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Abiotic controls on nitrogen fixation and respiration in selected woody debris from the Pacific Northwest, U.S.A.[1]

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Abstract: We estimated the effects of temperature, moisture, and oxygen concentration on nitrogen fixation and respiration in woody debris and used this information to model seasonal variation in these processes. We measured acetylene reduction and CO_2 evolution of wood samples to determine the relative effect of these abiotic factors on nitrogen fixation and respiration. The interactions of these abiotic factors were examined in a model to test whether temperature alone can be used as a predictor of seasonal changes in nitrogen fixation and respiration rates in woody debris. Nitrogen fixation rates were optimum near 30°C, whereas respiration rates were optimum over a broader range, from 30°C to 50°C. Nitrogen fixation and respiration rates were greatest above 175% and 100% wood moisture content, respectively, with little activity below 50%. Nitrogen fixation was optimum at 2% O_2, with activity much reduced above and below this concentration. Respiration was optimal when O_2 exceeded 1%. In our simulations, annual nitrogen fixation and respiration rates were 7.8 and 1.7 times greater, respectively, when only temperature limitation was included than when moisture and oxygen limitations were also included. Therefore, seasonal interactions of abiotic factors need to be considered when estimating annual nitrogen fixation and respiration rates.
Keywords: asymbiotic nitrogen fixation, respiration, woody debris.

Résumé : Nous avons estimé les effets de la température, de l'humidité et de la concentration en oxygène sur la fixation d'azote et la respiration dans des débris ligneux afin d'utiliser ces informations pour modéliser les variations saisonnières de ces processus. Nous avons mesuré la réduction de l'acétylène et l'évolution des teneurs en CO_2 sur des échantillons de bois pour déterminer l'effet de ces facteurs abiotiques sur la fixation de l'azote et la respiration. Les interactions des facteurs abiotiques ont été examinées à l'aide d'un modèle permettant de vérifier si la température seule peut servir à prédire les changements saisonniers des taux de fixation d'azote et de respiration dans les débris ligneux. Les meilleurs taux de fixation d'azote ont été observés à une température voisine de 30°C, alors que les taux optimaux de respiration sont survenus sur une gamme plus étendue de températures, soit entre 30°C et 50°C. Les plus hauts taux de fixation d'azote et de respiration ont été obtenus lorsque l'humidité des débris ligneux était respectivement supérieure à 175 et 100 %, l'activité étant faible à moins de 50 % d'humidité. La fixation de l'azote était optimale à 2 % d'O_2; de part et d'autre de cette concentration, l'activité était sensiblement réduite. Quant à la respiration, c'est à une concentration d'oxygène de plus de 1 % qu'elle se faisait le mieux. Dans les simulations où la température est le seul facteur limitatif, le taux de fixation annuelle d'azote était 7,8 fois supérieur et celui de la respiration 1,7 fois plus élevé que dans les simulations incluant aussi comme facteurs limitatifs l'humidité et l'oxygène. Ainsi, il est important de tenir compte des interactions saisonnières des facteurs abiotiques dans l'estimation des taux de fixation annuelle d'azote et de respiration.
Mots-clés : fixation asymbiotique d'azote, respiration, débris ligneux.

Nomenclature: Hitchcock & Cronquist, 1973.

Introduction

In the highly productive forest ecosystems of the Pacific Northwest, both tree and fungal growth are limited by the lack of available nitrogen (Cowling & Merrill, 1966; Gessel, Cole & Steinbrenner, 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 potential annual inputs from symbiotic nitrogen fixation (Cromack, Delwiche & McNabb, 1979). 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, Delwiche & McNabb, 1979; Sollins *et al.*, 1987; Hicks, 2000).

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 & Feller, 1989; Wei & Kimmins, 1998). 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. Sharp (1975) measured the effect of temperature from 15°C to 55°C on nitrogen fixation in decayed *Fagus sylvatica* (European beech) 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 acety-

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lene reduction and *Pinus contorta* (lodgepole pine) wood moisture at contents below 90%. In a study of nitrogen fixation in *Pseudotsuga menziesii* (Douglas-fir) 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 increase nitrogen fixation rates significantly 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 effects of temperature, moisture, and oxygen concentration on respiration than on nitrogen fixation in woody debris; however, this work did not involve the substrates and range of environmental conditions found in Pacific Northwest woody debris (Jensen, 1967; Griffin, 1977; Boddy, 1983; Scheffer, 1986). 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). The response of respiration to temperature is similar to that of nitrogen fixation, but with a slightly higher optimum (Boddy, 1983; Chen *et al.*, 2000).

The objective of this study was to estimate equation parameters that relate the effect of temperature, moisture, and oxygen concentration to nitrogen fixation and respiration rates in selected woody debris from the Pacific Northwest. We also examined the interactions of log temperature, moisture, and oxygen content in a model to demonstrate that annual estimates of nitrogen fixation may need to account for moisture and oxygen content as well as temperature.

Methods

STUDY AREA

Samples of woody debris were taken from the H. J. Andrews Experimental Forest and the 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 1,000 to 1,500 m by *Pseudotsuga menziesii* and *Tsuga heterophylla* (western hemlock; Franklin & Dyrness, 1988). Cascade Head is in the Oregon Coast Range and borders the Pacific Ocean. Forests are dominated by *Picea sitchensis* (Sitka spruce) and *Tsuga heterophylla* with Haplo-humult soils predominating in the area (Franklin & 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 (one cross-section was removed from each log sampled) 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 (approximately 4 mm × 4 mm × 70 mm) of the cross-sections were removed, placed in screw-topped culture tubes, and stoppered with serum bottle caps.

Subsamples from the cross-sections were screened for acetylene reduction and respiration activity prior to testing for abiotic effects. Portions of four cross-sections with moderate to high activity were then selected and sampled for subsequent tests of abiotic effects. Prescreening eliminated samples with rates near or below the limit of detection that would make it difficult to observe a response to an abiotic factor.

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. Sample moisture content was at least 100% and was not adjusted unless the effect of moisture content was being tested. Initial CO_2 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 the samples were incubated 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, as this was near the optimum observed by Silvester *et al.* (1982). Sample moisture was at least 100% and was not adjusted unless the effect of moisture content was being tested. Samples were incubated at 30°C for 24 hours. 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.* (1993) previously tested this method to check for effects from sample preparation time, oxygen concentration, incubation time, and air exposure. From these tests, they concluded that the method did not introduce significant experimental error. Acetylene reduction rates were verified on a subset of wood samples

using $^{15}N_2$, and these samples had an average acetylene reduced to $^{15}N_2$ fixed ratio of 4.4 (Hicks, 2000). We used the average ratio of 4.4 to convert acetylene reduction rates to nitrogen fixation rates.

After respiration and acetylene reduction were measured, the samples were weighed, dried at 80°C for 24 hours, 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%). For moisture contents, we created a range of moisture conditions by drying previously wetted wood in sealed containers over various amounts of Drierite® 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. 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).

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 (1986). 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 was an average of one to eight sub-samples depending on the number of samples that fell within each moisture range. Each of the woody 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

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

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:

$$Y = a - (a[1 - e^{-bX}]^c) \quad [2]$$

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:

$$Q10 = a*e^{(-b*X)} \quad [3]$$

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

$$Y = Q10^{(X - REFTEMP)/10} \quad [4]$$

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

SEASONAL INTERACTIONS

We examined the importance of including temperature, moisture, and oxygen effects when estimating seasonal nitrogen fixation rates by using a dead wood nitrogen fixation model developed with the response curves from this study (Hicks, 2000; available online at http://www.fsl.orst.edu/lter/pubs/webdocs/models/nfixwood.cfm). This model tracks daily nitrogen fixation and respiration rates in a log composed of five layers. Daily air 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.

When interpreting model results a word of caution is in order. We used this model to explore possible interactions between these factors. It was therefore primarily used as a heuristic as opposed to a predictive tool.

Results

TEMPERATURE RESPONSE

Nitrogen fixation and respiration had different responses to temperature (Figure 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 dropped more rapidly above 30°C than below. Both functions precisely fit the nitrogen fixation data (adjusted R^2 = 0.97 and 0.96, respectively; Table I). Respiration rates of *Pseudotsuga menziesii* bark and *Picea sitchensis* wood reached optimums at 40°C and 30°C respectively, while *Abies amabilis* (Pacific silver fir) wood respiration levelled off from 40°C to 65°C. Overall, the Chapman-Richard's equation provided a better fit to the respiration data than did the Q10 equation, having an adjusted R^2 of 0.86 compared to 0.76 for the Q10 equation (Table I). However, from 0°C to 30°C both equations fit the data equally well with an adjusted R^2 of 0.82.

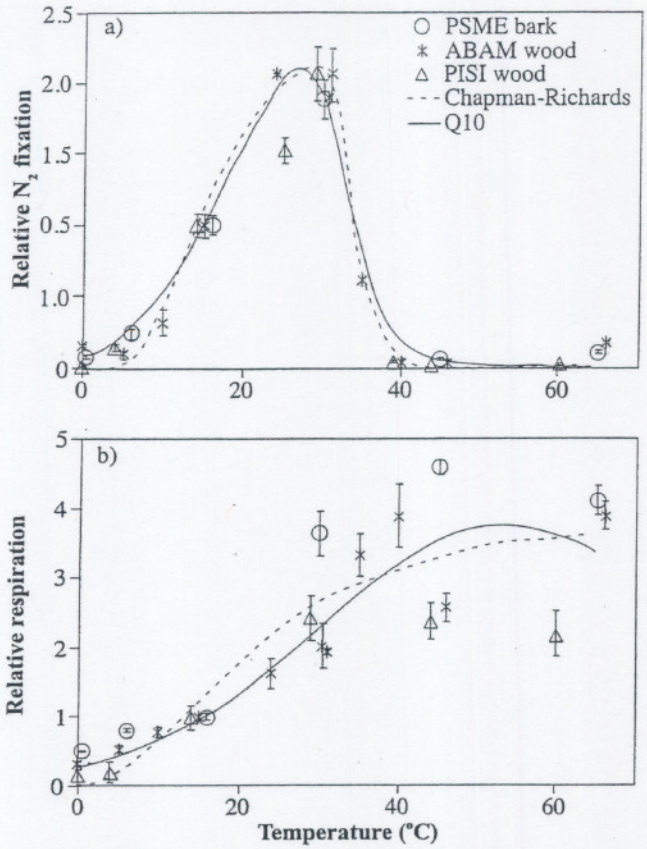

FIGURE 1. The effect of temperature on a) nitrogen fixation and b) respiration in *Pseudotsuga menziesii* (PSME) bark, *Abies amabilis* (ABAM) wood, and *Picea sitchensis* (PISI) wood. Error bars represent the standard error from the eight samples used at each temperature.

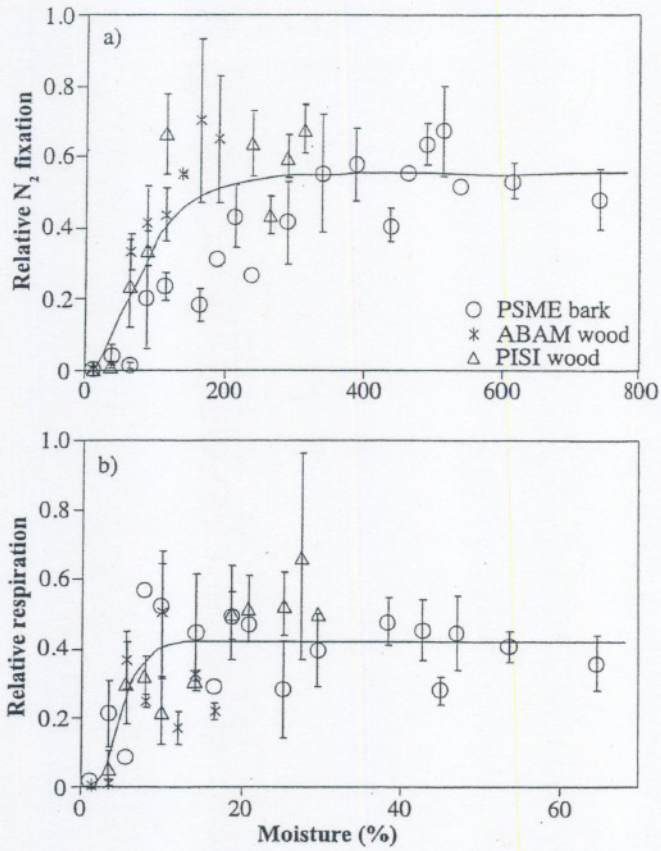

FIGURE 2. The effect of moisture on (a) nitrogen fixation and (b) respiration in *Abies amabilis* (ABAM) bark and wood and *Picea sitchensis* (PISI) wood. Error bars represent the standard error from the one to eight samples used at each moisture content range.

MOISTURE RESPONSE

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

OXYGEN RESPONSE

Nitrogen fixation and respiration rates responded differently to oxygen concentration (Figure 3a,b). Nitrogen fixation rates were optimum at 2% oxygen and decreased more steeply below this concentration than above. Respiration rates also rose rapidly, but 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 I).

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 declines during the warm, dry summer, while oxygen concentration increases, having an inverse relationship with wood moisture (Figure 4a). When temperature is the only abiotic vari-

TABLE I. 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.

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

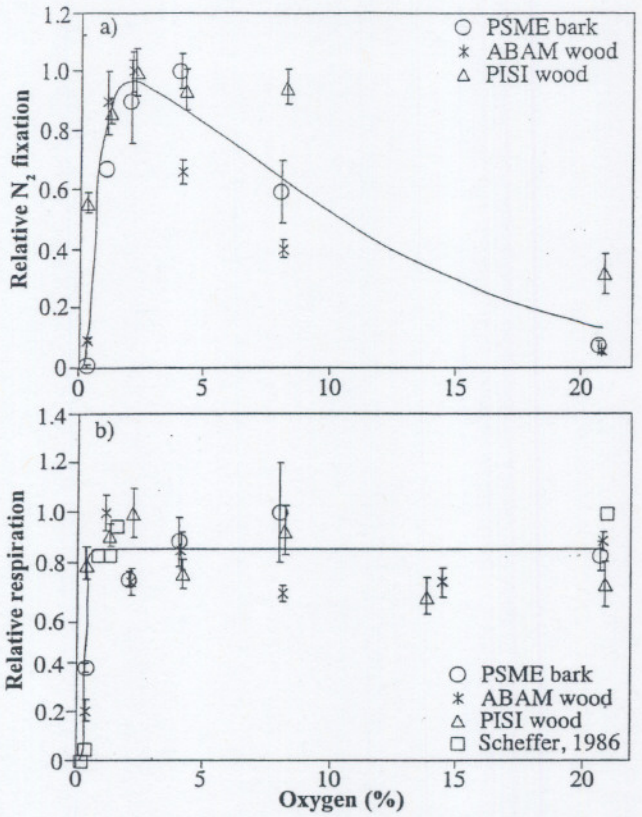

FIGURE 3. The effect of oxygen concentration on a) nitrogen fixation and b) respiration in *Pseudotsuga menziesii* (PSME) bark, *Abies amabilis* (ABAM) wood, and *Picea sitchensis* (PISI) wood. Error bars represent the standard error from the eight samples used at each oxygen concentration. The Scheffer (1985) data were not used to fit the curve, but are displayed for comparative purposes.

able controlling nitrogen fixation in the model, daily rates closely track temperature changes (Figure 4b). When 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 II). When moisture and temperature are included in the simulation, annual nitrogen fixation rates are about one-third the rates when only temperature is included. When all abiotic controls are included, fixation rates are nearly eight-fold lower than the rates when only temperature is included. Annual respiration is less sensitive than nitrogen fixation to the inclusion of moisture and oxygen in the simulations, because respiration is less inhibited than nitrogen fixation at low wood moisture contents and oxygen concentrations.

Discussion

TEMPERATURE RESPONSE

Both the Chapman-Richard's and modified Q10 equations provided a precise fit to the nitrogen fixation data

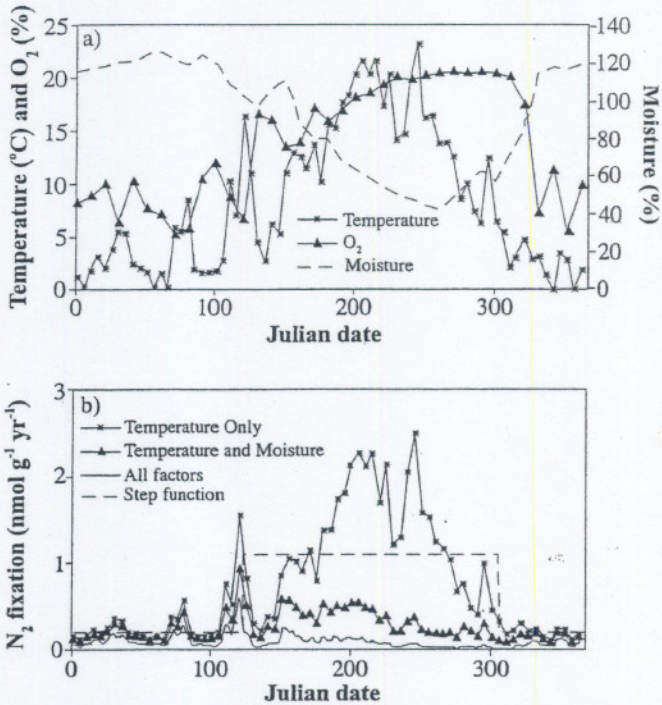

FIGURE 4. Seasonal changes in a) average log temperature, moisture, and oxygen content and b) nitrogen fixation rates from a simulation model using a 50-cm diameter, decay-class-one *Tsuga heterophylla* log and meteorological data from the H. J. Andrews Experimental Forest.

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

Abiotic Factors Included	Nitrogen Fixation ($nmol \cdot g^{-1} \cdot yr^{-1}$)	Respiration ($\mu mol \cdot g^{-1} \cdot yr^{-1}$)
Temperature	273.8	168.2
Temperature & Moisture	97.2	103.1
Temperature, Moisture & Oxygen	35.3	102.9

(Table I); however, the Q10 function has been widely used and is slightly easier to interpret. While the Chapman-Richard's equation provided a better overall fit to the respiration data (Table I), the modified Q10 equation did an equal or better job of modeling the temperature data from 0°C to 30°C. In addition, the modified Q10 equation declined above 50°C. Even though the data did not demonstrate an obvious decline, respiration rates must cease eventually as temperature increases, and the modified Q10 function is therefore more physiologically realistic than the Chapman-Richard's function, which did not decrease above the optimum temperature. In the Pacific Northwest, wood temperatures rarely exceed 30°C except in settings such as clearcuts, 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 were 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 sylvatica* veneers and found rates to be highest at 35°C. The response of nitrogen fixation in litter to temperature has also been studied. 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 drop sharply above 27°C. O'Connell and Grove (1987) measured the response of nitrogen fixation in litter from southwestern Australia to temperature and found rates to peak at 25°C and drop sharply above 28°C. The optimal temperature for nitrogen fixation appears to be lower for litter than for woody debris.

Our results for the response of respiration to temperature were 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 *Pseudotsuga 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).

In this study the response of wood respiration to temperature, particularly for the *Abies amabilis* samples, was somewhat different than other observed responses. Respiration rates in *Abies amabilis* 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 minutes) 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 $mol \cdot g^{-1} \cdot hr^{-1}$) and similar at 30, 50, and 60°C (0.9-1.0 $mol \cdot g^{-1} \cdot hr^{-1}$). Marra and Edmonds (1996) have observed temperatures over 55°C in woody debris from clearcuts in western Washington. Thus, a Q10 response does not occur above 40°C, and some 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 I). 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 I), because this function describes an average response and is theoretically realistic.

Our results for the response of nitrogen fixation to moisture were 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 to 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 100-200% moisture content.

The general response of respiration to moisture is similar to the response of nitrogen fixation, except under conditions where oxygen diffusion may be limiting. Respiration rate can decline at high moisture contents as the result of reduced oxygen diffusion rates.

Our results for the response of respiration to moisture were similar to those found in other studies. Boddy (1983) measured the response of respiration to moisture content in branch dead wood and found respiration rates to cease below 30% moisture content and increase from 30% to an optimum. 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% O_2, 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, although the adjusted R^2 for the respiration response is somewhat low (Table I). Both functions capture the average response of the data and are theoretically realistic.

The response of nitrogen fixation to oxygen concentration we observed was 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 we observed were 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 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 3b). Therefore, we feel this curve does an adequate job of capturing the average response of respiration from several woody substrates and fungal species from the Pacific Northwest.

SEASONAL INTERACTIONS

In the Pacific Northwest, wood temperature, moisture, and oxygen content fluctuate throughout the year (Harmon & Sexton, 1995; Hicks, 2000). 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 rates (Hicks, 2000; Figure 4).

Using temperature as the only variable to control nitrogen fixation rates could produce 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 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 are greatly limited by moisture and oxygen conditions, annual nitrogen fixation and respiration rates will be over- or underestimated, respectively.

Conclusion

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 of physical processes in wood 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.

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Literature cited

Boddy, L., 1983. Carbon dioxide release from decomposing wood: Effect of water content and temperature. Soil Biology and Biochemistry, 15: 501-510.

Chen, H., M. E. Harmon, R. P. Griffiths & W. Hicks, 2000. Effects of temperature and moisture on carbon respired from decomposing woody roots. Forest Ecology and Management, 138: 51-64.

Cowling, E. B. & W. Merrill, 1966. Nitrogen and its role in wood deterioration. Canadian Journal of Botany, 44: 1539-1554.

Cromack, K., C. C. Delwiche & D. H. McNabb, 1979. Prospects and problems of nitrogen management using symbiotic nitrogen fixers. Pages 210-223 *in* J. C. Gordon, C. T. Wheeler & D. A. Perry (ed.). Proceedings of Symbiotic Nitrogen Fixation in the Management of Temperate Forests. Oregon State University, Corvallis, Oregon.

Cushon, G. H. & M. C. Feller, 1989. Asymbiotic nitrogen fixation and denitrification in a mature forest in coastal British Columbia. Canadian Journal of Forest Research, 19: 1194-2000.

Flanagan, P. W. & A. K. Veum, 1974. Relationships between respiration, weight loss, temperature, and moisture in organic residues on tundra. Pages 249-277 *in* A. J. Holding (ed.). Soil Organisms and Decomposition in Tundra. Tundra Biome Steering Committee, Stockholm.

Franklin, J. F. & C. T. Dyrness, 1988. Natural Vegetation of Oregon and Washington. 2nd edition. Oregon State University Press, Corvallis, Oregon.

Gessel, S. P., D. W. Cole & E. W. Steinbrenner, 1973. Nitrogen balances in forest ecosystems of the Pacific Northwest. Soil Biology and Biochemistry, 5: 19-34.

Griffin, D. W., 1977. Water potential and wood-decay fungi. Annual Review of Phytopathology, 15: 319-329.

Griffiths, R. P., M. E. Harmon, B. A. Caldwell & S. E. Carpenter, 1993. Acetylene reduction in conifer logs during early stages of decomposition. Plant and Soil, 148: 53-61.

Harmon, M. E. & J. Sexton, 1995. Water balance of conifer logs in early stages of decomposition. Plant and Soil, 172: 141-152.

Harmon, M. E., J. F. Franklin, F. J. Swanson, P. Sollins, S. V. Gregory, J. D. Lattin, N. H. Anderson, S. P. Cline, N. G. Aumen, J. R. Sedell, G. W. Lienkaemper, K. Cromack, Jr. & K. W. Cummins, 1986. Ecology of coarse woody debris in temperate ecosystems. Advances in Ecological Research, 15: 133-302.

Heath, B., P. Sollins, D. A. Perry & K. Cromack, Jr., 1988. Asymbiotic nitrogen fixation in litter from Pacific Northwest forests. Canadian Journal of Forest Research, 18: 68-74.

Hendrickson, O. Q., 1991. Abundance and activity of nitrogen-fixing bacteria in decaying wood. Canadian Journal of Forest Research, 21: 1299-1304.

Hitchcock, C. L., & A. Cronquist, 1973. Flora of the Pacific Northwest. University of Washington Press, Seattle, Washington.

Hicks, W. T., 2000. Modeling nitrogen fixation in dead wood. Ph.D. thesis. Oregon State University, Corvallis, Oregon.

Highley, T. L., S. S. Bar-Lev, T. K. Kirk & M. J. Larsen, 1983. Influence of O_2 and CO_2 on wood decay by heartrot and saprot fungi. Phytopathology, 73: 630-633.

Jensen, K. F., 1967. Oxygen and carbon dioxide affect the growth of wood-decaying fungi. Forest Science, 13: 384-389.

Jurgensen, M. F., M. J. Larsen, S. D. Spano, A. E. Harvey & M. R. Gale, 1984. Nitrogen fixation associated with increased wood decay in Douglas-fir residue. Forest Science, 30: 1038-1044.

Marra, M. L. & R. L. Edmonds, 1996. Coarse woody debris and soil respiration in a clearcut on the Olympic Peninsula, Washington, U.S.A. Canadian Journal of Forest Research, 26: 1337-1345.

Moore, A. E., 1986. Temperature and moisture dependence of decomposition rates of hardwood and coniferous leaf litter. Soil Biology and Biochemistry, 18: 427-435.

O'Connell, A. M., 1990. Microbial decomposition of litter in eucalyptus forests of southwestern Australia: An empirical model based on laboratory incubations. Soil Biology and Biochemistry, 22: 153-160.

O'Connell, A. M. & T. S. Grove, 1987. Seasonal variation in C_2H_2 reduction (N_2-fixation) in the litter layer of eucalypt forests of southwestern Australia. Soil Biology and Biochemistry 19: 135-142.

Roskoski, J. P., 1981. Nitrogen fixation in hardwood forests of the Northeastern United States. Plant and Soil, 34: 33-44.

SAS, 1986. SAS Language Guide for Personal Computers, Version 6. SAS Institute Inc., Cary, North Carolina.

Scheffer, T. C., 1986. Oxygen requirements for growth and survival of sapwood-staining fungi. Canadian Journal of Botany, 64: 1957-1963.

Sharp, R. F., 1975. Nitrogen fixation in deteriorating wood: The incorporation of $^{15}N_2$ and the effect of environmental conditions on acetylene reduction. Soil Biology and Biochemistry, 7: 9-14.

Silvester, W. B., 1989. Molybdenum limitation of asymbiotic nitrogen fixation in forests of Pacific Northwest America. Soil Biology and Biochemistry, 21: 283-289.

Silvester, W. B., P. Sollins, T. Verhoeven & S. P. Cline, 1982. Nitrogen fixation and acetylene reduction in decaying conifer boles: Effects of incubation time, aeration, and moisture content. Canadian Journal of Forest Research, 12: 646-652.

Sit, V. & M. Poulin-Costello, 1994. Catalog of Curves for Curve Fitting. Research Branch, Ministry of Forests, Victoria, British Columbia.

Sollins, P., S. P. Cline, T. Verhoeven, D. Sachs & G. Spycher, 1987. Patterns of log decay in old-growth Douglas-fir forests. Canadian Journal of Forest Research, 17: 1585-1595.

Spano, S. D., M. F. Jurgensen, M. J. Larsen & A. E. Harvey, 1982. Nitrogen-fixing bacteria in Douglas-fir residue decayed by *Fomitopsis pinicola*. Plant and Soil, 68: 117-123.

Wei, X. & J. P. Kimmins, 1998. Asymbiotic nitrogen fixation in harvested and wildfire-killed lodgepole pine forests in the central forests of British Columbia. Forest Ecology and Management, 109: 343-353.