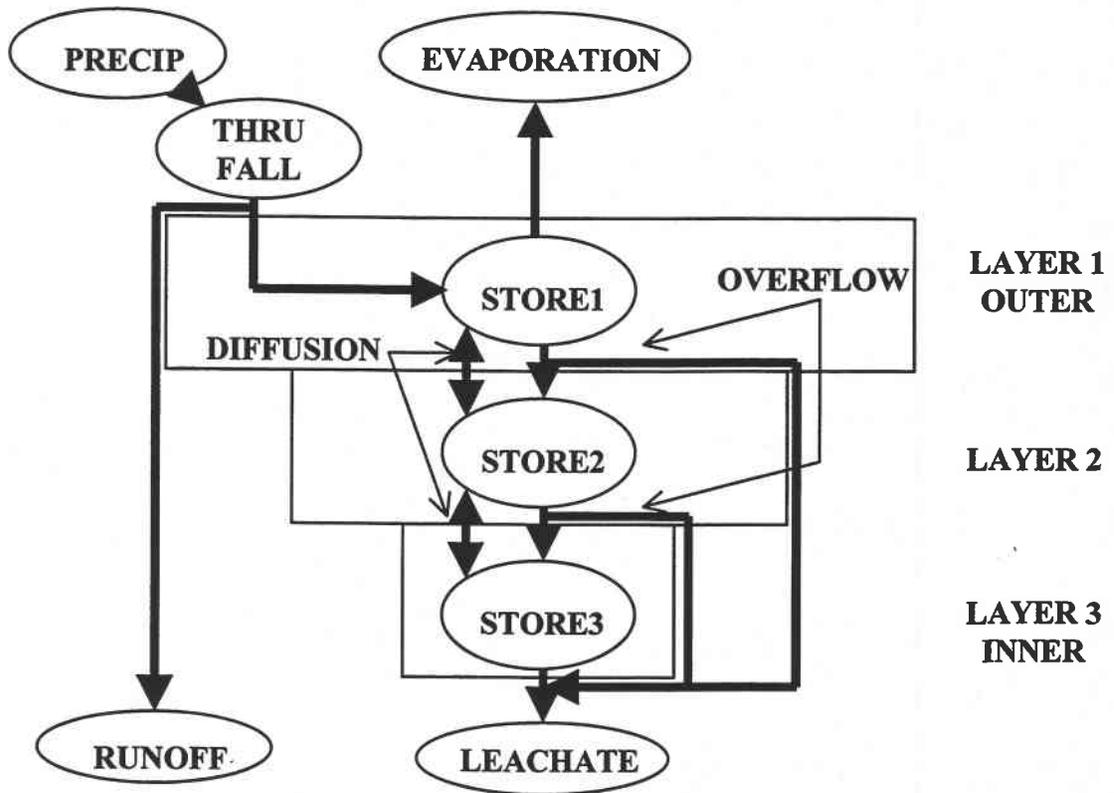


Figure 5.4. A diagram of the method used to model log moisture content.

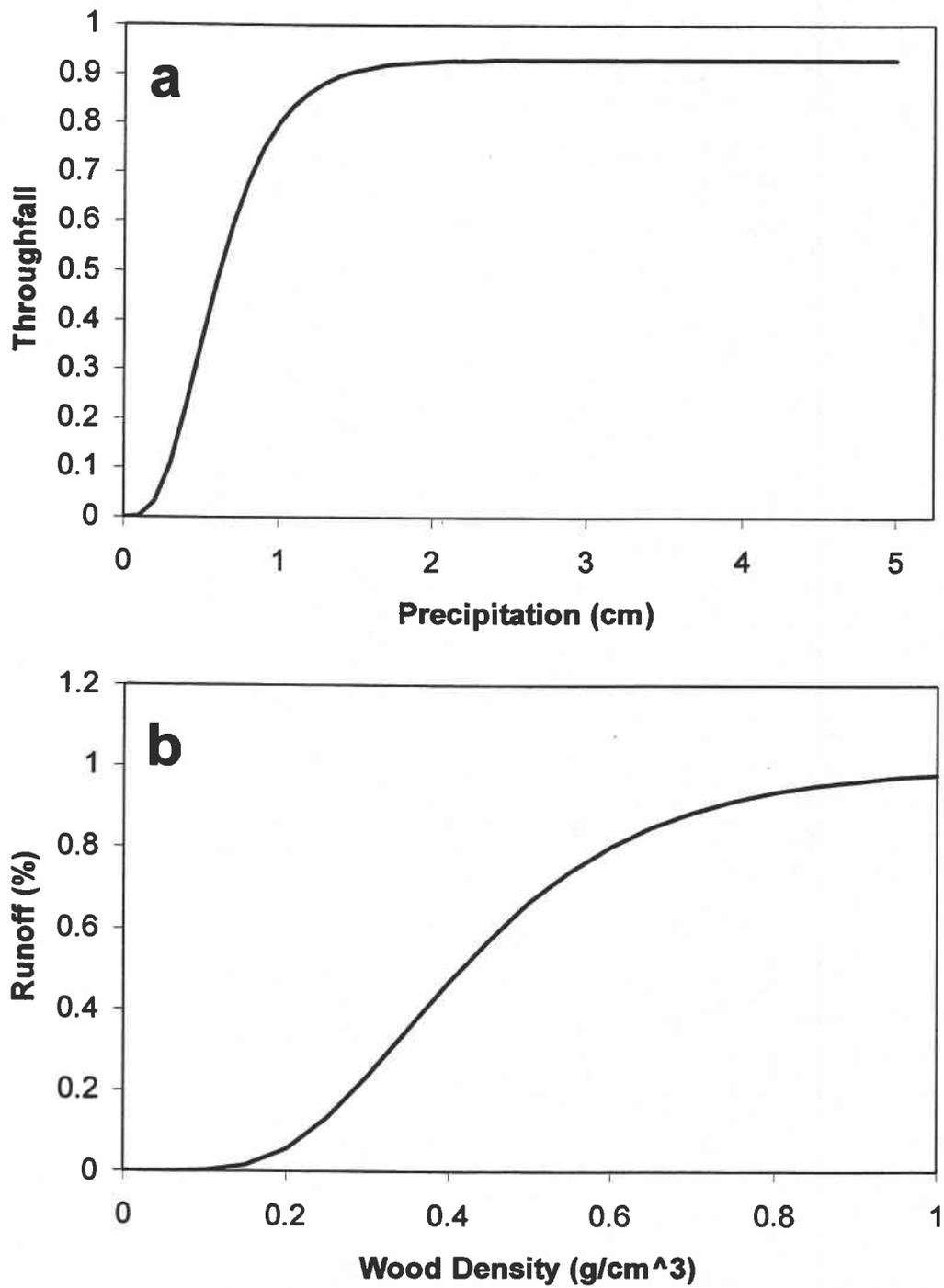


Figure 5.5. The relationship of (a) precipitation and throughfall, and (b) wood density and throughfall runoff.

where PROSA is used to convert PRECIP from mm/area to liters.

We use a "tipping bucket" approach to move excess water in the outer layer into internal layers. Conceptually, each layer is a bucket. If the amount of water entering the outermost bucket is greater than the bucket can hold, the excess (OVFLO, $l \cdot d^{-1}$) moves into the next layer or leaches out of the log. The amount of overflow that leaches out is determined using the same equation as used for runoff except for sound heartwood. When heartwood is at 80 to 100% of its initial density, all overflow runs off and leaches from the log. We model heartwood in this manner because field observations indicate that sound heartwood does not wet up to its maximum moisture content even when exposed to saturated sapwood (Harmon and Sexton, 1995). Possibly, heartwood does not reach maximum moisture contents until decomposition produces cracks and channels in the heartwood. The amount of water that enters the next inner layer is then determined by subtracting leachate from overflow. For the innermost layer, all overflow is considered leachate.

Second, we dry the log. Evaporation (EVAP, $l \cdot d^{-1}$) only occurs in the outer layer. We used the evaporation component of the soil moisture model of Huang *et al.* (1996) to determine evaporation:

$$EVAP = EVAPPOT * (MOIST / MAXMST).$$

where EVAPPOT is the potential evaporation in liters per day and MAXMST is the maximum moisture content of a layer as described below. EVAPPOT is determined

using a model of soil evaporation that requires mean monthly temperature and day length (Thornthwaite, 1948).

Next, we diffuse water between layers. Moisture diffusion (MDIFF, $l \cdot d^{-1}$) is expressed as:

$$MDIFF = MDMX * MGID * MDDIX * MDTIX * LOGSA$$

where MDMX is the maximum moisture diffusion rate ($l \cdot m^{-2} \cdot d^{-1}$), MGID is the moisture gradient between layers, MDDIX and MDTIX are the indices relating moisture diffusion and wood density and temperature, respectively. MGID is determined by comparing the fractions of actual to potential water stores for two adjacent layers. Figure 5.6a demonstrates the linear relationship of density and moisture diffusion as described by:

$$MDDIX = 1 - MDDIXA * DENSE$$

where MDDIXA is a constant (Table 5.1). Figure 5.6b demonstrates the relationship between temperature and moisture diffusion that is expressed as:

$$MDTIX = MDTIXA * (TEMP^{MDTIXB})^{0.5}$$

where MDTIXA and MDTIXB are constants (Table 5.1). When TEMP is less than zero MDTIX is assumed to be zero. MDMX, MDDIXA, MDTIXA, and MDTIXB were

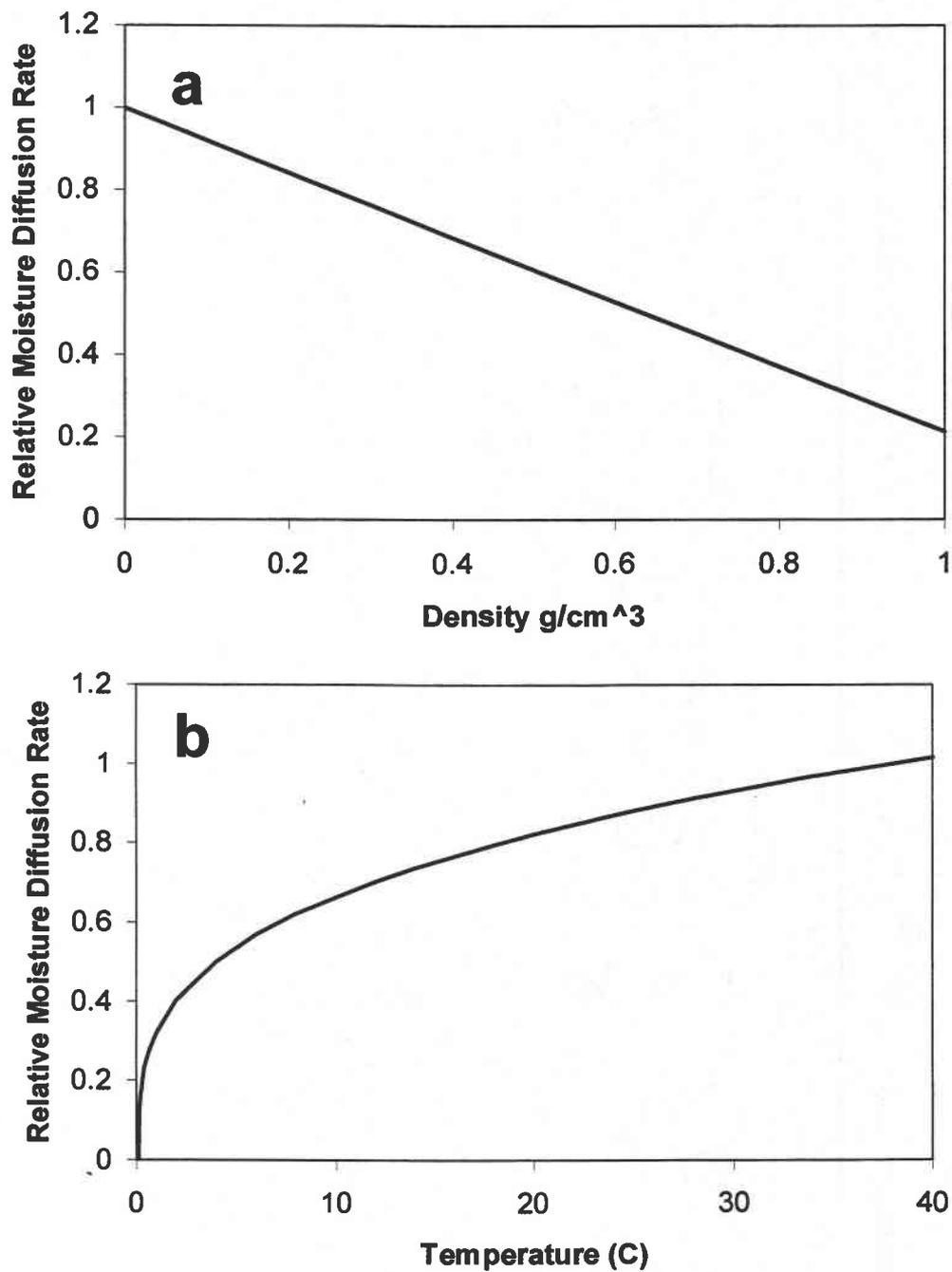


Figure 5.6. The relationship of the moisture diffusion rate and (a) wood density and (b) temperature.

determined from an unpublished experiment of moisture movement between wood blocks (Harmon, unpublished data). Because of the manner in which this experiment was performed, the effect of wood thickness on moisture diffusion rate could not be determined. The equations should be valid as long as layer thickness is 8mm or greater.

After accounting for infiltration, evaporation, and diffusion, the moisture content of a layer (MOIST) is calculated by:

$$\text{MOIST} = (\text{H}_2\text{OV} / \text{LMASS}) * 100.$$

The maximum moisture content for a layer (MAXMST) is a function of layer density as described by the following negative exponential equation:

$$\text{MAXMST} = \text{MMHITE} * (\text{EXP}(-\text{DENSE} * \text{MAXCOM}))$$

where MMHITE and MAXCOM are parameters that determine the height and steepness of the curve, respectively, based on data from Harmon & Sexton (1995, Table 5.1, Figure 5.7). This relationship reflects the fact that as wood density increases the amount of pore space that can store water decreases.

Moisture Effects

The moisture effects indices determine the effect of daily log moisture content on

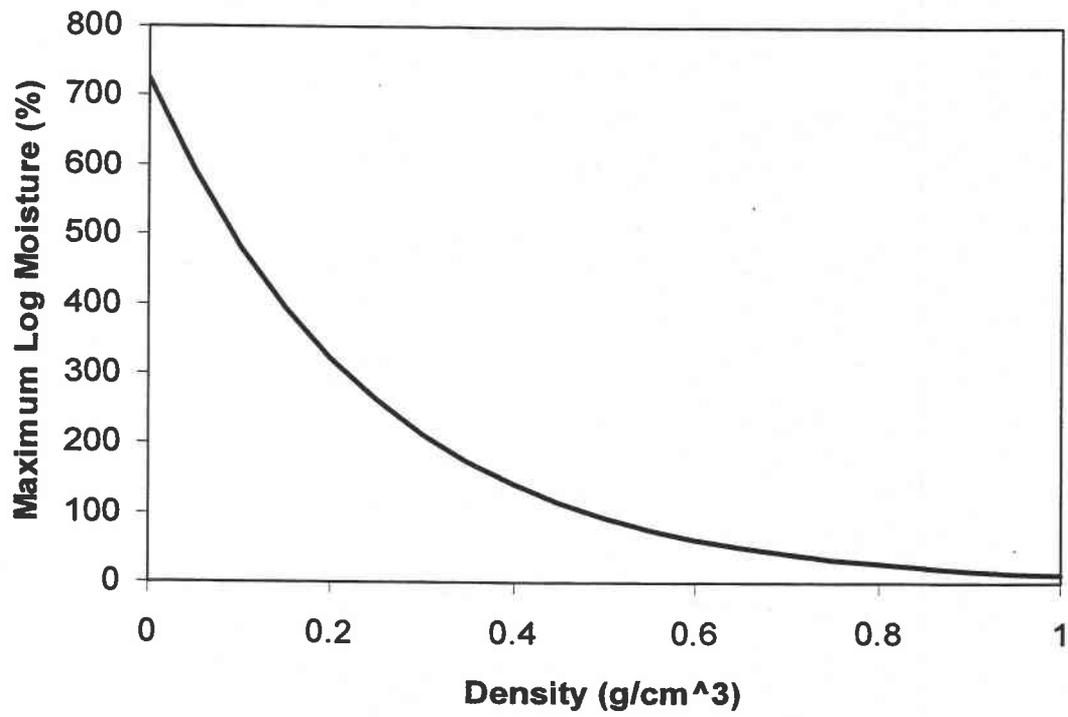


Figure 5.7. The relationship of wood density and the maximum log moisture content.

nitrogen fixation and respiration. A lack of moisture generally prevents respiration and nitrogen fixation in wood when levels fall below the fiber saturation point (Griffin, 1977, Figure 5.8). At this point microorganisms cannot overcome the matric potential of water stored in wood fibers. High log moisture content can also indirectly inhibit respiration presumably by slowing diffusion of oxygen (Boddy, 1983; Flanagan & Veum, 1974; Griffin, 1977). However, by incorporating an oxygen index for nitrogen fixation in our model, we directly account for the latter effect (see *Log Oxygen Content* section). The nitrogen fixation and the respiration moisture indices are solved using Chapman-Richards equations:

$$\text{MSTIDN} = (1 - \text{EXP}(-\text{SHPMN} * \text{MOIST}))^{\text{LAGMN}}$$

$$\text{MSTIDR} = (1 - \text{EXP}(-\text{SHPMR} * \text{MOIST}))^{\text{LAGMR}}$$

where SHPMN, LAGMN, SHPMR and LAGMR are parameters that determine the shape of the curve and are generated from data collected on various substrates (Chapter 2; Table 5.1).

Log Oxygen Content

Daily log oxygen content (O₂, %) is needed to alter the indices which control the effect of oxygen on nitrogen fixation and respiration (see *Oxygen Effects*). We modeled oxygen content mechanistically using respiration and oxygen diffusion. These processes

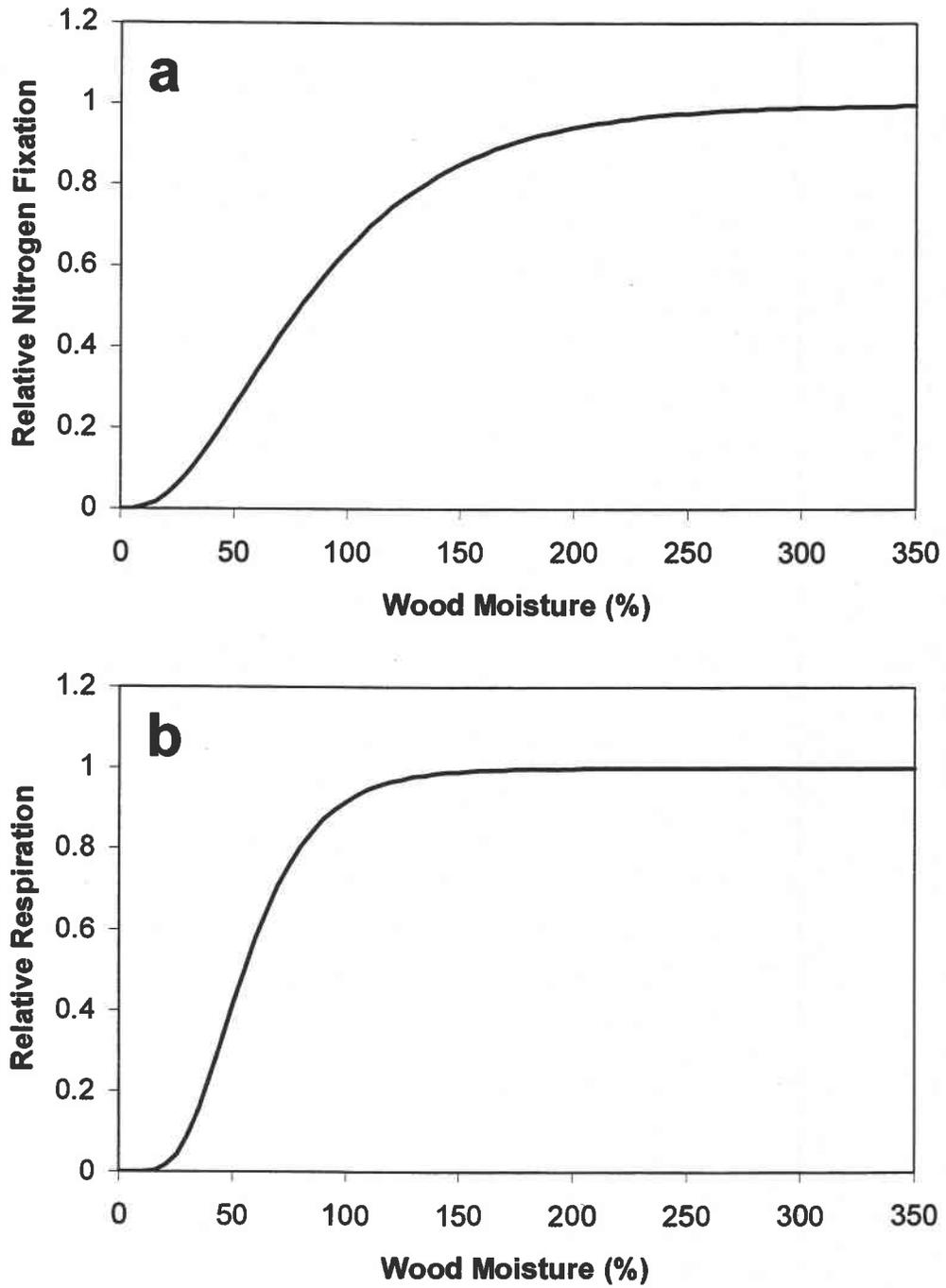


Figure 5.8. The effect of wood moisture on (a) nitrogen fixation and (b) respiration rate.

are most accurately described using molar quantities. Thus, we keep track of daily changes in the oxygen content in a layer in moles, and use this molar value to determine percent oxygen content for each time step.

We determine the moles of oxygen (O2MOL, mol) present in a layer on a daily basis with the following equation:

$$O2MOL = MO2GAS + MO2H2O + DIFF - RESPC$$

where RESPC is the moles of oxygen respired per day, DIFF is the moles of oxygen diffusing into the layer per day (see *Oxygen Diffusion* section), MO2GAS is the moles of oxygen in gaseous form, and MO2H2O is the moles of oxygen dissolved in water.

MO2H2O is determined from the amount of water in the layer (H2OV) and the concentration of oxygen in the water (CO2H2O). CO2H2O is calculated with Henry's Law using the partial pressure of oxygen and the Henry's Law constant (k , $\text{mol}\cdot\text{l}^{-1}\cdot\text{atm}^{-1}$) which varies with temperature (Weast, 1973):

$$k = (-2.5 \cdot 10^{-5}) \cdot \text{TEMP} + 0.002.$$

MO2GAS is determined from the ideal gas equation. The volume of gas in the layer (GASV) used in the ideal gas equation is the difference of H2OV and H2OVM.

Finally, we backcalculate O2 from O2MOL. First, we determine the amount of O2MOL that is contained in gas (XMOL) using Henry's Law and the ideal gas equation.

$$OCON = (R * TEMPK * k * H20V) / GASV$$

where R is the ideal gas constant and TEMPK is the temperature in Kelvin. Then:

$$XMOL = O2MOL / (OCON + 1).$$

Log oxygen content (O2) can then be determined from the ideal gas equation.

Oxygen Index for Nitrogen Fixation

This function describes the effect of daily log oxygen concentration (O2, %) on nitrogen fixation rate (Figure 5.9a). The response of nitrogen fixation to oxygen has two components. The first portion describes the increase in activity as oxygen concentration rises due to the demands of these aerobically respiring nitrogen fixers for energy. This index rises from zero when oxygen is absent to one at an optimum. The following Chapman-Richards equation describes this increase:

$$O2N1 = (1 - \text{EXP}(-\text{SHPON1} * O2))^{\text{LAGON1}}$$

where O2N1 is the first component of the oxygen index for nitrogen fixation; SHPON1 and LAGON1 are parameters that determine the shape of the curve and are generated from data collected on various substrates (Chapter 2, Table 5.1). The second component

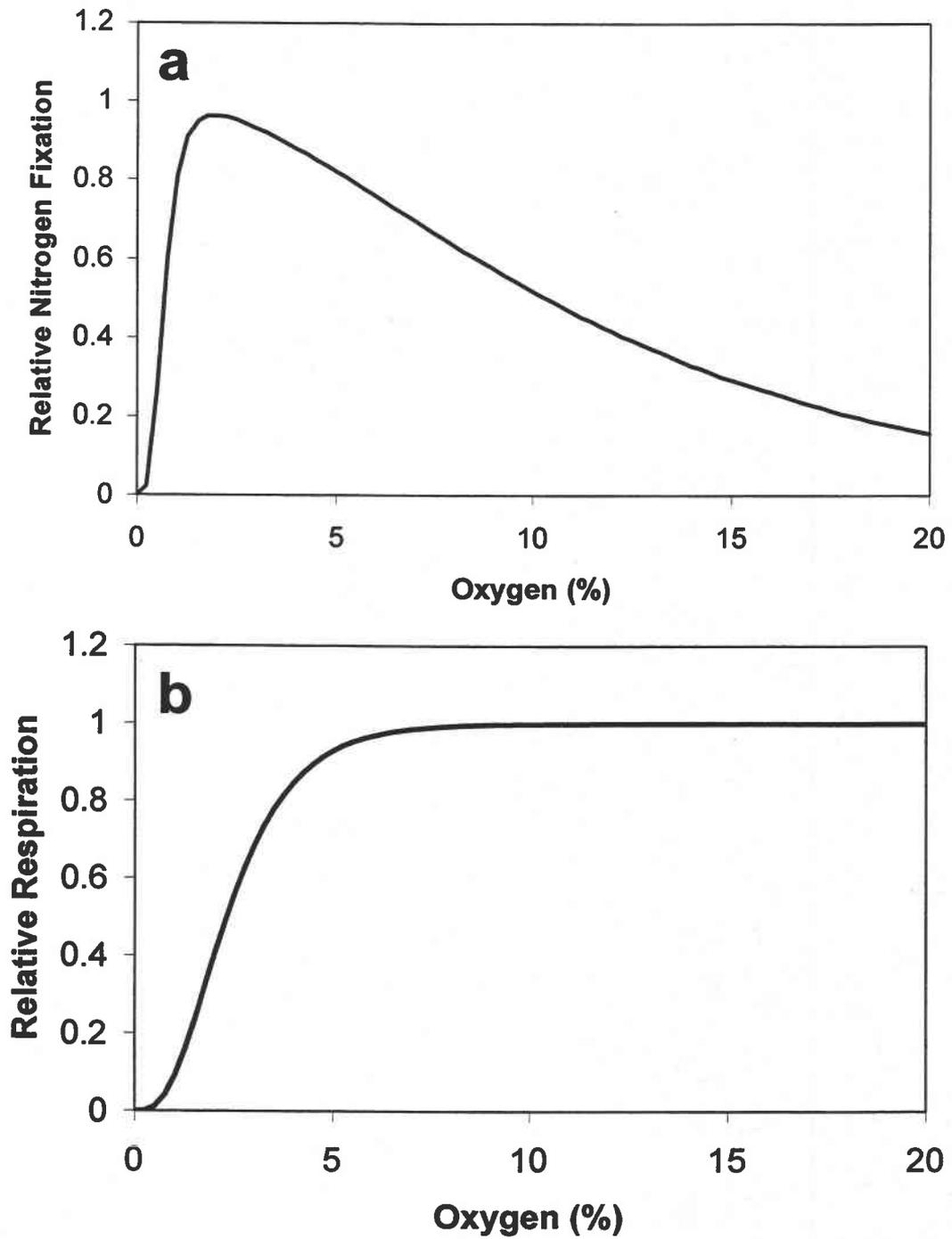


Figure 5.9. The effect of oxygen concentration on (a) nitrogen fixation and (b) respiration rate.

of the oxygen response describes the inactivating effect of rising oxygen concentrations on nitrogenase (Silvester *et al.*, 1982). The index starts at one then declines to zero after reaching the optimum:

$$O2N2 = 1 - ((1 - \text{EXP}(-\text{SHPON2} * O2))^{\text{LAGON2}})$$

where O2N2 is the second component of the oxygen index for nitrogen fixation.

SHPON2 and LAGON2 are parameters that determine the shape of the curve and are generated from data collected on various substrates (Chapter 2, Table 5.1).

The nitrogen fixation response to oxygen index results from combining these two components:

$$O2IDXN = O2N1 * O2N2.$$

Oxygen Index for Respiration

This index describes the effect of oxygen on respiration rate by simulating the increase and subsequent leveling of aerobic respiration activity that occurs as oxygen concentration rises (Figure 5.9b). Unlike nitrogen fixation, respiration is not inhibited by atmospheric oxygen concentrations. Thus, the index starts at zero when oxygen is absent and rises to one. The following Chapman-Richards equation describes this effect:

$$O2IDXR = (1-EXP(-SHPOR*O2))^LAGOR$$

where SHPOR and LAGOR are parameters that determine the shape of the curve and are generated from data collected on various substrates (Chapter 2; Scheffer, 1985; Table 5.1). We modified the response of respiration to oxygen to include the additional inhibiting effect of CO₂, by limiting respiration below 5% O₂ (Highley, 1983).

Oxygen Diffusion

We incorporate oxygen concentration and diffusion in our model to provide a mechanistic means for evaluating the effect of oxygen on nitrogen fixation and respiration. High wood moisture has been shown to indirectly inhibit respiration by reducing oxygen diffusion, and this moisture effect could be used to estimate the inhibitory effect of low oxygen concentration on respiration (Boddy, 1983; Chen *et al.*, 2000). Because nitrogen fixation and respiration respond differently to oxygen concentration, we could not use an inhibiting effect of high moisture to model both responses to oxygen.

The oxygen diffusion rate (DIFF, mol·d⁻¹) is controlled by log moisture, density and oxygen content:

$$DIFF = DIFMAX*LOGSA/THIK*MSTIDD*DENIDD*O2IDXD$$

where DIFMAX ($\text{mol O}_2 \cdot \text{m}^{-1} \cdot \text{d}^{-1}$) is the maximum diffusion rate of oxygen through wood (Chapter 4). Three indices describe the effect of log moisture (MSTIDD), density (DENIDD) and oxygen content (O2IDX) on the oxygen diffusion rate. Indices range from zero to one and are used to reduce DIFMAX when any conditions controlling diffusion are limiting.

Moisture Index for Oxygen Diffusion

Increasing wood moisture decreases oxygen diffusion rates for two reasons. Wood fibers and decomposed material swell as their moisture content increases up to the fiber saturation point. Cracks and air spaces shrink, decreasing the area of air space available for diffusion. Once wood fibers become saturated additional water fills the remaining pore spaces. Oxygen moves slower in wood saturated in this manner, because the oxygen diffusion rate constant is four orders of magnitude lower in water compared to air (Harmon *et al.*, 1986; Bird, 1960).

We modeled the effect of water on diffusion in the following way. The moisture index for oxygen diffusion (MSTIDD) is assumed to remain at one as long as log moisture content is below the fiber saturation point. As moisture content increases above the fiber saturation point, MSTIDD is related to the fraction of pore space filled with air (FPSA, Figure 5.10a) as expressed by:

$$\text{MSTIDD} = 10^{(-\text{MIDDA} + \text{MIDDB} * \text{FPSA})}$$

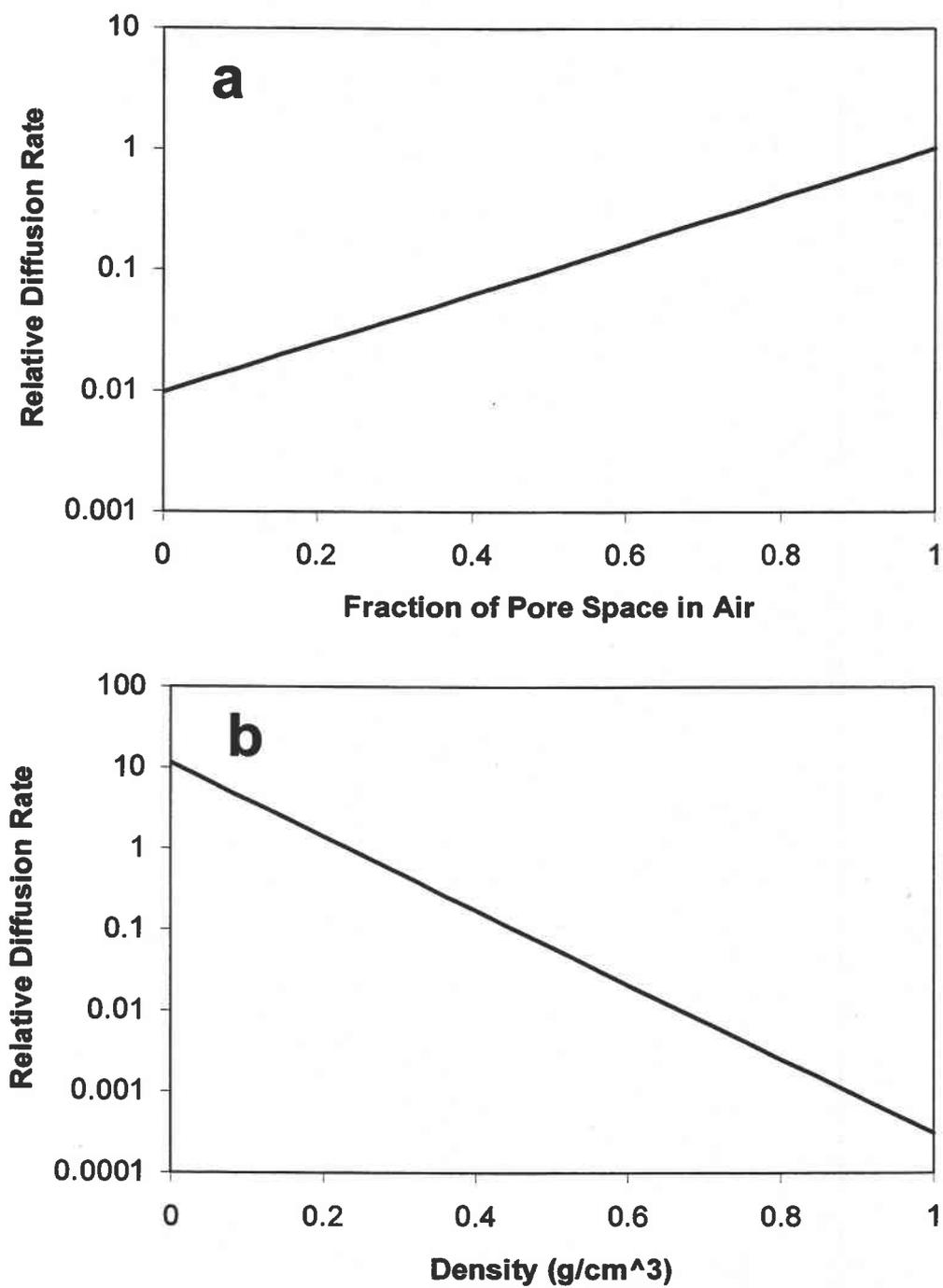


Figure 5.10. The relationship of the oxygen diffusion rate and (a) the fraction of pore space in air and (b) wood density.

where MIDDA determines the y-intercept and MIDDB controls the rate of increase of the curve. Parameter values were determined from measurements of oxygen diffusion through wood cores of varied moisture (Chapter 4, Table 5.1). FPSA is determined from the following equation:

$$\text{FPSA} = (\text{GASVM} - \text{H}_2\text{OV}) / \text{GASVM}.$$

FPSA is used as a metric of wood moisture because it is independent of wood density.

Density Index for Oxygen Diffusion

As wood density increases, oxygen diffusion rates decrease because denser wood has less pore space available for oxygen diffusion (Figure 5.10b). We used a negative exponential equation to estimate the effect of wood density on oxygen diffusion:

$$\text{DENIDD} = 10^{(\text{DIDDA} - \text{DIDDB} * \text{DENSE})}$$

where DIDDA and DIDDB are parameters that alter curve height and slope steepness respectively. Parameter values were determined from measurements of oxygen diffusion through wood cores of varied density (Chapter 4, Table 5.1).

Oxygen Gradient Index for Oxygen Diffusion

According to Fick's Law, oxygen diffusion should decrease linearly as the oxygen gradient from the outside to the inside of the log decreases. The following equation is used to describe this effect:

$$O2IDX = (O2OUT - O2) / O2OUT$$

where O2OUT is the oxygen concentration external to the log.

Methods

Uncertainty Analysis

We used uncertainty analysis to evaluate the sensitivity and degree of confidence in parameters. Our goal was to identify parameters that are estimated with low confidence and to which the model is highly sensitive (Table 5.2). Parameters that have low estimate confidence and low sensitivity are not as critical, because they have little influence on model output. Precise parameters that have high sensitivity are also of less concern given the resolution of estimates. Finally, the parameters of least interest are those with low sensitivity and high estimate confidence.

We tested the relative influence of all model parameters on the estimate of the amount of nitrogen fixed annually (NFI) in a decay class two, *Tsuga heterophylla* log

by recording the percent change in NFIX after increasing the parameter by 10%. After identifying sensitive parameters with a low estimate confidence, we further tested model sensitivity to these parameters by increasing and decreasing parameters by 5, 10, and, 20% to see if the response was linear or curvilinear.

The uncertainty of the parameter estimate was crudely estimated by assigning parameters to the low or high uncertainty category. If we felt the parameter estimate was within 10% of the real value, the parameter was assigned to the low uncertainty category, while parameters that were not estimated this well were assigned to the high uncertainty category.

Table 5.2. Criteria used to identify parameters of greatest concern.

		Parameter Sensitivity	
		HIGH	LOW
Uncertainty Of Parameter Estimate	H I G H	High Concern Low confidence in parameter estimate and parameter variation greatly impacts model output	Low Concern Low confidence in estimate but parameter variation minimally impacts model output
	L O W	Low Concern Parameter variation greatly impacts model output but high confidence in parameter estimate	Low Concern Low confidence in estimate and parameter variation minimally impacts model output

Model Validation

Unfortunately, there is little available data for direct validation of predicted nitrogen fixation rates; however, we compared predictions of respiration rate, temperature, moisture, and oxygen concentration to independent field data (Chapter 4). Studies of nitrogen fixation in dead wood are relatively scarce, with only two estimates of the annual amount of nitrogen fixed in wood at the stand scale in the Pacific Northwest (Silvester *et al.*, 1982; Sollins *et al.*, 1987). In addition, no method has been developed to measure absolute nitrogen fixation rates in logs in the field without removing a portion of the sample and incubating it in conditions that probably do not resemble those of the log. Thus, even if the data on nitrogen fixation in dead wood existed, we would be limited to relative comparisons.

Model Experiments

Climate sensitivity

To begin understanding how variations in climate in the Pacific Northwest and possible future changes in climate would affect nitrogen fixation rates in dead wood, we simulated annual nitrogen fixation rates in a 50 cm diameter, decay class two, *Tsuga heterophylla* log in a variety of Pacific Northwest locations (Table 5.3). We used a decay class two, *Tsuga heterophylla* log because it has relatively high activity and sensitivity to

changes in precipitation. To simulate climate change we ran the model with adjusted meteorological data from each site. Daily temperatures were increased by 2°C and daily precipitation was decreased by 10%.

Log level

To gain an understanding of the contribution of nitrogen fixation to the nitrogen budget of a log, we used our model to estimate how much nitrogen is fixed over the 200 year lifetime of a 1.5 Mg, 50 cm, *Pseudotsuga menziesii* log decaying at a rate of 0.02 yr⁻¹. The log was assumed to initially contain 0.1% nitrogen or a store of 1.5 kg N (Harmon and Sexton, 1995).

Table 5.3. The annual amount of nitrogen fixed (nmol·g wood⁻¹·yr⁻¹) using 1998 meteorological data and under a scenario where temperatures are 2°C higher and precipitation is 10% lower in a *Tsuga heterophylla*, decay class two log for several sites covering a range of climate types in the Pacific Northwest.

Site	Lat.	Elevation (m)	Annual Temp. (°C)	Annual Precip. (cm)	N ₂ Fixed	N ₂ Fixed (+2°C,-10%)
Ashland, OR	42°13'	560	11.7	78	22	25
Cascade Head Exp. Forest, OR	45°02'	50	11.0	282	170	227
Wind River Exp. Forest, WA	45°52'	346	9.6	272	120	156
H.J. Andrews Exp. Forest, OR	44°14'	1018	8.2	256	121	153
Pringle Falls Exp. Forest, OR	43°41'	1278	7.0	92	16	18
Mt. Rainier, WA	46°47'	1654	3.6	329	41	53

Stand level

We estimated the amount of nitrogen fixed at the stand level for the three sites where wood was sampled to parameterize our model (Chapter 3). We used woody debris biomass estimates for the H. J. Andrews from Sollins *et al.* (1987) while biomass estimates from Wind River and Cascade Head are from Harmon (unpublished). Woody debris biomass was 143 Mg, 167 Mg, and 153 Mg for H. J. Andrews, Wind River, and Cascade Head, respectively. Woody debris biomass was primarily *Pseudotsuga menziesii* and *Tsuga heterophylla* at H. J. Andrews and Wind River, while Cascade head also had substantial amounts of *Picea sitchensis*.

Relative Importance

We used model output for estimating nitrogen fixation rates in wood over a hypothetical 500-year succession and literature values to estimate nitrogen fixation inputs from other sources. In this analysis we assumed *Alnus rubra* and *Ceanothus velutinus* only occurred early in succession, lichens only after 150 years, and wood, soil, and litter were present throughout. Nitrogen inputs from precipitation and soil were assumed to remain constant throughout succession (2.5 and 0.5 kg N ha⁻¹ yr⁻¹, respectively).

Results and Discussion

Uncertainty Analysis

Model output was relatively insensitive (*ie.*, less than a 5% change in NFIX) to most of the parameters (Table 5.1). We have high confidence in some parameter estimates that the model was sensitive to (*e.g.*, REFTMP and RESPMAX). The remaining parameters, that the model is sensitive to and we are not highly confident in our estimate of, fall into three groups: 1) the maximum nitrogen fixation rate (NFIKMAX), 2) parameters related to generating log moisture content (SHPRUN, MMHITE, MAXCOM, and EVAPOT), and 3) parameters related to oxygen diffusion (DIFMAX, MIDDB, and DIDDB). Altering these parameters of most concern by various amounts generally produced linear changes in NFIX, although EVAPOT and DIDDB produced slightly curvilinear changes in NFIX (Figure 5.11).

As expected, altering NFIKMAX by a given percent results in the same relative change in NFIX (Figure 5.11). Nitrogen fixation rates are highly variable and vary with the woody tissue, degree of decay, and species (Chapter 3; Sollins *et al.*, 1987). Despite the many logs we sampled to generate our table of NFIKMAX values, we are not highly confident in our values. The accuracy and applicability of our model would therefore improve with additional data from different species and sites.

In general, altering the parameters that influence log moisture content increases NFIX if it leads to greater log moisture, and decreases NFIX if it leads to lower log moisture (Figure 5.11a). Changing the parameters that influence log moisture content produces a similar magnitude change in NFIX when compared to altering NFIKMAX.

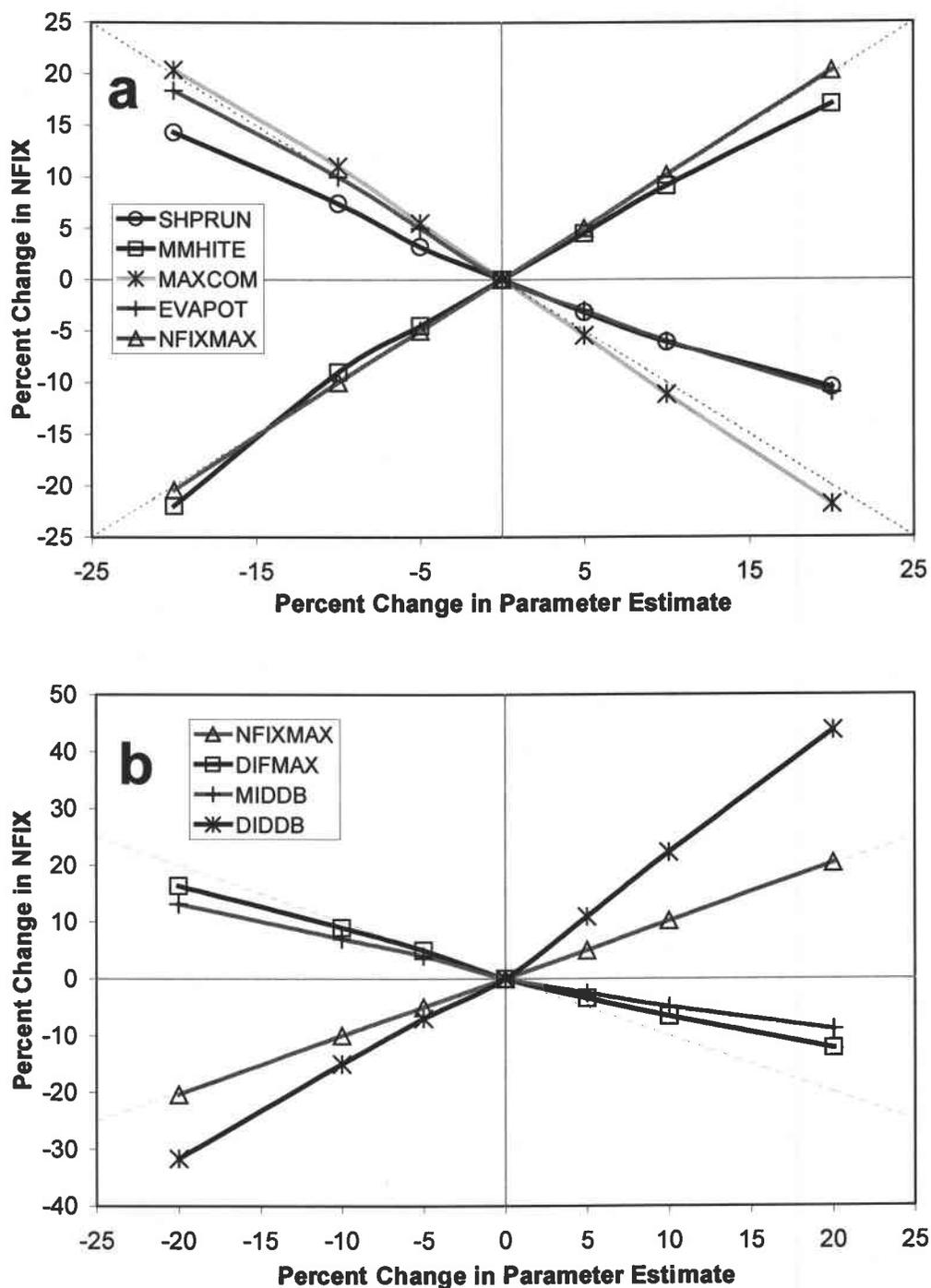


Figure 5.11. The effect of altering several parameters related to (a) generating log moisture content and (b) oxygen diffusion on the annual amount of nitrogen fixed (NFIX, $\text{nmol} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$).

Unfortunately, very little is known about evaporation and runoff of water from logs (Harmon and Sexton, 1995). The parameters involved in generating the maximum moisture content (MMHITE and MAXCOM) of logs are relatively accurate; however, logs early in decay do not seem to reach these moisture contents under field conditions. Future research should focus on developing better estimates of evaporation and runoff, as well as evaluating the discrepancy between lab generated maximum moisture contents and the maximum moisture contents observed in the field.

In general, altering the parameters that influence oxygen diffusion increases NFIX if it leads to lower rates of oxygen diffusion, and decreases NFIX if it leads to higher rates of oxygen diffusion (Figure 5.11 a). Changing the parameters that influence rates of oxygen diffusion produces a similar magnitude change in NFIX when compared to altering NFIXMAX except for DIDDB, which produced a greater change. DIDDB determines the exponential rate of decrease in the diffusion rate as wood density increases, thus changes in DIDDB can have a proportionately greater effect on NFIX than other parameters. Future research should focus on improving our estimates of MIDDB and DIDDB, as well as investigating the role of longitudinal oxygen diffusion, which we did not include in our model.

Model Validation

Our model produced a seasonal pattern of wood respiration rate similar to observed rates obtained from soda lime measurements of respiration by logs at Wind

River Experimental Forest in Washington (Figure 5.12, Figure 5.13a, Chapter 4). Both curves peak in summer when temperatures both are warmest and are lowest in the winter months. The relationship of the predicted and observed data is significant despite the low correlation coefficient ($p = 0.01$, $r^2 = 0.35$). The low degree of correlation is not surprising because the soda lime respiration measurements were highly variable and are not an absolute measure of respiration rate.

Model estimates of average daily log temperature closely track daily air temperature in 50 cm diameter logs (Figure 5.14). Unpublished data of log temperature measurements in logs close to 50 cm in diameter indicates that log temperatures are generally within 1°C of average daily air temperature. In 100 cm diameter logs, modeled log temperatures are often up to 5°C different from air temperature. The larger ratio of mass to surface area as log diameter increases should produce similar results in actual logs. Thus, our model appears to reasonably estimate log temperature.

Our model produced a comparable pattern of seasonal changes in wood moisture content in comparison to moisture contents obtained from time domain reflectometry (TDR) in logs at Wind River Experimental Forest in Washington (Figure 5.13b, Figure 5.15, Chapter 4). Average annual moisture contents were similar and increased with decay class for actual and modeled results (Table 5.4). In addition, seasonal fluctuations in moisture content in decay classes four and five are similar in magnitude and timing. However, our model does predict greater seasonal fluctuations in moisture content than observed for decay classes one through three. The greatest practical difference between our model and actual data is in decay class one, where the lower average predicted moisture contents might produce underestimates of respiration and nitrogen fixation

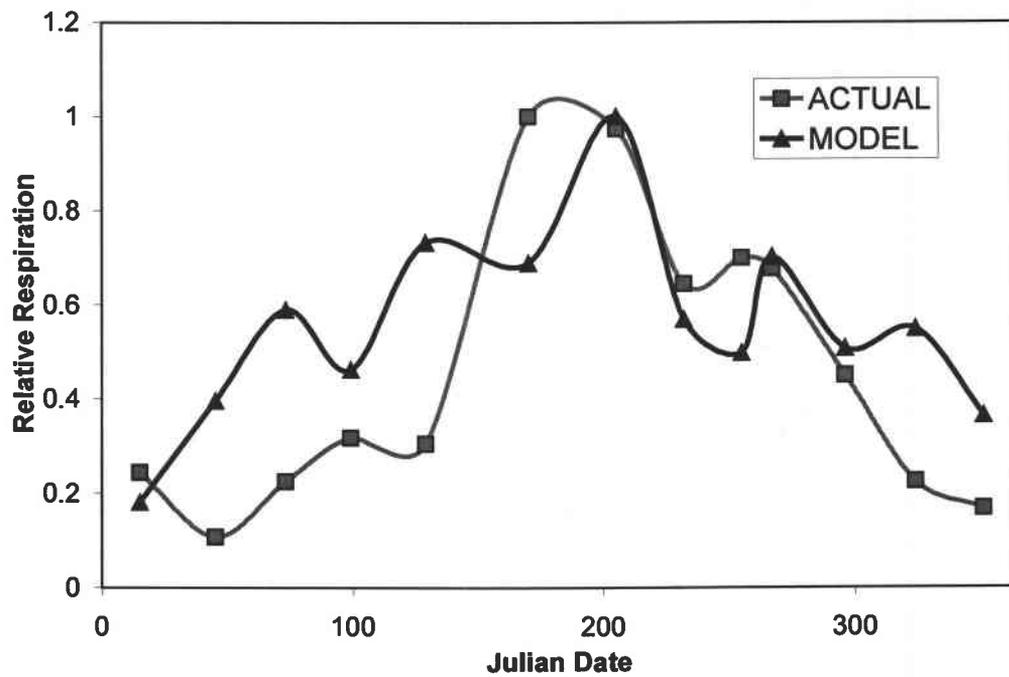


Figure 5.12. A comparison of relative respiration rates from field data and the model.

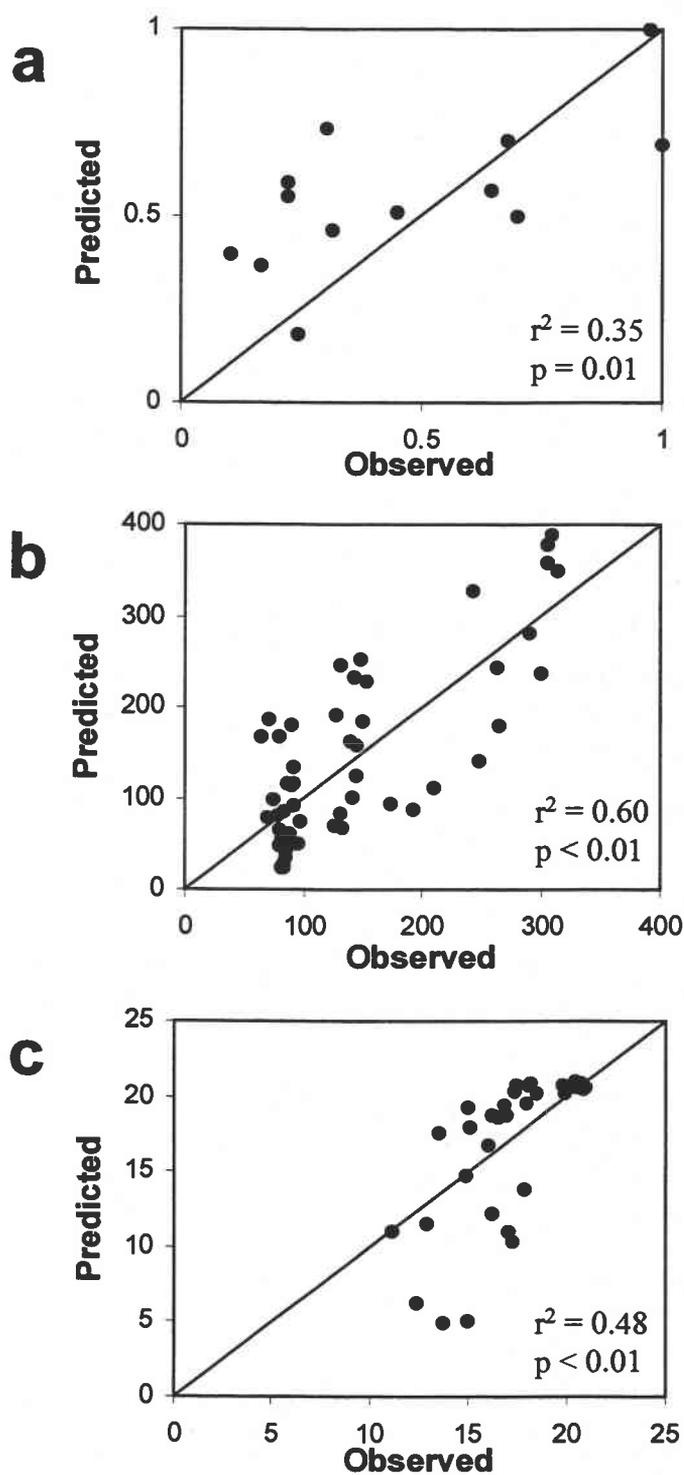


Figure 5.13. Predicted versus observed plots for comparing data from the model and the field, respectively, for (a) respiration (b) moisture content and (c) oxygen concentration.

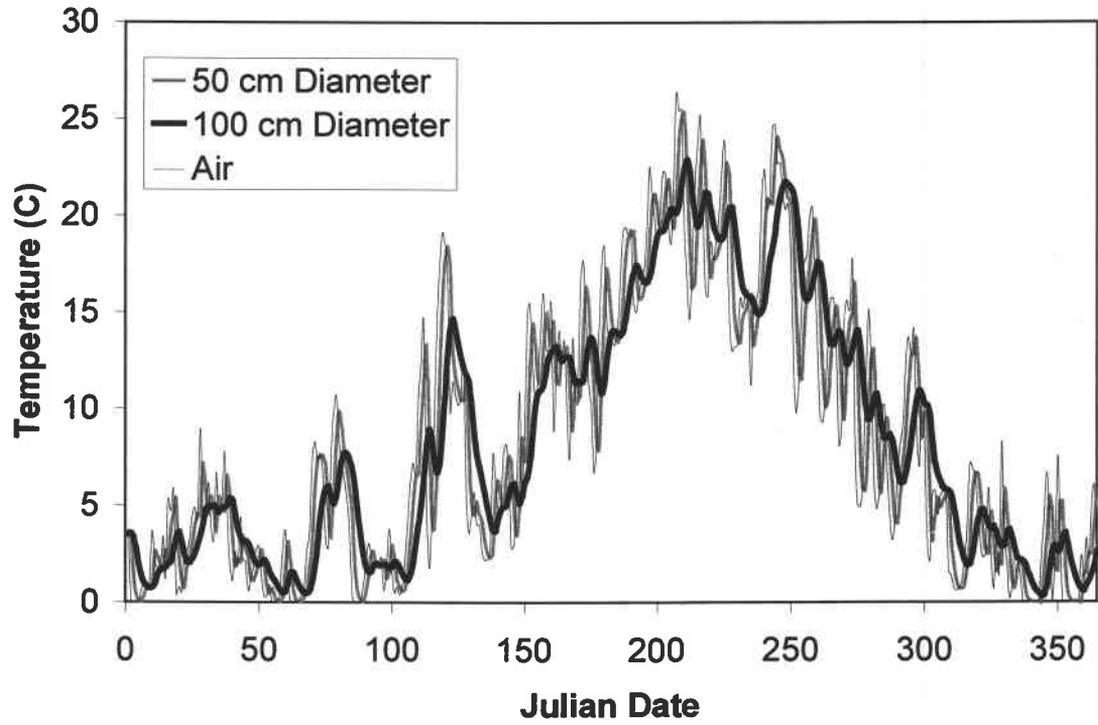


Figure 5.14. The influence of log diameter on average log temperature.

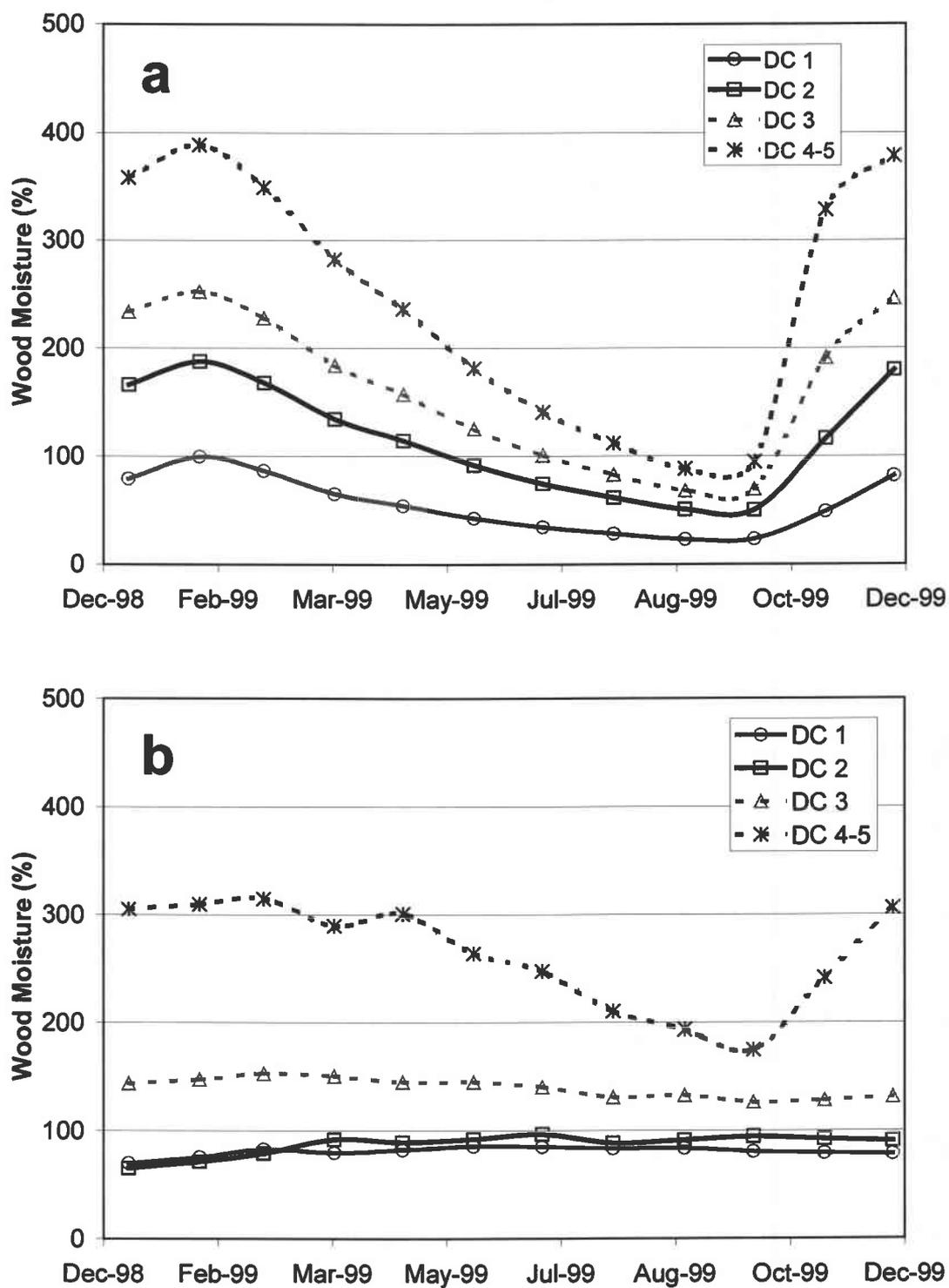


Figure 5.15. A comparison of wood moisture content from (a) a model and (b) field data.

throughout much of the year. Harmon and Sexton (1995) also found little seasonal variation in the moisture content of sound heartwood. The wetting and moisture diffusion characteristics of sound heartwood may explain the discrepancy between modeled and actual data.

Table 5.4. Average annual log moisture content and oxygen concentration by decay class for field data and model results.

Decay Class	Log Moisture Content (%)		Log Oxygen Concentration (%)	
	Actual	Model	Actual	Model
1	81	56	15.2	14.3
2	87	116	16.6	15.9
3	140	161	19.7	20.1
4	254	244	20.6	20.6
5	274	251	20.8	20.4

Average and seasonal oxygen concentrations were similar for modeled and actual data from oxygen monitoring tubes placed in logs at Wind River Experimental Forest in Washington (Figure 5.13c, Figure 5.16, Table 5.4). Average log oxygen concentrations are very close in magnitude and increase with decay class for both actual and modeled data. The only differences of consequence occurred in decay class one logs where the model underestimates of oxygen concentration from Julian dates 10-100 could result in overestimates of nitrogen fixation. Respiration would not be affected, as it is not inhibited in our model until oxygen falls below 5%.

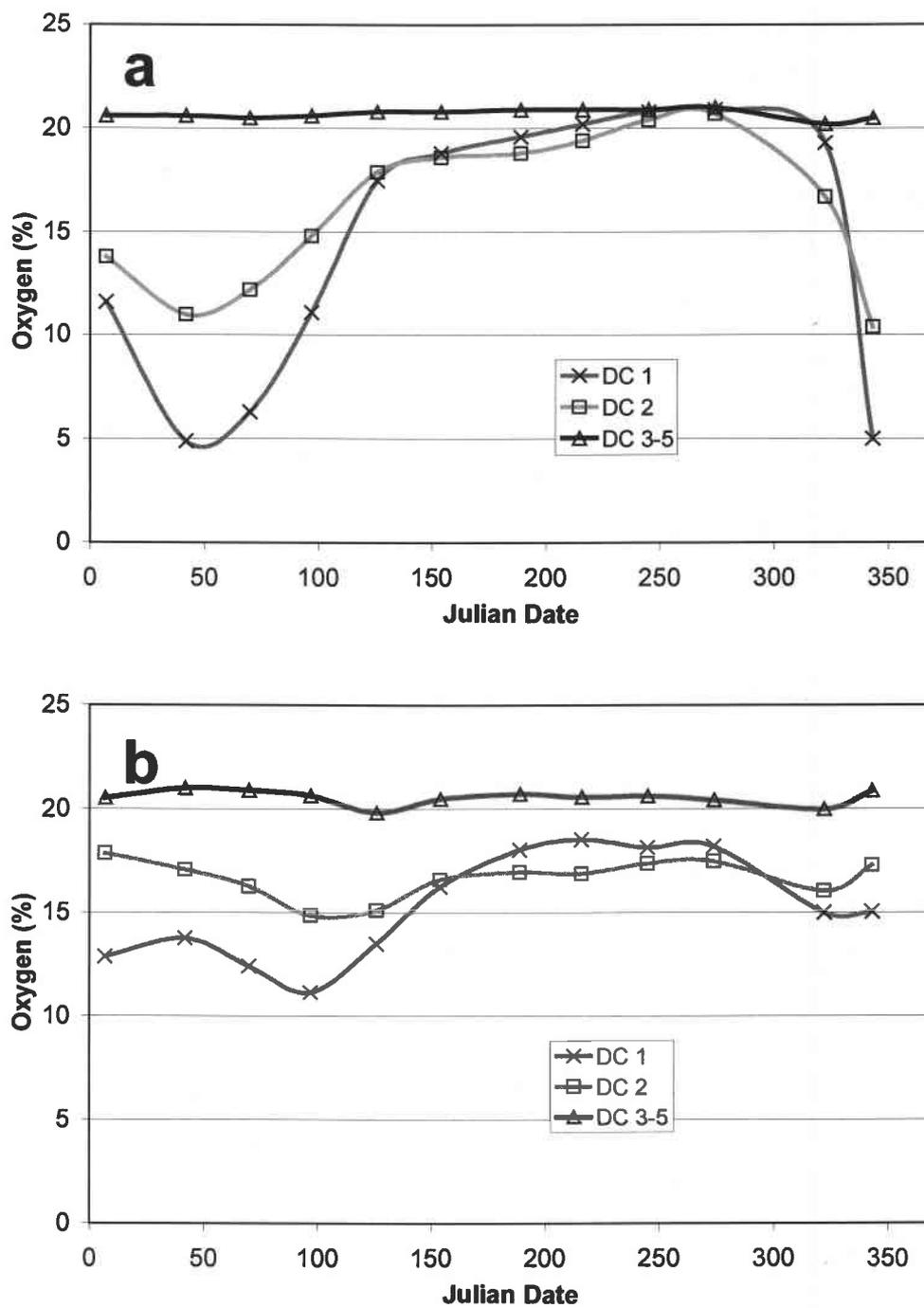


Figure 5.16. A comparison of log oxygen concentration from (a) model and (b) field data.

Model Experiments

Climate sensitivity

Nitrogen fixation rates in a decay class two *Tsuga heterophylla* log varied among the different sites (Table 5.3). The highest nitrogen fixation rate was in the warm and wet Cascade Head Experimental Forest, and the lowest fixation rate was in the cool and dry Pringle Falls Experimental Forest east of the Cascades. Despite the high annual temperature for Ashland, OR, nitrogen fixation rates were relatively low because of the dry climate in the Klamath Range. We would expect the highest nitrogen fixation rates in woody debris in the Pacific Northwest to be in warm, moist site near the coast such as Cascade Head. The model simulations indicate that dry interior sites east of the Cascades and in the Klamaths probably have the lowest rates of nitrogen fixation per gram of woody debris.

Predicted changes in the climate may affect nitrogen fixation rates. It is estimated that the Pacific Northwest will become warmer and drier (Hanson *et al.*, 1988). Their models indicate a temperature rise of 2-5°C in mean temperature and little change in precipitation. In addition, the annual pattern of relatively dry summers and mild, wet winters will persist.

In our simulation of climate change, log level nitrogen fixation rates in woody debris increased at all sites (Table 5.3). Increases were greatest at sites with abundant precipitation (*e.g.*, Cascade Head), while in the dry interior regions east of the Cascades and in the Klamaths rates only increased slightly (*e.g.*, Ashland and Pringle Falls). Changes in the amount of nitrogen fixed per hectare are more difficult to predict because

this depends on the amount of woody debris available. Changes in disturbance regime and tree productivity will undoubtedly affect woody debris biomass and these changes have the potential to alter nitrogen fixation rates to a greater degree than changes in temperature and precipitation (Franklin *et al.*, 1991).

Log level

Over the 200-year lifetime of the simulated *Pseudotsuga menziesii* log, 0.4 kg of nitrogen were fixed, which was equivalent to 28% of the initial amount of nitrogen in the log. Considering the limiting role nitrogen plays in wood decay (Cowling and Merrill, 1966), our results suggest that nitrogen fixation is playing a significant role in the nitrogen cycle and decomposition of logs.

Stand level

Nitrogen fixation rates at the stand level varied among the different sites. The annual amount of nitrogen fixed was highest at Cascade Head ($1.2 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$), followed by Wind River ($0.8 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$), and the H. J. Andrews ($0.7 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$). The warmer and wetter climate at Cascade Head probably explains most of the difference between Cascade Head and the other two sites as the biomass of logs and nitrogen fixation activity of species was not so different. These results are somewhat lower than two estimates of the amount of nitrogen fixed in woody debris at the H. J. Andrews of 1.0

kg N·ha⁻¹·yr⁻¹ and 1.4 kg N·ha⁻¹·yr⁻¹ by Sollins *et al.* (1987) and Silvester *et al.* (1982), respectively. Sollins *et al.* (1987) sampled the range of log species and decay classes to a much greater degree than Silvester *et al.* (1982) and is probably a more realistic estimate.

Relative Importance

The low annual rates of nitrogen fixation in woody debris we have predicted have to be evaluated in a successional and landscape context to understand the relative importance of this process as a nitrogen input. Although the maximum annual rates of symbiotic nitrogen fixers are higher, asymbiotic nitrogen fixers in wood and soil can contribute significant amounts of nitrogen because of their wide extent. Nitrogen fixation is carried out by asymbiotic microorganisms in wood, litter, and soil, and by microorganisms in symbiotic relationships with plants and other organisms. Asymbiotic nitrogen fixers generally contribute up to 1 kg N ha⁻¹ yr⁻¹, while symbiotic fixers such as *Alnus rubra* and *Ceanothus velutinus* can contribute 100 kg N ha⁻¹ yr⁻¹ or more (Table 5.5). However, symbiotic fixers are restricted to certain stages of succession and areas of the landscape, whereas asymbiotic fixers are not.

In our analysis, nitrogen inputs from *Alnus* and *Ceanothus* peak early in succession at 100 and 50 kg N ha⁻¹ yr⁻¹, respectively, then rapidly decline (Figure 5.17). These species fix nitrogen rapidly as biomass increases initially. Then biomass and fixation rates decline rapidly as the species are shaded and are outcompeted by larger, longer-lived conifers. Lichen nitrogen inputs are assumed to begin after 100 years, rise to a maximum of 4 kg N ha⁻¹ yr⁻¹ at 200 years, and then remain constant (Neitlich, 1993).

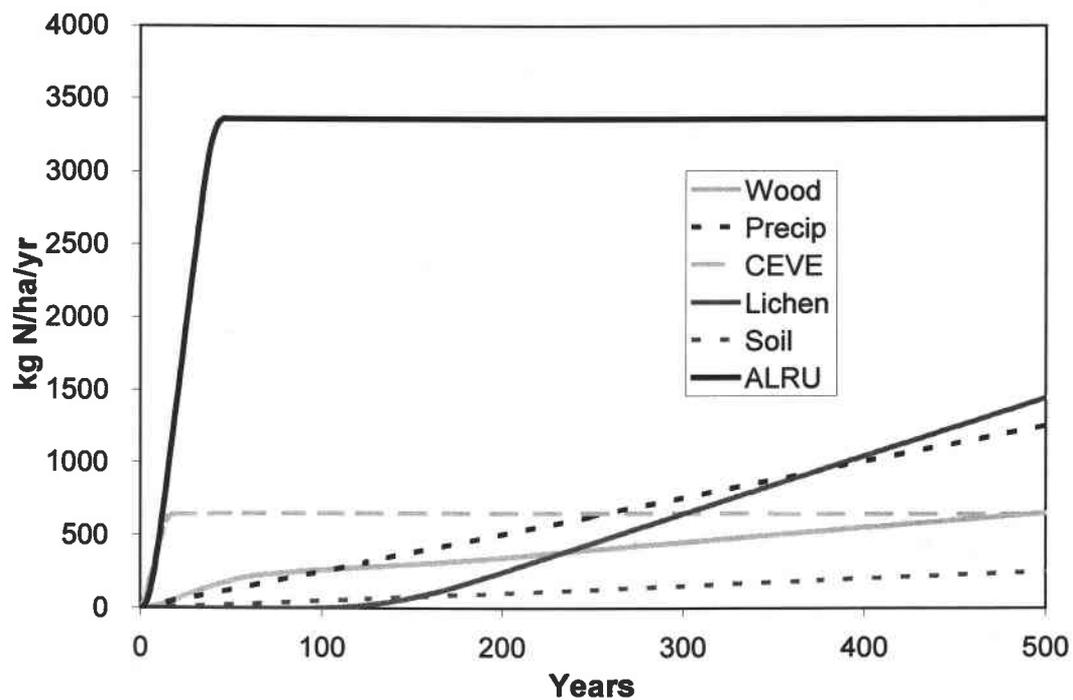


Figure 5.17. Cumulative nitrogen inputs to Pacific Northwest forests over 500 years of secondary succession.

Lobaria biomass follows this same pattern. Woody debris nitrogen inputs also follow the pattern of woody debris biomass with a peak near $4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ early in succession created by the death of the previous stand; a decrease as the initial wood mass decomposes and tree death is negligible; and a final leveling off at $1 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ as aging trees die and are replaced.

Table 5.5. A comparison of annual forest nitrogen inputs in the Pacific Northwest

Nitrogen Source	Annual Input ($\text{kg N ha}^{-1} \text{ yr}^{-1}$)	Reference
Precipitation and Dust	2-3	Sollins, 1980
Wood	1.0	Sollins <i>et al.</i> , 1987
Litter and Soil	0.5	Heath <i>et al.</i> , 1987
Lichens (<i>Lobaria oregana</i>)	0-21	Neitlich, 1993
<i>Alnus rubra</i>	0-200	Bormann <i>et al.</i> , 1994
<i>Ceanothus velutinus</i>	0-100	Conard <i>et al.</i> , 1985

During one cycle of secondary succession, a hypothetical stand in the central Cascades of Oregon received $7090 \text{ kg N ha}^{-1}$ over 500 years from the following sources: precipitation and dry deposition (18%); symbiotic fixation by *Alnus rubra* (48%) and by lichens (21%); and asymbiotic fixation in woody debris (9%) and in soil and litter (4%). In stands without symbiotic fixers, however, asymbiotic inputs provide 42% of total nitrogen inputs. Given that, across the landscape *Alnus rubra* occurs only in low elevation, recently disturbed sites; *Ceanothus velutinus* occurs primarily in high elevation, recently disturbed sites; and nitrogen fixing lichens occur in low elevation stands over 150 years old, their inputs at the landscape scale may be quite restricted

(Sollins *et al.*, 1987). Because asymbiotic nitrogen fixation occurs in all forests, their relative nitrogen contribution must be greater than maximum annual rates would suggest.

Conclusions

At this point, our model is primarily a synthesis and learning tool. Therefore our model is best used for examining relative differences, developing theory, synthesizing, and directing research. Our model is not recommended at this point for determining absolute values for nitrogen fixation rates. Current methods for estimating actual nitrogen fixation rates in woody debris are also limited in their absolute accuracy. Thus, any model of this process will be limited in this manner. However, as a synthesis and learning tool our model is a useful step towards understanding and predicting nitrogen fixation rates in dead wood and the controlling mechanisms.

Key areas for future research include further surveying of nitrogen fixation activity, and investigations of the processes that control oxygen diffusion and log moisture content. We need to verify the relationships of oxygen diffusion with wood density and moisture content. In addition, better methods for estimating evaporation and runoff of water from logs are needed.

Low-level chronic nitrogen inputs from asymbiotic nitrogen fixation in woody debris, soil, and litter may be important to nitrogen deficient Pacific NW forests. Managed stands with reduced levels of woody debris may be losing a significant nitrogen input.

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6. Summary

1. Nitrogen fixation and respiration in woody debris are influenced by wood temperature, moisture and oxygen concentration. Nitrogen fixation and respiration had similar responses to temperature with nitrogen fixation being optimum near 30°C and respiration being optimum over a broader range from 30°C to 50°C. Nitrogen fixation and respiration responded similarly to wood moisture content with little to no measurable activity below 50%, and optimal activity above 175% to 100% for nitrogen fixation and respiration, respectively. Nitrogen fixation rates were optimized at 2% O₂ with rates much reduced above and below this concentration. Respiration rates were optimal when O₂ exceeded 1%.

2. Past studies of nitrogen fixation in dead wood generally have used seasonal variations in temperature to predict the annual amounts of nitrogen fixed in woody debris, ignoring limitations of other abiotic factors. In our simulations, annual nitrogen fixation and respiration rates were 7.8 and 1.7 times greater, respectively, when only temperature limitations were included as compared to when all three abiotic controls were used. Therefore, seasonal interactions of abiotic factors need to be considered when estimating annual N₂ fixation and respiration rates.

3. The average AR: ¹⁵N₂ conversion ratio increased as acetylene reduction and ¹⁵N₂ fixation rates increased. For example, the average AR: ¹⁵N₂ ratio increased with temperature from 3.6 at 10°C to 4.9 at 30°C. Increased nitrogen fixation rates may result

in increased rates of inhibitory processes, such as hydrogen evolution, that can inhibit nitrogen fixation but not acetylene reduction.

4. Nitrogen fixation and respiration in woody debris were significantly influenced by the degree of decay of the wood, and the woody tissue type, but not by the species. Actual nitrogen fixation and respiration rates were significantly higher at a warmer, wetter coastal site when compared to two interior sites, but potential rates were not significantly different. Patterns of microbial colonization and abundance, resource quality, and climate probably explain most of the patterns observed in our study.

5. In both the radial and longitudinal directions, the oxygen diffusion coefficient (D_{O_2}) in wood increased exponentially as the fraction of pore space in air (FPSA) increased and as density decreased. D_{O_2} in the longitudinal direction was 1.4 to 34 times greater than for the radial direction.

6. In the field, mean O_2 concentrations in logs were not significantly different between species. In contrast, mean O_2 concentrations were significantly lower in decay class one and two logs as compared to decay class three through five logs. Higher density wood in decay class one and two logs probably explain these differences. Mean O_2 concentrations only decreased with radial depth in decay class two logs.

7. Seasonal field O₂ levels did not consistently vary with log moisture, respiration, or air temperature. Low O₂ concentrations in observed in November 1998 may result from increased precipitation following the summer drought.

8. The comparison of the results from our model of oxygen diffusion in the radial direction and field data indicate that *in vivo* measurements of radial oxygen diffusion underestimate field oxygen concentrations and diffusion rates. Cracks and passages in decay class five logs and longitudinal oxygen diffusion in decay class one logs may account for this discrepancy.

9. In our logs, oxygen concentrations were rarely as low as 2%, indicating anaerobic conditions are not as common in logs as we previously thought. Oxygen limitations on decomposition may occur in relatively sound and/or water soaked wood, but probably not in decayed logs in a terrestrial setting.

10. Uncertainty analysis of our model of nitrogen fixation in woody debris indicates that the focus of future research should be on improving estimates of the maximum nitrogen fixation rate, parameters involved in regulating log moisture content, and parameters involved in estimating oxygen diffusion rates.

11. In comparison to independent data, our model reasonably estimated seasonal patterns of log temperature, moisture, oxygen content, and respiration.

12. Our model estimates an annual nitrogen fixation rate of $0.7 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ for an old-growth stand at the H. J. Andrews, which is reasonably close to an independent estimate of $1.0 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ made for the same stand. Model output indicates that a decay class two, *Tsuga heterophylla* log fixes the most nitrogen in warm wet sites such as those near the coast, and the least in dry sites east of the Cascades and in the Klamath Range.

Raising the annual temperature by 2°C and decreasing precipitation by 10% caused nitrogen fixation rates to increase at all sites. Increases were greatest in warm wet sites and least in dry sites.

13. Despite low annual rates of asymbiotic nitrogen fixation in wood, soil, and litter, asymbiotic nitrogen fixation can contribute 9% to 42% of a stands nitrogen inputs over succession when symbiotic fixers such as *Alnus rubra* and *Lobaria oregana* are present and absent, respectively. Managed stands with reduced levels of woody debris and litter may be losing a significant nitrogen input.

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