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# Acetylene reduction in conifer logs during early stages of decomposition*

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**Abstract**

Acetylene reduction was examined periodically for as long as 68 months in the outer and inner bark, sapwood, and heartwood of decaying logs of western hemlock [*Tsuga heterophylla* (Raf.) Sarg.] western redcedar (*Thuja plicata* D. Don), Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco], and Pacific silver fir (*Abies amabilis* Dougl. ex Forbes) in the western Oregon Cascade Mountains. Tissues from freshly cut logs from sound trees were unable to reduce acetylene. However, after 18 months of decomposition, acetylene reduction was found in all log tissues except heartwood. Over the 68-month study period, no significant relationship between reduction rate and tissue moisture was found. Acetylene reduction rates differed significantly among tissues, log species, and time of exposure to decomposers. Although acetylene reduction generally showed a steady increase with time, tissues of some species showed a more complex, nonlinear pattern of change. Although the amount of nitrogen fixed is low compared to the total present in decaying logs, it might be an important source of readily available nitrogen for the microbiota responsible for decomposition.

## Introduction

Todd et al. (1975) estimated that as much as 75% of total nitrogen coming into temperate deciduous forests is from symbiotic and asymbiotic nitrogen fixation; Bormann et al. (1977) calculated the input at 68%. Granhall and Lindberg (1980) reported that from 5% to 10% of the nitrogen required by vegetation in temperate coniferous forests is supplied by nitrogen fixation, and Sollins et al. (1980) found that 58% of the nitrogen entering an old-growth Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] ecosystem came from nitrogen fixation by lichens. Such estimates suggest that the long-term productivity of temperate coniferous forests may depend on nitrogen fixation because nitrogen is, in most cases, the most limiting nutrient (Cole and Rapp, 1981).

Many studies show that a significant fraction of nitrogen comes from asymbiotic fixation in both living (Van der Kamp, 1986) and dead trees (Cornaby and Waide, 1973; Jurgensen et al., 1984, 1989; Larsen et al., 1978; Silvester et al., 1982; Weber and Sundman, 1986). The amount of nitrogen fixed in logs (downed, dead trees), may be important for microorganisms that degrade wood, although it may be small relative to the total nitrogen present. Of the variables affecting nitrogen-fixation rate in logs, the one most consistently and directly correlated with it is the state of decomposition (Cornaby and Waide, 1973); Jurgensen et al., 1984; Todd et al., 1975). In general, higher nitrogen-fixation rates are found in older wood, regardless of tree

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species. The type of decay fungi in decomposing logs also appears to effect the rates, which may be controlled by the availability of substrate produced during the decay process (Jurgensen et al., 1989). Most recent studies have examined only a few decay stages or used a chronosequence to infer temporal trends.

This study is part of a larger investigation being conducted within intact old-growth Pseudotsuga-Tsuga forests at the H.J. Andrews Experimental Forest, on the west slope of the Cascade Mountains in Oregon (Carpenter et al., 1988; Harmon, 1992; Kelsey and Harmon, 1989; Zhong and Schowalter, 1989). Two time-series studies of log decomposition that were installed in 1985 (Carpenter et al., 1988; Harmon, 1992) and 1987 (Kelsey and Harmon, 1989) provided us an opportunity to examine changes in acetylene reduction periodically over the first 68 months of log decomposition. In addition to acetylene reduction, temporal patterns of log moisture content, density, nutrient content, carbon chemistry, respiration and leaching loss, as well as the structure of invertebrate and fungal communities are being studied (Carpenter et al., 1988; Harmon, 1992; Kelsey and Harmon, 1989; Zhong and Schowalter, 1989). Thus, much published and unpublished data provide a larger context in which to place temporal patterns of acetylene reduction.

In this study, we sampled logs of four conifer species to determine yearly changes in nitrogen-fixation rates of asymbiotic nitrogen-fixing organisms (measured as acetylene reduction). Specifically, we examined acetylene reduction as a function of tree species, tissue type, time of log exposure to decomposers, and log moisture content.

## Methods and materials

### *Study area*

The logs used in this study are on Log Decomposition Site Number 1 (Harmon, 1992) in the H.J. Andrews Experimental Forest near Eugene, Oregon. Elevation at the site is 1065 m, mean annual temperature 8.9°C and mean annual precipitation 229 cm. The habitat type is classified as *Tsuga heterophylla-Abies amabilis/ Rhodendron macrophyllum-Berberis nervosa*. Soils are deep, well-drained Typic Dystrochrepts.

### *Field sampling*

The first time series, established in September 1985, is described in detail by Harmon (1992). Living trees of four species, western hemlock [*Tsuga heterophylla* (Raf.) Sarg.], western redcedar (*Thuja plicata* D. Don), Douglas-fir, and Pacific silver fir (*Abies amabilis* Dougl. ex Forbes), were felled within and near the H.J. Andrews Experimental Forest and cut into 500 logs (5.5 m long, 45–60 cm in diameter). The logs were then transported and evenly distributed on the forest floor at six old-growth study sites (4–10 ha each). Temporal patterns of nitrogen fixation have been studied at only one of those sites (Harmon, 1992). The second time series was established in March 1987 at Log Decomposition Site number 1 as part of an effort to characterize initial tannin, phenol, and pentane extractives of the four tree species (Kelsey and Harmon, 1989). One tree of each of the four species was felled next to Log Decomposition Site Number 1, and the boles were bucked into 5.5 m lengths. Diameters of these logs were similar to those in the first series.

The initial condition of each log was characterized at the beginning of each time series (see Harmon, 1992 for methods) by total volume and volume and density of each tissue type (i.e., outer bark, inner bark, sapwood, heartwood). Each year following establishment of the study, a subsample of logs has been examined for changes in these variables.

Long-term temporal patterns of acetylene reduction were examined in cross sections from logs in the first time series at 18, 22, 30, 56, and 68 months after felling, and in the second time series at 0, 4, 24, 36, and 48 months after felling. All except the 4- and 22-month samples were taken during spring (March to May). The 4- and 22-month samples were taken in July.

At each sampling time, two cross sections approximately 10 cm thick were cut from each log and transported in plastic bags to a laboratory at Oregon State University within 6 h of

cutting. Cross sections were stored there in plastic bags at 5°C until subsamples could be taken (within 2 days).

*Laboratory procedures*

In the laboratory, each cross section was cut into three pie-shaped segments for removal of pieces of outer and inner bark, sapwood, and heartwood. Pieces of each tissue were cut into match-sized pieces approximately 100 mm long and transferred to covered plastic trays until samples were prepared for incubation. Two g (wet wt) of wood or bark were placed into screw-topped culture tubes (20 × 125 mm) were then stoppered with serum bottle caps. Samples were processed serially rather than in batches so that the timing for each was the same.

For acetylene reduction, tube headspace was purged with Ar for at least 3 min at >130 cc./min; then 1 mL of headspace was removed and replaced with 1 mL of laboratory air, giving a final $O_2$ concentration of 1%. The final acetylene concentration was 10% in all subsamples except in the controls that did not contain acetylene. Acetylene reduction assays were run at 15°C, the mean air temperature at the study site during late spring, summer and early fall (Harmon, 1992). The tissue samples were incubated for 24 h. Ethylene was assayed on a Hewlett Packard model 5830 gas chromatograph fitted with a flame ionization detector. After incubation, 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.

Since our methods differed slightly from those of previous studies (Cornaby and Waide, 1973; Larsen et al., 1978; Silvester et al., 1982), we tested the effect of sample preparation time, $O_2$ concentration and incubation time on acetylene reduction rates of the samples. The effect of exposing prepared wood samples to laboratory air was determined at room temperature for 30 min. The time required to prepare samples was usually <15 min. No differences were observed between those samples and samples exposed to air for <5 min. In the test of effects of $O_2$ concentration and incubation time, tissue samples were incubated for 9, 20, 24, 31, 48, 79, 103, 192 and 264 hr at 0%, 1%, 2%, 5%, 10%, and 20% atmospheric $O_2$. As reported by others (Baker and Attiwill, 1984; Cornaby and Waide, 1973; O'Connell and Grove, 1987; Tjepkema, 1979; Van der Kamp, 1986), the response was linear in most cases (41 of 44 in our study) during the first 24 h in the presence of 1% $O_2$. Rates after 24 h at this $O_2$ concentration were greater than or equal to those at higher oxygen concentrations. Finally, we measured ethylene production in the absence of acetylene. None was observed. From these observations, we conclude that the procedures did not introduce experimental error.

*Data analysis*

The statistical analysis was conducted at two levels of resolution. At the first, (tissue level) each tissue type was examined, and a replicate was the individual tissue sample. At the second (log level), a weighted mean value of acetylene reduction rates for an entire-pie-shaped segment was used, the weighted mean being the acetylene reduction rate for each tissue, weighted by the density and proportional volume of each tissue in the cross section. At each level of resolution, we performed statistical tests separately for each time series.

On the tissue level, a three-way randomized factorial analysis of variance was used to test the effects of species of log, tissue type, time, and their interactions on acetylene reduction rates. Because the complexity of interpreting higher order interactions, we also tested species and time effects for each tissue type with a two-way randomized factorial analysis of variance. On the log level, a two-way randomized factorial analysis of variance was used to test whether species of log, time, and their interactions affected acetylene reduction rates. Since acetylene reduction rates at both tissue and log levels were non-normally distributed, we used natural logarithmic transformations before the analyses.

In the final set of analyses, we examined the relationship of moisture content and acetylene reduction rates. Separate polynomial regressions with moisture content as the independent variable were tested for each tissue type. Since

acetylene reduction did not occur before 18 months, initial and 4-month samples were excluded from this analysis.

Statistical analyses were conducted with SAS procedure GLM for unbalanced designs (SAS Institute Inc., 1985). Comparisons are described here as "significant" at $p \leq 0.05$, "highly significant" at $p < 0.01$.

## Results

### *Tissue-level patterns*

During the first 4 months of decomposition, no acetylene reduction was detected in the logs. The rates of nitrogen fixation, as estimated by acetylene reduction, were at or near the limits of detection ($0.01\ \text{nmol} \times \text{g}^{-1} \times \text{h}^{-1}$) at time 0 and 4 months in logs of all four conifer species.

Heartwood was the only tissue type in which acetylene reduction, as shown by ethylene production did not generally differ between species or increase with time (Fig. 1). Analysis of variance for this tissue in the first time series indicated no significant effect of time or species. In the second time series, the time effect was highly significant because of an increase in production above background levels at 36 months; however, that peak may have little ecological meaning, since low rates of acetylene reduction were measured in the second series at 48 months. The low level of nitrogen fixation in heartwood is paralleled by low loss of mass and low respiration rates (unpublished data).

Acetylene reduction rates in sapwood (Fig. 2) were significantly higher than those in heartwood, but lower than those in outer or inner bark. In the first time series, the effect of time was highly significant, although the effect of species was not. In the second time series, neither time nor species had significant effect. For the combined data set, time had highly significant effect but species did not. Although acetylene reduction in western redcedar showed a linear temporal response, the other three species showed peaks between 18 and 24 months that were most clearly apparent in Douglas-fir sapwood (Fig. 2). Although the overall increase in acetylene reduction rates corresponds to the

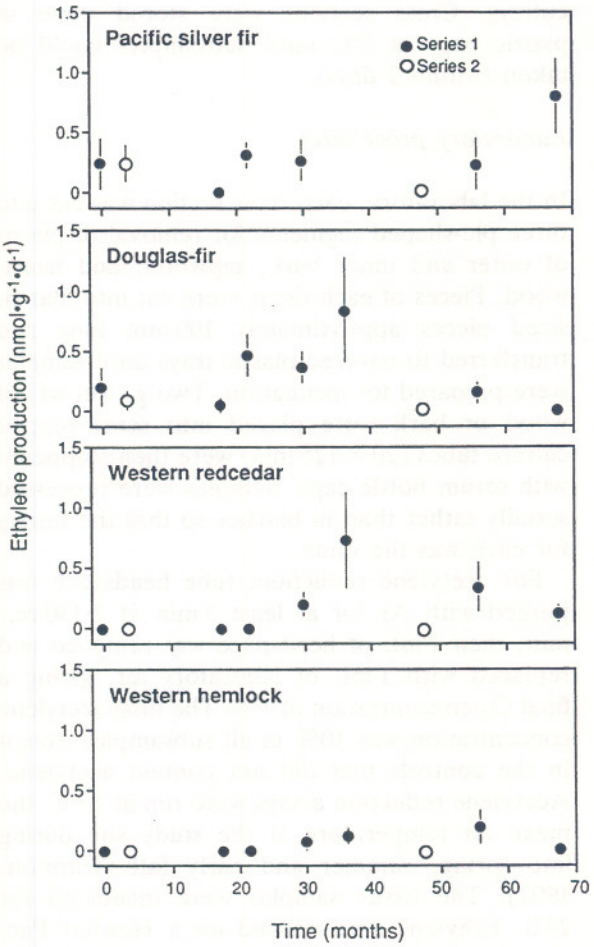


*Fig. 1.* Acetylene reduction as shown by ethylene production rates for heartwood of four conifer species. Vertical lines indicate standard errors about the mean.

increasing decay of sapwood, the cause of the 18- to 24-month peaks is not known.

Outer bark (Fig. 3) generally supported higher rates of acetylene reduction than sapwood or heartwood, but lower rates than inner bark. In both time series, the effect of time was highly significant. The effect of species was highly significant in the first time series, significant in the second. The overall temporal pattern for the four species was for acetylene reduction rates to increase from time 0 to a plateau between 24 and 30 months, which may mirror the colonization of the exterior zone of the log. Assuming that the average acetylene reduction rates between 30 and 68 months are the maximum rates, outer bark of Pacific silver supported the highest

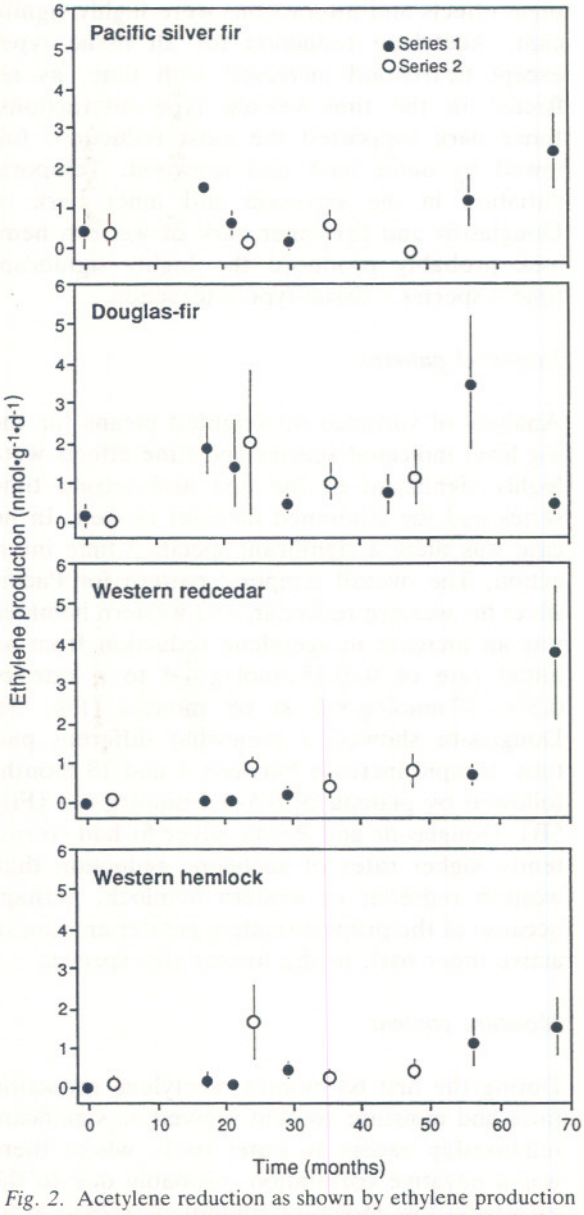


*Fig. 2.* Acetylene reduction as shown by ethylene production rates for sapwood of four conifer species. Vertical lines indicate standard errors about the mean.

rates $3.23 \pm 0.47$ nmol/g × d, mean ± standard error), followed by Douglas-fir ($2.32 \pm 0.65$ nmol/g × d), western redcedar ($1.39 \pm 0.35$ nmol/g × d) and western hemlock ($1.17 \pm 0.18$ nmol/g × d).

Generally, inner bark had the highest acetylene-reduction rates of the four tissue types examined (Fig. 4). In both time series, there

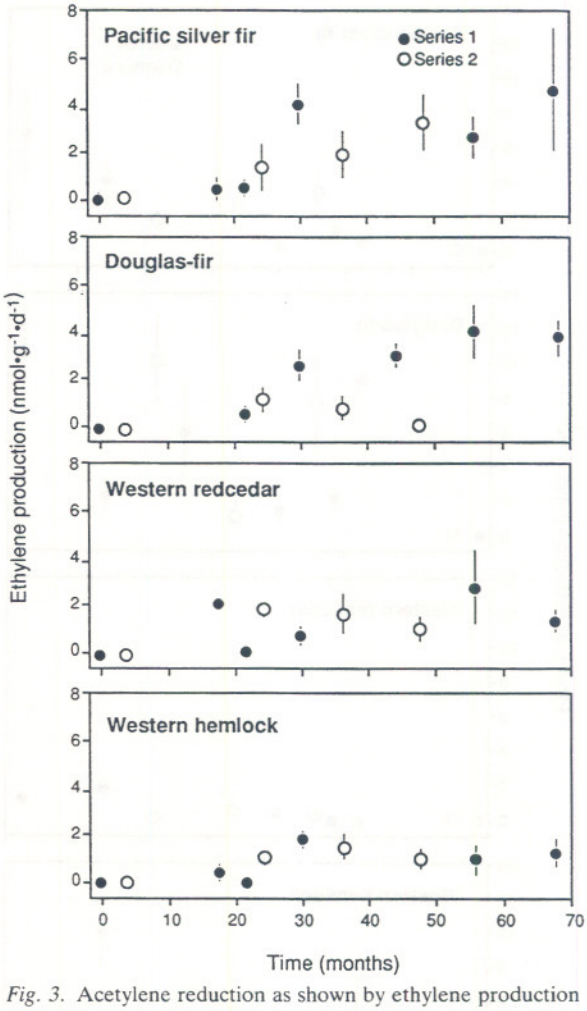


*Fig. 3.* Acetylene reduction as shown by ethylene production rates for outer bark of four conifer species. Vertical lines indicate standard errors about the mean.

were highly significant time, species, and time × species interactions. In Pacific silver fir and western redcedar, the increase with time appeared to parallel that of outer bark, with a rapid increase followed by a plateau of $2.86 \pm 1.10$ and $0.775 \pm 0.29$ nmol/g × d, respectively (Fig. 4). While the 30- to 68-month average was high for both Douglas-fir ($5.39 \pm 1.75$ nmol/g × d) and western hemlock ($4.19 \pm 1.66$ nmol/g × d), the temporal pattern appeared more complex than a simple plateau (Fig. 4). It is not clear whether the fluctuations between 18 and 68 months represent high spatial or temporal variation. Regardless, highly significant time × species interactions resulted from this variation.

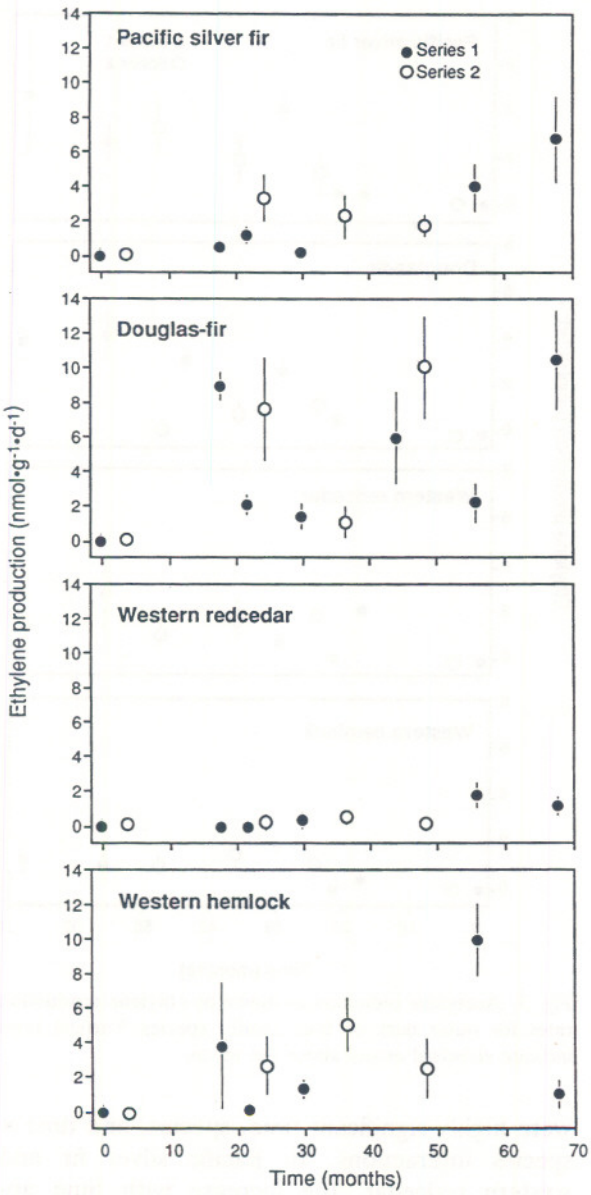


*Fig. 4.* Acetylene reduction as shown by ethylene production rates for inner bark of four conifer species. Vertical lines indicate standard errors about the mean.

Interpretation of the analysis of variance that tested the effects of species, time, tissue type, and their interactions was complicated. In the first time series, all main effects and interactions were highly significant except the time × species interaction, which was significant. In the second time series, neither species nor time × species interactions were significant, but all the other main effects and interactions were highly significant. Acetylene reduction for all tissue types except heartwood increased with time, as reflected in the time × tissue type interactions. Inner bark supported the most reduction, followed by outer bark and sapwood. Temporal variation in the sapwood and inner bark of Douglas-fir and the inner bark of western hemlock probably produced the highly significant time × species × tissue-type interaction.

*Log-level patterns*

Analysis of variance of weighted means for the log level indicated species and time effects were highly significant in the first and second time series and the combined data set as well. In no case was there a significant species × time interaction. The overall temporal pattern for Pacific silver fir, western redcedar, and western hemlock was an increase in acetylene reduction from an initial rate of 0–0.15 nmol/g × d to a rate of 0.57–1.77 nmol/g × d at 68 months (Fig. 5). Douglas-fir showed a somewhat different pattern: a rapid increase between 4 and 18 months followed by plateau of 0.5–1.5 nmol/g × d (Fig. 5B). Douglas-fir and Pacific silver fir had consistently higher rates of acetylene reduction than western redcedar or western hemlock, perhaps because of the proportionately greater amount of active inner bark in the former two species.

*Moisture content*

During the first 68 months, acetylene reduction rates and moisture content showed no significant relationship except in outer bark, where there was a negative correlation, probably due to the extremely low moisture content of <28% at 22 months, a value close to the limit of microbial tolerances (Griffin, 1977). Moisture content of inner bark and sapwood, the most active tissues, remained well above that low value during the course of the study; therefore, we would not expect to find moisture limitation in these tissues. Heartwood moisture contents were often <35% and close to the value measured at the beginning of the time series (Harmon, 1992); therefore, low acetylene reduction rates in heartwood were mostly likely due to lack of decompo-

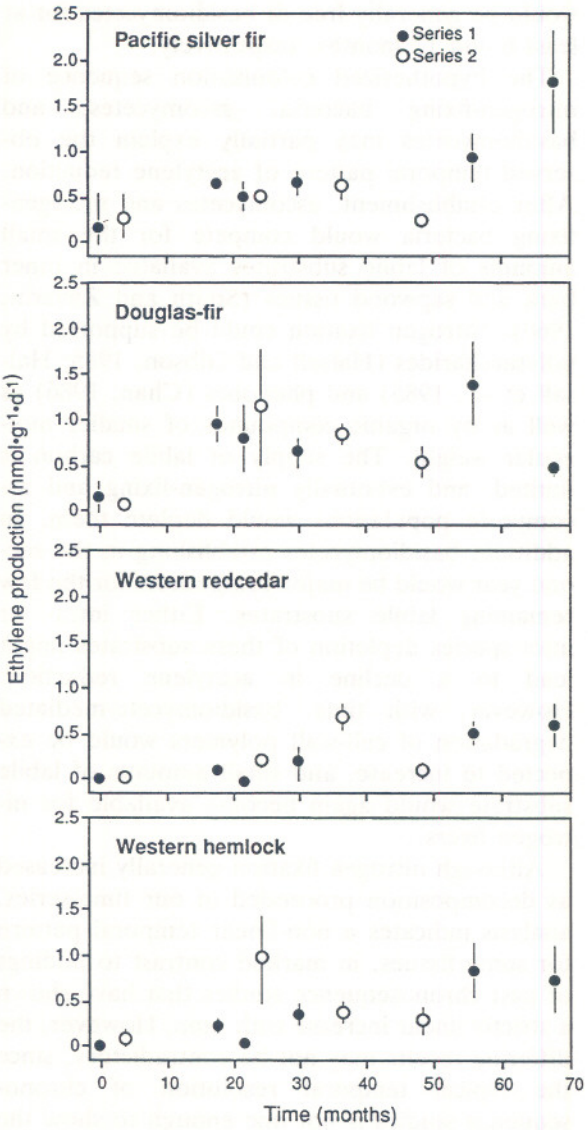


*Fig. 5.* Acetylene reduction as shown by ethylene production rates for entire logs of four conifer species. Vertical lines indicate standard errors about the mean.

sition rather than to moisture limitation. While reductions in microbial activity are often associated with excess moisture, we did not observe this effect in our laboratory incubations.

## Discussion

Optimum rates of nitrogen fixation had apparently not yet been reached at 68 months, since the observed acetylene reduction was less than that reported by others for decaying woody tissues. For example, acetylene reduction rates reported for decaying Douglas-fir wood by Larsen et al. (1978), Silvester et al. (1982), and Jurgensen et al. (1984) were an order of magnitude greater than those we observed. Results most comparable to ours were estimates of summer acetylene reduction rates for a chronosequence of Douglas-fir,western redcedar, and western hemlock (Sollins et al., 1987), which ranged from 0.69 to 3.41 nmol/g × d for class I logs, similar to the 0.5 to 2.4 nmol/g × d we measured in the earliest stages of decay. In a more recent study (Jurgensen et al., 1989) in which acetylene production rates in white and brown rotten coniferous wood were compared, the rates for brown-rot fungi (0.64 to 7.47 nmol/g × d) were close to the rates we observed; however, the rates observed in white-rot fungi were higher, ranging from 3.55 to 46.65 nmol/g × d.

We hypothesize that acetylene reduction was low initially because colonization had not yet occurred. In the first spring after felling, nitrogen-fixing bacteria were most probably introduced into the logs as the result of tunneling by insect genera, including both ambrosia beetles (*Trypodendron*) and bark beetles (*Dendroctonus* and *Dryoetes*) (Zhong and Schowalter, 1989). Bridges (1981) isolated nitrogen-fixing bacteria from bark beetles, which suggests beetle tunneling as a probable mechanism by which logs are colonized by nitrogen-fixing bacteria. The fact that Pacific silver fir and Douglas-fir in our study had the most bark beetle galleries (Zhong and Schowalter, 1989) and the highest rates of acetylene reduction suggests that the beetles played a major role in introducing nitrogen-fixing bacteria to logs.

As decay continues to spread through the logs, we anticipate that acetylene reduction rates for entire logs will increase. Even after 68 months, heartwood acetylene reduction rates were not much greater than in freshly cut logs. Since heartwood comprises from 40% to 75% of the log mass of these four species (Harmon, 1992), a modest increase in nitrogen-fixation rates of that tissue type could greatly increase the overall average for logs. As decomposition progresses to

include more heartwood, we predict that the differences in acetylene reduction rates between species will increase.

Perhaps the most interesting feature of the temporal pattern in the two time series was the temporary peak in acetylene reduction in Douglas-fir sapwood and inner bark between 18 and 24 months and in western hemlock sapwood at 24 months. This pattern is in marked contrast to patterns observed for other tissue types in this study and in past chronosequence studies, which have generally shown a steady increase of the rate of nitrogen fixation and the activity of decay fungi with time.

There are at least two possible explanations for the temporal patterns we observed. One possibility is that events occurring in short-term temporal patterns, such as weather events that occurred just before sampling, obscured the long-term pattern. It is not clear, however, why the sapwood and inner bark would respond to short-term factors in some species but not in others. Also, one would expect that the outer bark, the tissue type most exposed to short-term weather variations, would be more responsive to changes than sapwood. A more detailed examination of seasonal variation in nitrogen fixation will be required to test this explanation.

Another explanation is that the interaction of nitrogen-fixing bacteria, ascomycetes, and wood-decaying basidiomycetes varies with time and among species of logs. There is no reason to assume that the interaction among microbial populations is constant with time (Jurgensen et al., 1984; Larsen, et al., 1978; Silvester et al., 1982). Supplemental data from the same logs indicate that the colonization of ascomycete and basidiomycete fungi did not occur simultaneously. Beetles introduced many ascomycete but few basidiomycete fungi into the logs during the first year (Carpenter et al., 1988). The low activity of basidiomycetes that year was shown by the lack of basidiocarps, which were not formed until the end of the second year. Moreover, the major wood-degrading basidiomycete genus in the time series (*Naematoloma*), produces fruiting bodies and spores in late autumn (Smith et al., 1970), at least 6 months after beetle tunneling begins (Zhong and Schowalter, 1989). Thus, it is plausible that logs felled in March and September would be generally free of basidiomycetes for at least 6 and 12 months, respectively.

The hypothesized colonization sequence of nitrogen-fixing bacteria, ascomycetes, and basidiomycetes may partially explain the observed temporal pattern of acetylene reduction. After establishment, ascomycetes and nitrogen-fixing bacteria would compete for the small amounts of labile substrates available in inner bark and sapwood tissues (Smith and Zavarin, 1960). Nitrogen fixation could be supported by polysaccharides (Halsall and Gibson, 1985; Halsall et al., 1985) and phenolics (Chan, 1986) as well as by organic compounds of smaller molecular weight. The supply of labile carbon is limited, and eventually nitrogen-fixing and ascomycete populations would deplete them. In addition, basidiomycetes establishing in the second year would be major competitors for the few remaining labile substrates. Either intra- or inter-species depletion of these substrates might lead to a decline in acetylene reduction. However, with time, basidiomycete-mediated degradation of cell-wall polymers would be expected to increase, and small amounts of labile substrate would again become available for nitrogen fixers.

Although nitrogen fixation generally increased as decomposition proceeded in our time-series, analysis indicates a non-linear temporal pattern for some tissues, in marked contrast to findings of past chronosequence studies that have shown a strictly linear increase with time. However, the differing results may not be contradictory, since the typical temporal resolution of chronosequence studies is not fine enough to show the early peak observed in this study. The complex temporal patterns may be caused by short-term temporal cycles or by the different colonization patterns of decomposer groups and their changing interactions during succession.

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