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Nitrogen fixation in root-colonized large woody residue of Oregon coastal forests

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Abstract

Coarse woody residues are conspicuous features of the forest floor in coastal Oregon forests. They provide habitats for plants, animals, and a diversity of microorganisms. Live plants are partially or completely rooted in the woody residues. This study provides baseline information on nitrogenase activities (nitrogen fixation) and populations of nitrogen-fixating organisms in root-colonized and noncolonized woody residues on forest and clearcut sites. Coarse woody residue of decay classes IV–V were sampled at three sites of Douglas-fir stands having varying amounts of understory vegetation. Nitrogen-fixation activity in woody residues was detected on all three sites. The woody residues at lower elevation sites near the coast had the least nitrogen-fixing activity and nitrogen-fixing bacterial populations. Plant colonized and noncolonized woody residues had significantly higher nitrogenase activity than the adjacent soils, but the activity between the colonized and noncolonized woody residues did not differ significantly.

Keywords: Coarse woody residues; Nitrogen fixation; Forest disturbance; Bacteria; Fungi; Harvesting

1. Introduction

Coarse woody residues are conspicuous features of the forest floor in coastal Oregon forests. The methods used to recruit and remove woody residues impact numerous related biological activities (Cromack et al., 1978). They provide habitats for plants, animals, and a diversity of microorganisms (Maser and Trappe, 1984; Crawford et al., 1990). The existence of asymbiotic nitrogen fixation in decayed wood and litter was first postulated by Cowling and Merrill (1966) then demonstrated by Cornaby and Waide (1973), Sharp and Millbank (1973), Larsen et al. (1978, 1982), Tjepkema (1979), Hendrickson (1988), Cushon and Feller (1989), Harvey et al. (1989) and Griffiths et al. (1993). Asymbiotic bacteria in coarse woody debris fix about 1 kg of N_2 per hectare per year, perhaps an important long-term source of nitrogen input into some forest ecosystems (Silvester et al., 1982; Dawson, 1983; Heath et al., 1988).

Many plants, such as western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) or huckleberry (*Vaccinium* spp), form mycorrhizae with fungal species when

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partially or completely rooted in well decayed woody residue (Kropp and Trappe, 1982). Selective absorption of nutrients by mycorrhizae and exudation of energy-rich carbon substrates by plants, coupled with lower oxygen tension by respiration often create in the mycorrhizosphere, a selective stimulation of microaerophilic nitrogen-fixing bacteria that make nitrogen readily available to plants. Decaying wood is a nutritionally poor substrate compared to mineral forest soils. The plants rooted in woody residues must extract sufficient nutrients for survival and growth. Nitrogen-fixing bacteria have been found to be intimately associated with mycorrhizae (Li and Hung, 1987; Li et al., 1992). However, nitrogen fixation in the mycorrhizosphere of the coarse woody residue has not been critically investigated. The study was designed to provide baseline information on the amount of nitrogen fixation by asymbiotic bacteria in large woody debris before and after tree harvest and to determine the influence of root activity on nitrogen-fixation rates in large woody debris at three sites in coastal Oregon forests.

2. Materials and methods

2.1. Study sites

Coarse woody residues were sampled at three stands of old-growth Douglas-fir and nearby harvested (5–10 years) areas in the Oregon Coast Range. One site was located at 547 m elevation along the Woods Creek Road on Marys Peak about 24 km southwest of Philomath. This old-growth stand consists of large Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (300–500 years old) with varying amounts of western hemlock and several other coniferous and broad-leaved species in the overstory and understory (Franklin et al., 1981). The second site was at 183 m elevation at Cascade Head Experimental Forest near Lincoln City and was dominated by 60-year old stands of western hemlock and Sitka spruce (*Picea sitchensis* (Bong.) Carr.). The third site, a Bureau of Land Management (BLM) property, was located at 492 m elevation about 34 km west of Eugene. This site had 60-year old stands of mixed Douglas-fir, western hemlock, and western redcedar (*Thuja plicata* Donn ex D. Don) with varying amounts of *Vaccinium* spp., *Polystichum munitum* Kaulf.) Presl, *Berberis nervosa* Pursh, and *Acer macrophyllum* Pursh in the understory. Each site had forested and harvested areas. Previous studies estimated there were approximately 5.5 mg ha^{-1} of woody residue contents on the Woods Creek site. However, our sites are estimated to have approximately 50 t ha^{-1} for forested old-growth areas and 20 t ha^{-1} for harvested sites (materials over 25 mm in size). Table 1 describes general climatic conditions at the time of sampling which were determined using methods described by Johnson and Curl (1972) and Xiwei and Arp (1993).

2.2. Log decay classification system and analysis procedures

Logs were classified according to the following five-class system widely used in the Pacific Northwest (Maser and Trappe, 1984) (Table 2). Five decaying logs in class IV or V partially colonized by western hemlock and other vegetation were randomly selected at each site within plots of forested and harvested treatments. Selection of logs for sampling was used as previously described by Crawford et al. (1990). Sampling was conducted in the fall and spring of 1989. Ten replicate wood samples, five each from root-colonized and non-colonized, were removed from woody residues. Samples were removed using 95% ethanol rinsed hand trowels. Nitrogenase activity, measured by acetylene reduction methods (McNabb and Geist, 1979), was assayed by placing the material in glass jars (450 ml volume) with 10% acetylene atmosphere and incubated at field temperature in the dark for 6–12 h. Ethylene production was measured with a Hewlett Packard 5830A gas chromatograph fitted with a flame-ioniza-

Table 1
Climate data for May and November

Location	Temperature (°C)		Precipitation (cm)	
	May	Nov	May	Nov
Cascade Head	11.7	18.4	12.32	24.86
Bureau of Land Management	13.4	8.3	7.67	13.33
Woods Creek	12.2	7.2	4.85	11.25

Data are from Climatological Data, Oregon, Vol.95/5, 1989.

tion detector and a 2 m × 2.1 mm stainless steel column packed with Poropak R on 80–100 mesh on chromosorb W. The oven temperature was adjusted to 70°C; detector and injector temperatures were adjusted at 100°C. The nitrogen carrier gas flow rate was adjusted to 40 ml min^{-1}. Samples in glass jars incubated in ambient air were included for controls. Blank samples were used to convert for C_2H_4 contained in C_2H_2 gas. Both endogenous C_2H_4 production and background C_2H_4 levels were checked regularly. Rates reported are net C_2H_2 reduction rates after subtraction of background levels and endogenous rates. After acetylene reduction was measured, samples were oven-dried to determine moisture content and dry weights. A similar experiment was conducted for the mineral soil adjacent to each log. The litter and duff materials were removed and mineral soils were collected at a depth of 15 cm using 95% ethanol rinsed hand trowels at each site.

The most probable number technique (MPN) was used to estimate numbers of N_2 – fixing bacteria in all wood samples as well as in mineral soil. Eight serial dilutions (10^{-1}–10^{-8}) were made from 1 g samples with sterile distilled water. Five replicates of 10 ml nitrogen-deficient medium (Rennie, 1981) in test tubes were made for each dilution and incubated at 30°C for 3–7 days. Acetylene was injected into each tube at 10% of the total gas volume. After 24 h, gas samples were taken from each tube and analyzed for ethylene formation. Tubes not exhibiting ethylene formation were assumed not to have N-fixing bacteria present. The numbers of nitrogen-fixing bacteria in the wood or soil samples were estimated using the technique of Alexander (1982).

3. Statistical analysis

The study included three sites. Each site had a forest and harvested subarea. Forest-harvested subunits were than subsampled by five logs with adjacent soil. Each log-soil unit was subsampled at two media classes and measured at two seasons. Accordingly, the study design was a randomized block, split-split-plot design with an analysis format that provides a test of location where location is the statistical blocking factor. This test is properly interpreted as a measure of the efficiency of blocking, not as a test of the differences among locations.

4. Results

The average mean temperature for the spring sampling sites was 12.4°C with the average mean temperature for the fall sampling sites being 8°C (Table 1). Precipitation mean average for the sites was higher at 16.5 cm in the fall compared to 8.3 cm in the spring (Table 1). The highest rates of nitrogenase activities were detected in noncolonized woody residues (0.72 nmol of ethylene per g h^{-1}) in the fall on forested sites followed by nitrogenase activities in root-colonized woody residues (0.67 nmol of ethylene per g h^{-1}). On average, nitrogenase activity in root-colonized woody residues (0.47 nmol of ethylene per g h^{-1}) on harvested sites was slightly higher than nitrogenase activity in noncolonized woody residue (0.43 nmol of ethylene per g h^{-1}) of harvested sites. Soil nitrogenase activity was higher on forested sites (0.24 nmol of ethylene per g h^{-1}) than

Table 2
Characteristics of fallen Douglas-fir trees in five decay classes [a]

Characteristics	I	II	III	IV	V
Bark	Intact	Intact	Trace	Absent	Absent
Twigs, 0.3 cm	Present	Absent	Absent	Absent	Absent
Texture	Intact	Intact to partly soft	Hard, large pieces	Small, soft blocky pieces	Soft powdery
Shape	Round	Round	Round	Round to oval	Oval
Wood color	Original	Original	Original	Light brown to faded	Red brown to reddish brown
Invading roots	None	None	—	Sapwood	Heartwood
Portion on ground	Elevated	Elevated, slight sag	Sagging near ground	Heartwood on ground	Heartwood

[a] Adapted from Maser and Trappe (1984).

Table 3
Fall nitrogenase activities, measured by acetylene reduction, of the coarse woody residues on the forested (f) and harvested (h) sites (nmol ethylene per $g\,h^{-1}$)

Sampling sites and elevations	Root-colonized woody residue		Noncolonized woody residue		Adjacent mineral soil	
	f	h	f	h	f	h
Cascade Head, 183 m	0.22	0.31	0.24	0.22	0.01	0.24
Bureau of Land Management, 492 m	0.94	0.45	0.47	0.30	0.30	0.01
Woods Creek Rd., 547 m	0.85	0.64	1.45	0.78	0.41	0.05
Average	0.67	0.47	0.72	0.43	0.24	0.1

Data are means of five replicates.

Table 4
Spring nitrogenase activities, measured by acetylene reduction, of the coarse woody residues on the forested (f) and harvested (h) ites (nmoles ethylene per $g\,h^{-1}$)

Sampling sites and elevation	Root-colonized woody residue		Noncolonized woody residue		Adjacent mineral soil	
	f	h	f	h	f	h
Cascade Head, 183 m	0.22	0.39	0.11	0.20	0.00	0.32
Bureau of Land Management, 492 m	0.62	0.61	0.87	0.68	0.11	0.33
Woods Creek Rd., 547 m	1.36	1.07	1.41	1.14	0.24	0.14
Average	0.73	0.69	0.80	0.67	0.12	0.26

Data are means of five replicates.

Table 5
ANOVA (fall samples)

Source	DF	F value	$Pr > F$
Soil versus other	1	9.64	0.0145 [a]
Rhizosphere versus non-rhizosphere	1	0.00	0.9570
Location	2	3.26	0.2350
Forested/harvested	1	1.78	0.3140

Type III mean square error term used from SAS GLM procedure.
[a] Significant at $P < 0.05$.

Table 6
ANOVA (spring samples)

Source	DF	F value	$Pr > F$
Soil versus other	1	12.77	0.0073 [a]
Rhizosphere versus non-rhizosphere	1	0.02	0.8956
Location	2	11.01	0.0833
Forested/harvested	1	0.00	0.9606

Type III mean square error term used from SAS GLM Procedure.
[a] Significant at $P < 0.05$.

Table 7
Enumeration of nitrogen-fixing bacteria per gram of woody residues

Sampling sites and elevation	Root-colonized	Non-colonized soil	Adjacent mineral
Cascade Head, 183 m	12(9) [a]	3(0)	0(9)
Bureau of Land Management, 492 m	90(336)	472(244)	353(104)
Woods Creek Rd., 543 m	112(123) [b]	137(123) [b]	146(126) [b]

[a] Nitrogen-fixing bacterial populations in coarse woody residues of harvested sites are indicated within the brackets. Data is mean of three replicates.
[a] Numbers × 1000.

on harvested sites (0.10 nmol of ethylene per $g\,h^{-1}$) (Table 3). Spring nitrogenase activity was highest in noncolonized woody residues (0.79 nmol of ethylene per $g\,h^{-1}$) on forested sites followed by rates in root-colonized woody residues (0.73 nmol of ethylene per $g\,h^{-1}$) on forested sites. Nitrogenase activity was higher in root-colonized woody residues (0.69 nmol of ethylene per $g\,h^{-1}$) on harvested sites than nitrogenase activity in noncolonized woody residues (0.67 nmol of ethylene per $g\,h^{-1}$) on harvested sites. Soil nitrogenase activity was higher in harvested sites (0.26 nmol of ethylene per $g\,h^{-1}$) than soil of forested sites (0.12 nmol of ethylene per $g\,h^{-1}$) (Table 4). Generally, the highest rates of nitrogenase activities were detected during the spring than activities in the fall (Tables 5 and 6). The enumeration of nitrogen-fixing bacteria per gram of woody residue was highest in noncolonized woody residue on the BLM site. The average mean population was highest in soil and noncolonized woody residues at the Woods Creek site (Table 7). The average mean nitrogen-fixing populations were highest in soils followed by populations in noncolonized woody residues.

5. Discussion

Nitrogen-fixation activity in woody residue was detected on all three experimental sites. In addition, nitrogen-fixation activity was detected in soils on both the harvested and unharvested experimental sites with the exception of forested soil at Cascade Head Experimental Forest in the spring. Nitrogen-fixation activity also was low in soil at the Cascade Head Experimental Forest in the fall. In general, both forested and harvested sites at Woods Creek showed higher activity and populations of nitrogen-fixing microbes in the woody residues and soils in comparison to other experimental sites. The Cascade Head Forest site contained few nitrogen-fixing microbes in comparison to two other experimental sites. Root-colonized and non-colonized woody residues had higher activity and were significantly different from the adjacent mineral soil. Activity between the colonized and non-colonized in woody residue did not differ significantly. Presumably, the non-colonized woody residues still contained an available carbon source for nitrogenase activity (Harvey et al., 1989; Jurgensen et al., 1989).

The Cascade Head Experimental Forest site is located on the fringes on the Oregon Coast. The nitrogen-fixing organisms at the site could be intolerant to salinity levels brought in constantly by oceanic moisture. Studies conducted by Burleigh and Dawson (1991) and Reddell et al. (1986) show that sodium chloride can inhibit in-vitro growth and sporulation of nitrogen-fixing *Frankia* with similar growth inhibited in soil. With malate semi-solid medium (Zuberer, 1987), nitrogen-fixing *Azospirillum* also was detected in soils at all sites except forested soils at the Cascade Head Experimental Forest site. Oxygen concentration in coarse woody debris can be as low as 2% (Paim and Becker, 1963). Thus, most of the nitrogen-fixing organisms in woody residues would be microaerophilic.

Even though the nitrogen-fixing rates are small compared to those of actinorrhizal and leguminous plants (Stowers, 1987), the nitrogen input on a long-term basis may add significantly to the nitrogen budget of both the forest and clearcut sites where cover of coarse woody residues is sparse. Coarse woody residues on the forest floor increase soil organic matter, partly because the well-decayed wood is rich in residual lignin. Soil organic matter is important in maintaining water retention capacity, nutrient supply, and source of mycorrhizal fungi and conifer seedlings (Kropp, 1982; Harmon et al., 1986). Coarse woody residues create and maintain structural and biological diversity that contributes to forest long-term productivity, because animals, organisms, structure pathways, and ecosystem functions are interdependent. Removal of coarse woody residues would reduce these relationships with concurrent reduction of ecosystem processes performed by the coarse woody residues. The results suggest that retention of coarse woody residues during harvesting may be of significance to the future of forest ecosystem long-term productivity.

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