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Substrate controls on nitrogen fixation and respiration in woody debris from the Pacific Northwest, USA

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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 three sites in 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 using acetylene reduction and CO_2 evolution, respectively. We also directly measured nitrogen fixation with $^{15}N_2$ on a subset of samples to determine an average actylene reduced to $^{15}N_2$ fixed (AR:$^{15}N_2$) ratio of 4.4. Over the range of wood decay examined, actual and potential nitrogen fixation and respiration rates peaked in moderately decayed wood. 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* (Mirb.) Franco, *Tsuga heterophylla* (Ref.) Sarg., or *Picea sitchensis* (Bong.) Carr. for nitrogen fixation or respiration. Nitrogen fixation and respiration rates were highest in bark, lower in sapwood, and lowest in heartwood. Patterns of microbial colonization and abundance, resource quality, and climate probably explain most of the patterns observed in our study.

Keywords: Woody debris; Nitrogen fixation; Respiration

1. 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 and Merrill, 1966; Gessel et al., 1973; Spano et al., 1982; Bormann et al., 1994). Nitrogen fixation is an important source 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; Hicks, 2000).

Many substrate 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,

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Sollins et al. (1987) found no significant differences between the three species they examined. Griffiths et al. (1993) found that nitrogen fixation rates varied with wood tissue during the first 6 years of decomposition with the highest to lowest rates found in inner bark, outer bark, sapwood, and heartwood, respectively. Larsen et al. (1978); Jurgensen et al. (1984) found nitrogen fixation rates increased as wood decay progressed, whereas Harvey et al. (1989); 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 substrate 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* (Douglas-fir), *Tsuga heterophylla* (western hemlock), and *Thuja plicata* Donn (western redcedar) when examining the entire range of wood decay; however, Carpenter et al. (1988) found *P. menziesii* to have higher respiration rates than *T. 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 to 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.

Our primary objective 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. To our knowledge, this study is the most comprehensive examination of substrate level influences on nitrogen fixation and respiration in dead wood over a broad geographic range. Effects of other substrate factors such as decomposer species were not investigated because they are considered to be of secondary importance. Also, these factors are incorporated into the samples used for this study. The rates measured in this paper were used to parameterize a nitrogen fixation simulation model (Hicks, 2000).

2. Methods

2.1. Study area

Samples of woody debris were taken from sites in 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 to 1500 m by *P. menziesii* and *T. heterophylla* (Franklin and Dyrness, 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 *P. menziesii* and *T. 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 *P. sitchensis* and *T. heterophylla* with Haplohumult soils predominating in the area (Franklin and Dyrness, 1988).

2.2. 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 sampled *P. menziesii*, *T. heterophylla*,

Abies amabilis (Dougl.) Forbes (Pacific silver fir), and *Thuja plicata* logs at the H.J. Andrews Experimental Forest; *P. menziesii* and *T. heterophylla* logs at the Wind River Experimental Forest; and *P. sitchensis* (Sitka spruce) and *T. heterophylla* logs at Cascade Head. Logs that could be identified to the species level were selected randomly from within each site. Logs were assigned to a decay class with decay class one being least decayed and five the most decayed (Harmon and Sexton, 1996). A single cross section was cut from the middle of each log sampled. A total of 93 logs were sampled at the three sites with at least two replicates for each combination of species and decay class. 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 6 years of wood decay at the H.J. Andrews Experimental Forest. Subsequent unpublished resampling has extended this database to cover the first 12 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 (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. Four subsamples were removed from each cross section and the results from these four subsamples were averaged to produce the log level result used in statistical analysis. "Actual" acetylene reduction and respiration measurements were started within 24 h 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 that 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 min to allow them to adjust to the incubation environment. Samples for respiration tests were incubated in lab air at 15 °C. 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. A final reading was taken after incubating for at least 2 h.

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. (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.

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, and expressing results as a percent of oven dry weight.

To convert acetylene reduction data to the actual amount of nitrogen fixed, we directly measured nitrogen fixation with $^{15}N_2$ on a subset of samples ($n = 24$) and calculated the AR:$^{15}N_2$ ratio. Acetylene reduction rates, using the above methods, were first measured on two different substrates (*A. amabilis* and *P. 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 at.% $^{15}N_2$ was added to produce a headspace with 14 at.% $^{15}N_2$ except in the control wood samples that received no $^{15}N_2$. Wood samples were incubated for 2 days then removed, ground, and analyzed with a mass spectrometer to get the absolute and relative amounts of the nitrogen isotopes in the samples. We assumed activity rates were constant during the $^{15}N_2$ incubation periods. Initial and final headspace samples were also taken for nitrogen isotope ratio measurement to determine $^{15}N_2$ headspace concentrations and leakage rates. We used the average ratio for all samples (4.4) when converting AR values to dinitrogen fixed.

2.3. 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 (1986). 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 12 years of log decay. Only data from years nine through 12 were used to avoid periods early in decay when wood tissues were not fully colonized by decomposers and nitrogen fixers. The sample size for testing tissue level effects was 64.

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 <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

Table 1
P-values and significance of the means for each of the independent variables for the different experiments from ANOVA and ANCOVA tests

Dependent variable	Independent variable	P-values	
		ANOVA	ANCOVA
AR:$^{15}N_2$ ratio	Species	<0.001[a]	
	Temperature	0.024[a]	
Potential N_2 fixation	Decay class	0.008[a]	
	Site	0.068	
	Species	0.722	
Actual N_2 fixation	Decay class	0.046[a]	0.062
	Site	0.022[a]	0.023[a]
	Species	0.413	0.425
	Wood tissue[b]		<0.001[a]
Potential respiration	Decay class	0.001[a]	
	Site	0.407	
	Species	0.354	
Actual respiration	Decay class	0.014[a]	0.003[a]
	Site	0.004[a]	0.004[a]
	Species	0.181	0.187
	Wood tissue[b]		<0.001[a]
Difference of potential and actual N_2 fixation	Decay class	0.027[a]	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

The P-values for the covariate moisture were not included in the table.

[a]Means for independent variables are considered to be significantly different from each other when $P<0.05$.

[b]Data for estimating nitrogen fixation and respiration means for wood tissues are from Griffiths et al. (1993) and subsequent unreported resampling (see Section 2).

situations where confidence limits may overlap but not enough to include the mean being compared.

3. Results

3.1. AR:$^{15}N_2$ ratio

The AR:$^{15}N_2$ ratio significantly differed between the two wood species and among the three incubation temperatures (Table 1, Fig. 1). *Picea sitchensis* had a mean AR:$^{15}N_2$ ratio of 5.2 as compared to a ratio of 3.5 for *Abies amabilis*. The average AR:$^{15}N_2$ 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.

3.2. Degree of decay

Potential and actual nitrogen fixation rates peaked in moderately decayed wood and were lowest in the most decayed wood (Fig. 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 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 nitrogen 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 (Fig. 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 wood (Fig. 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 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 higher in decay classes one through three and lower in decay classes four and five (Fig. 3a).

3.3. Site

Potential nitrogen fixation rates did not significantly vary among the three sites. In contrast, actual rates were significantly different (Table 1, Fig. 2b). Average actual nitrogen fixation rates decreased from 0.45

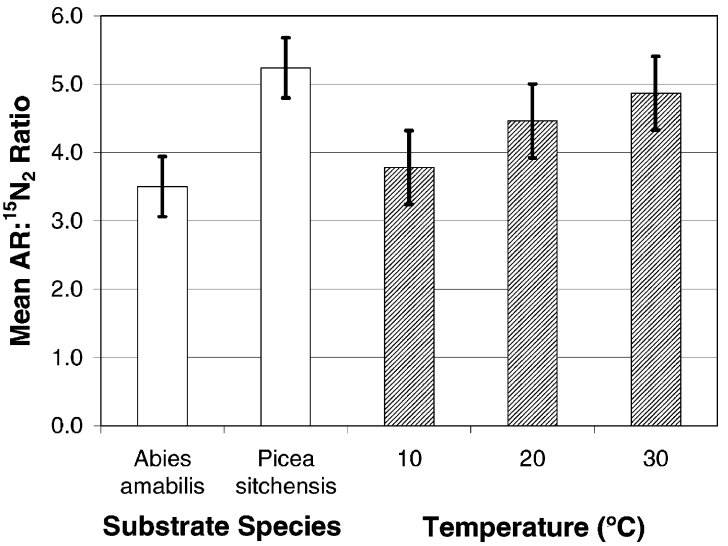

Fig. 1. The least squares mean of the ratio of acetylene reduced to dinitrogen fixed and 95% confidence limits for two different species of wood and three incubation temperatures.

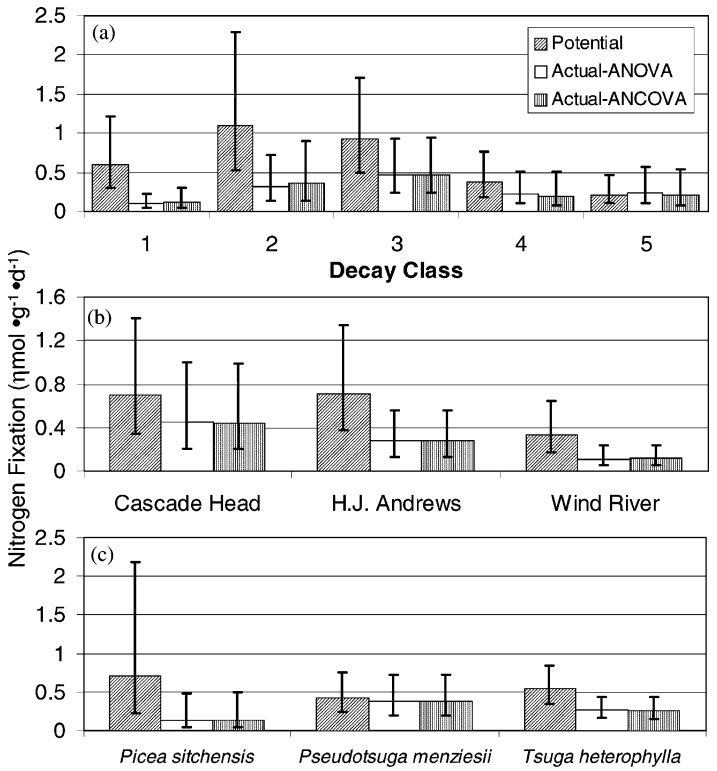

Fig. 2. Medians and 95% confidence limits for potential and actual nitrogen fixation rates for (a) five decay classes of wood where one is least and five most decayed; (b) three sites in the Pacific Northwest, and (c) three species of wood. For actual nitrogen fixation rates, medians from both the ANOVA and ANCOVA were reported. The ANOVA did not, while the ANCOVA did include moisture as a covariate.

ηmol g^{-1} d^{-1} at Cascade Head, to 0.27 ηmol g^{-1} d^{-1} at the H.J. Andrews, to 0.11 ηmol g^{-1} 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 μmol g^{-1} d^{-1} at Cascade Head, to 0.23 μmol g^{-1} d^{-1} at the H.J. Andrews, to 0.14 μmol g^{-1} d^{-1} at Wind River (Table 1, Fig. 3b).

3.4. Wood species

No significant differences were found among the three species examined for potential and actual nitrogen fixation and respiration rates (Table 1). *P. sitchensis* had the highest potential nitrogen fixation rates but the lowest actual rates (Fig. 2c). *T. heterophylla* had the highest potential respiration rates, while *P. menziesii* had the highest actual rates (Fig. 3c).

3.5. Woody tissues

Nitrogen fixation and respiration rates were significantly different among the four woody tissues (Fig. 4). Nitrogen fixation rates dropped from 0.72 ηmol g^{-1} d^{-1} in outer bark, to 0.57 ηmol g^{-1} d^{-1} in inner bark, to 0.24 ηmol g^{-1} d^{-1} in sapwood, to 0.09 ηmol g^{1} d^{-1} in heartwood. Respiration rates were highest in inner bark at 1.51 μmol g^{-1} d^{-1}, followed by outer bark at 1.14 μmol g^{-1} d^{-1}, then by sapwood at 0.88 μmolg^{-1} d^{-1}, and finally heartwood at 0.17 μmol g^{-1} d^{-1}.

3.6. Differences between actual and potential rates

Potential nitrogen fixation rates averaged 0.24 ηmol g^{-1} d^{-1} (86%) 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

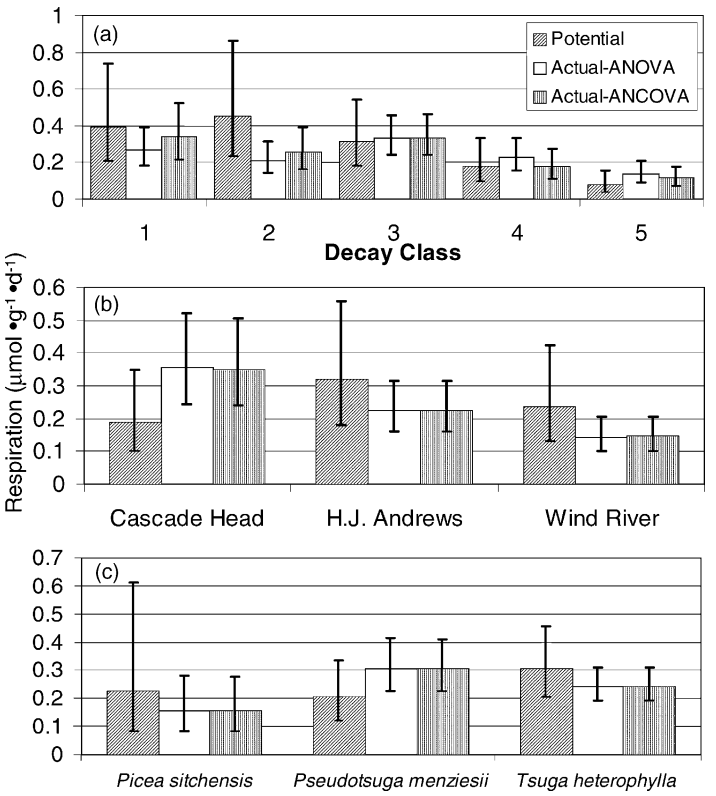

Fig. 3. Medians and 95% confidence limits for potential and actual respiration rates for (a) five decay classes of wood where one is least decayed and five most; (b) three sites in the Pacific Northwest, and (c) three species of wood. For actual respiration rates, medians from both the ANOVA and ANCOVA were reported. The ANOVA did, while the ANCOVA did not include moisture as a covariate.

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 1, Fig. 2a). There were no significant differences between potential and actual nitrogen fixation rates among the different sites and species tested (Table 1, Fig. 2b and c).

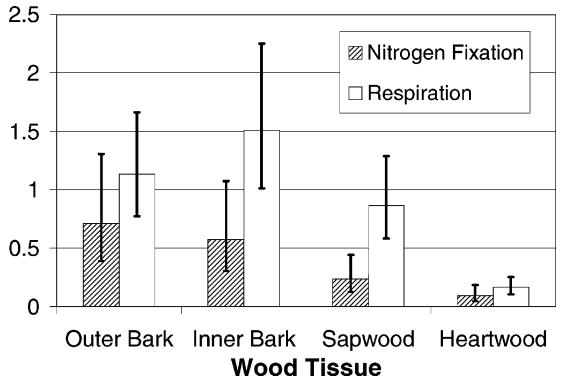

Fig. 4. Medians and 95% confidence limits for nitrogen fixation (ηmol g^{-1} d^{-1}) and respiration (µmol g^{-1} d^{-1}) rates for four wood tissues in years 9–12 of wood decay.

Potential respiration rates averaged 0.02 µmol g^{-1} d^{-1} (8%) 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 rates among the different decay classes, particularly in decay class two, but not when moisture was included as a covariate (Table 1, Fig. 3a). There were no significant differences between potential and actual respiration rates among the different sites and species tested (Table 1, Fig. 3b and c).

4. Discussion

4.1. AR:$^{15}N_2$ ratio

The differences in the AR:$^{15}N_2$ ratios between species and among the incubation temperatures share one striking similarity: the mean ratio increased as acetylene reduction and $^{15}N_2$ fixation rates increased. Average acetylene reduction and $^{15}N_2$ fixation rates were 2.4 and 1.7 times higher, respectively, for *P. sitchensis* when compared to *Abies amabilis*. For incubation temperature, acetylene reduction and $^{15}N_2$ 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:$^{15}N_2$ 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 $^{15}N_2$ fixation, such as those in *P. sitchensis* and at 30 °C, could 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 synthesis of amino acids that can affect nitrogenase (Hardy et al., 1973; Sprent, 1979). Because AR rates would not be affected by these inhibitory processes, AR:$^{15}N_2$ ratios should theoretically increase as nitrogen fixation activity increases.

In general, AR:$^{15}N_2$ ratios from studies of wood and soil are similar to the theoretical ratio of four to one (Bergersen, 1991). Hardy et al. (1973) found an average AR:$^{15}N_2$ 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 3.5–4.5 for incubations lasting 6 and 42 h, respectively. They hypothesized that the increase of the AR:$^{15}N_2$ ratio with time resulted from inhibition of nitrogen fixation by acetylene, and subsequent nitrogen depletion in the fixing organisms causing stimulation of nitrogenase activity. Roskoski (1981) found an unusually high AR:$^{15}N_2$ ratio of 8.5 for wood samples from the eastern deciduous forests of the United States. She measured AR and $^{15}N_2$ fixation on paired samples instead of the same sample and used a relatively long incubation period of 5 days. Using paired samples produced large variation in her data and long incubation periods are known to produce higher AR:$^{15}N_2$ ratios (Hardy et al., 1973; Silvester et al., 1982). In addition, the higher nitrogen fixation activity and the moisture conditions and size of her samples may have contributed to the relatively high ratio.

Our overall average AR:$^{15}N_2$ 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:$^{15}N_2$ ratio for 10 °C is 3.6. In addition, the substrates we used had relatively high nitrogen fixation rates. If higher fixation activity is associated with higher AR:$^{15}N_2$ 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 underestimates.

4.2. 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. First, nitrogen fixation and respiration rates are limited by colonization extent in early decay classes. 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. Second, log resource quality declines with increasing decay. It has long been noted that the rate of decomposition of litter and resource quality declines with time (Heal et al., 1997). Third, 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). Resource quality is highest in decay class one logs, 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); 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); 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 *P. menziesii* and *T. heterophylla* logs. The respiration rates they observed in *Thuja plicata*, which was not included in the species comparison portion of 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.

4.3. Site

Cascade Head Experimental Forest had the highest actual nitrogen fixation and respiration rates. The milder, wetter climate of Cascade Head on the Oregon Coast, as compared to Wind River or the H.J. Andrews from the interior Cascade Range may explain this difference. The mild climate of Cascade Head may prevent drastic declines of microorganism populations that may occur during the colder winters and dryer summers in the Cascade Range. The relatively low respiration and nitrogen fixation rates at Wind River are somewhat surprising, because 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 was 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.

4.4. Wood species

Although 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 *P. menziesii* and *A. amabilis* than in *T. plicata* and *T. heterophylla* at the H.J. Andrews during the first 6 years of log decay. Sollins et al. (1987) found no significant differences in nitrogen fixation or respiration rate among *P. menziesii*, *T. plicata*, and *T. 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).

4.5. Woody tissues

Tissue level patterns of nitrogen fixation during the 9–12 years of log decay were generally similar to patterns during the first 6 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 6 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 possibly the presence of extractives that inhibit decay (Harmon et al., 1986).

4.6. Differences between actual and potential rates

Higher potential as compared to actual nitrogen fixation rates were 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 microbial populations to adjust. The increase in activity was most pronounced in the difference between actual and potential nitrogen fixation rates in decay class one logs (Fig. 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 lower than actual rates for samples from Cascade Head, but this difference can adequately be explained by chance (Fig. 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 1; Fig. 3a).

The smaller difference between actual and potential respiration rates in comparison to the difference for nitrogen fixation rates may result from differential responses to wood moisture by these two processes. Hicks et al. (submitted for publication) found wood respiration rates to be less sensitive to low wood moisture as compared to nitrogen fixation rates. Many of the wood samples we measured had moisture contents in the 50–100% range where respiration is less inhibited than nitrogen fixation.

5. Conclusion

The mechanisms that control the variability in the AR:$^{15}N_2$ conversion ratio in woody debris are poorly understood. Mechanisms such as hydrogen evolution and end-product inhibition of nitrogen fixation may explain the positive correlation between the AR:$^{15}N_2$ conversion ratio and nitrogen fixation 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, 1981; 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.

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