AN ABSTRACT OF THE THESIS OF

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Microbial populations associated with the major substrates of the canopy of a 70 m old-growth Douglas fir were studied to determine potential activities. Seasonal samples from bark, foliage, epiphytic moss, lichens, and litter accumulations were collected to i) obtain population data, ii) to isolate the major groups of microorganisms present, iii) to measure enzymatic activities associated with cellulose and xylan degradation, and iv) to examine the potential for nitrogen fixation. Five hundred sixty-two bacterial isolates were tested for utilization of 25 carbohydrates associated with the canopy substrates, and for activities in the nitrogen and sulfur cycle transformations.

Total bacterial populations, reflecting seasonal temperature and moisture conditions, were lowest on bark and foliage (21 to 266 x 10^3 colony forming units (CFU/gm)) on moss and lodged litter (19 to 610 x 10^5 CFU/gm). Lichens contained intermediate numbers of bacteria (3.3 to 270 CFU/gm) while the flourescent pseudomonads and Streptomyces reflected 0.005 to 2.2 percent of the total plate counts.

The majority of the organisms isolated were classifed as species of Arthrobacter, Bacillus, Flavobacterium, and Xanthomonas. Isolates of Alcaligenes (=Achromobacter), Aeromonas, Chromobacterium, Micrococcus and Pseudomonas were less common).

No measurable rates of nitrogen fixation attributable to bacteria were detected by acetylene reduction. Isolates of Bacillus, Citrobacter, and Enterobacter were recovered sporadically. Eleven species in six genera of lichens containing a blue-green algal phycobiont showed positive acetylene reduction. One species, Lobaria oregana, accounted for 51 percent of the total lichen biomass of the canopy.

Cellulase and xylanase activity was routinely detected in moss and litter samples, and less frequently in lichens. There was a strong correlation between the two activies for moss ($r=0.94$) and litter ($r=0.81$).

MICROBIAL ECOLOGY OF AN OLD-GROWTH DOUGLAS FIR CANOPY

by

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MICROBIAL ECOLOGY OF AN OLD-GROWTH DOUGLAS FIR CANOPY

1. INTRODUCTION

The canopy of Pacific Northwest old-growth coniferous forest is an integral and vital part of the forest ecosystem and is composed of a complex, interacting association of living and decaying tree components and epiphytes. In addition to intact tree components such as bark, woody tissue, and foliage, there are a host of micro- and macroorganisms that inhabit the tree surfaces (Denison, 1973). A diverse assortment of animals including vertebrates, arthropods, nematodes, and protozoans have been found in the canopy. A similar diversity of plants are present: algae, fungi, mosses, lichens, and ferns. And, as in any system, there are a host of bacteria present associated with all of these substrates.

Isolated vertically from the forest floor by up to 30 m, the canopy is an ecosystem in its own right with inputs, outputs, and internal transformations. Inputs are in the form of sunlight to the phototrophs, atmospheric mineral inputs in gaseous, liquid,and particulate form, and organic compounds exuded from the tree foliage. Outputs to the forest floor can be as litterfall of various canopy components, vertebrate and invetebrate wastes, or leaching and transport of compounds by precipitation.

Research programs examining the components of and interactions within the coniferous canopy have focused primarily on tree structure, component biomass and diversity (Pike et. al., 1975, 1977), and foliar micro-

epiphytes (Sherwood and Carroll, 1974 and Bernstein and Carroll, 1977). Current research at both Oregon State University and the University of Oregon has focused on the ecology of canopy invertebrates, the leaching of materials from canopy substrates, micrometeorology of the canopy, and nitrogen fixation. One of the potentially important areas of investigation in the canopy system is the role of bacteria in canopy processes. Bacteria are the agents of many biologically-essential transformations in terrestial systems including decomposition of virtually any organic residue, biological nitrogen fixation, and cycling of nitrogenous and sulfur compounds (Alexander, 1977). The purpose of this paper is to report on an investigation of the microbial ecology of the major substrates in the canopy of an old-growth Douglas fir. The objectives of the research were:

1) to study magnitudes and fluctuations of bacterial and actinomycete populations seasonally and vertically on bark, foliage, lichens, moss, and litter accumulations in the canopy;

2) identify the major groups of microorganisms present and their activities against commonly occurring or environmentally-significant carbohydrates;

3) determine activities of extracellular enzymes capable of degrading cellulose and xylan, releasing sugars that would be available to microorganisms; and

4) identify and quantify any significant sources of nitrogen fixation in the canopy.

2. LITERATURE REVIEW

2.1 Research on the Douglas Fir Canopy

The old growth coniferous forests of the Pacific Northwest have recently been the focus of extensive investigation because of their economic importance. A large portion of this research has focused on nutrient cycling and, in particular, the recycling of tree components. In studying a range of old growth Douglas fir (Pseudotsuga menziesii (Mirb.) Franco) communities, Grier and Logan (1977) computed above ground biomasses ranging from 492 to 976 metric tons/ha with an annual net primary production of 6.2 to 10.1 metric tons/ha. Of this biomass approximately 6 Kg/ha is annually shed as litterfall composed of needles, woody tissue, reproductive structures and epiphytes (Abee and Lavender, 1972). This litterfall forms a litter layer on the forest floor ranging from 23 to 86 metric tons/ha and represents a nitrogen pool of 202-1345 Kg/ha (Youngberg, 1966). This litter gradually decomposes and releases nutrients to be recycled by the forest ecosystem.

The canopy ecosystem of old growth Douglas fir was first actively investigated by two Oregon State University Botany undergraduates, Diane Tracy and Diane Nielson, sponsored by a National Science Foundation-Undergraduate Research Participation study. Utilizing modified technical rock climbing techniques, methodology was developed to

allow ready access to the canopy and non-destructive study of the tree and its components (Denison et. al., 1972). Applying these techniques to a 65 meter Douglas fir in the H.J. Andrews Experimental Forest, Pike et. al. (1972) reported an epiphytic biomass of 18.3 Kg composed of mosses and liverworts (8.9 Kg) and lichens (9.4 Kg). Approximately 70 percent of the lichen biomass was Lobaria oregana (Tuck.) Mull. Arg., a lichen with a secondary Nostoc phycobiont and capable of fixing atmospheric nitrogen.

Denison (1973) described the old growth canopy as a diverse aggregation of plants, fungi, insects, and bacteria in a variety of associations. This system, isolated from the forest floor by up to 30 meters vertically and ranging to over 70 meters in height, "is a distinct habitat providing its own special conditions of moisture, light, temperature, and other qualities." Dension reported on initial nitrogen fixation rates (acetylene reduction and growth increments) by Lobaria oregana as contributing annually between 1.8 and 10 lbs. nitrogen/acre.

Pike et. al. (1977) utilized computer selected sampling to determine the biomasses of tree and epiphyte canopy components on a 400 year old Douglas fir. After the living woody tissue, the dominant biomasses were needles, 198 Kg; dead twigs, 78 Kg; lichens 13.1 Kg, and bryophytes, 4.7 Kg. Lobaria oregana was the dominant lichen and represented 78 percent of the lichen biomass. Microepiphyte (algal and fungal, but not bacterial) biomass was determined to be 190.9 m^2 or 6.8 percent surface area coverage for needles and 300.5 cm^3 cell volume for twigs.

A floristic survey of the canopies of eleven large old growth trees (Pseudotsuga menziesii, Tsuga heterophylla (Raf.) Sarg., and Pinus lambertiana Doug.) and nine smaller understory trees (Tsuga heterophylla, Taxus brevifolia Nutt., and Acer circinatum Pursh) by Pike et. al., (1975) generated a listing of 74 species of lichens, 25 species of mosses, and 6 species of hepatics. The study also described six distinctive epiphyte zones based on tree geometry.

2.2 Microbial Research in Forest Ecosystems

2.2.1 Microbial Populations

Research on aerial plant surfaces, reviewed by Preece and Dickinson (1971) and Dickinson and Preece (1976), has focused almost exclusively on pathogen interactions, non-tree plants, and tropical systems. Microbiological studies in temperate forest ecosystems have largely been limited to the litter and soil component. These have generally focused either on total populations or on taxonomic groups of bacteria, actinomycetes, and fungi. A partial listing of the more relevent of these investigations is presented in Table 1.

The soil of a Danish beech forest yielded plate counts of bacteria averaging 26.9×10^6/gm and composed principly of spore forming rods (Bacillus), Arthrobacter-like pleomorphic forms, and actinomycetes. The beech leaves had plate counts ranging from 25×10^4 to 39.5×10^6/gm and contained a distinctly different flora from that of the soil. The predominant phyllosphere forms were yellow-pigmented rods, non-pigmented non-spore forming rods, and fluorescent Pseudomonads. Spore-forming bacteria and actinomycetes either were rare or non-existant (Holm and Jensen, 1972).

In studies of deciduous woodland humus and soil in the United Kingdom (Gray et. al., 1974) the microbial populations and the major taxa comprising these populations varied widely in both amorphous humus and soil. The dominant taxa varied with substrate: ash litter, Arthrobacter and Pseudomonas; oak litter, Pseudomonas and members of the coli-aerogenes group; amorphous humus, Achromobacter (=Alcaligenes) and Mycobacterium; and soil, Bacillus (Hissett and Gray, 1973).

Goodfellow (1968), in a study of soil microflora beneath a 40 year old stand of Pinus nigra laricio in England, reported a wide taxonomic diversity of isolates. In order of decreasing frequency of occurrence the isolates from the A_1 horizon were: Bacillus (58%), Arthrobacter (13%), Flavobacterium and Pseudomonas (6%), Staphylococcus and Micrococcus (5%), Streptomyces (3%), Achromobacter and unidentified forms (2%), and fluorescent Pseudomonads (1%).

Wright and Bollen (1966) determined seasonal variations in litter and soil populations of actinomycetes, bacteria, and fungi under a young stand of Douglas fir in western Oregon. Their results showed an inverse relationship between the periods of peak plate counts of actinomycetes and bacteria compared with peak numbers of the fungal population.

Fungi of the genera Aspergillus, Mucor, Penicillium, Phomopsis, and Trichoderma have been isolated from Douglas fir needles in the initial stages of decomposition (Minyard and Driver, 1972) and from the litter and soil of alder, conifer, and mixed alder-conifer stands (Lu et. al., 1968; Wicklow et. al., 1974).

Fungal succession and populations on needles from an old-growth Douglas fir have also been examined. Bernstein and Carool (1977) measured epiphytic fungi, yeasts, and protococcoid algae in a microscopic survey of the needle surface. Sherwood and Carroll (1974) reported a well-defined successional sequence of fungi with needle age and they also described the epiphytic fungi of twigs.

2.2.2 Microbial Activities

In addition to information on populations and microbial taxa associated with temperate forest ecosystems, numerous investigators have examined specific microbial activities associated with litter and soil. Some of the parameters include respiration, activities of enzymes degrading plant matter, and the ability of bacterial isolates to utilize environmentally available carbohydrates.

Microbial respiration of the forest floor has been studied both singlely as seasonal variations in CO_2 evolution (Phillips, 1976); and in relation to various enzyme activities in coniferous litter (Spalding, 1977). In the former study, CO_2 evolution was most strongly associated with moisture content during the summer and fall months, and with temperature during the winter and spring months. In the latter study significant correlations were found between CO_2 evolution and the activities of amylase, cellulas, and xylanase in coniferous litter samples. There was also a strong linear relationship ($r=0.91$) between cellulase and xylanase activities.

Any microbial activity requires a source of driving energy which, most commonly, is produced by the metabolism of some form of carbohydrate. Table 2 lists those carbohydrates that are frequently found associated with the major components of the Douglas-fir canopy. They may occur

either in free or polymeric form. Also, phenolic degradation products of lignin (i.e. protocatechuic acid and vanillin) may be present and could serve as energy sources (Alexander, 1977).

The ability of forest litter and soil bacterial isolates to utilize these carbon compounds has been examined in a Scandinavian spruce forest (Niemela and Sundman, 1977); a pine forest soil (Goodfellow, 1968, 1969); and a deciduous hardwood forest soil (Hissett and Gray, 1973). The results of these studies have been combined in Table 3. Hexose monosaccharides were more widely utilized than pentoses or disaccharides, and polyols utilized least of all the simple carbohydrates. For the polysaccharides, starch was more frequently metabolized than cellulose. The percentage of isolates using lignin-related plenoics was highly variable between soils, but always below that of the simple sugars.

2.2.3 Nitrogen Cycling

Nitrogenis the major limiting factor in the growth and maintenance of northwest coniferous forests (Gessel et. al., 1951). The principal microbial nitrogen transformations are ammonification, which increases the amount of nitrogen ultimately available to plants; denitrification, which represents a net loss of nitrogen to the system; and nitrogen fixation, which represents a net nitrogen input. Two studies of coniferous forest soil bacteria indicated more isolates were capable of ammonification than possessed nitrate reductase. In a Douglas fir non-rhizosphere soil there were approximately twice as many ammonifiers as nitrate reducers (Neal et. al., 1975); while in a Scandinavian spruce forest there were 3.6 times more ammonifiers than nitrate reducers (Niemela and Sundaman, 1977).

Biological nitrogen fixation in temperate forests is limited to certain groups of bacteria and blue-green algae. For this review, I will exclude legumes and actinorrhizal associations since these are usually found only in naturally or artificially perturbed symtoms. Members of the Enterobacteraceae or Bacillaceae have been described as free-living agents or existing in loose association with eucaryotes. Blue-green algae are most commonly found as a symbiotic component of certain species of lichens.

The occurrence of free-living bacteria capable of nitrogen fixation in temperate forest soils is usually so limited that significant nitrogen input is not realized. Jurgensen and Davey (1971) described the presence of populations of anaerobic clostridia and facultative Bacillus polymyxa capable of growing on nitrogen-free media at 2400 and 490 cells/g, respectively, in a Douglas fir forest soil (from the Olympic Peninsula, WA). Roskoski (1977) estimated total nitrogen fixation means of 1.25 to .096 kg/ha/yr for 14 to 200 year old northeastern hardwood forests. Nitrogen fixing bacteria have frequently been found associated with living, diseased, and decaying wood in coniferous forests. Bacon and Mead (1971) isolated members of the genera Achromobacter, Bacillus and Erwinia from the sapwood of Ponderosa Pine and which were able to grow in a nitrogen-free media. Aho et. al. (1974) recovered nitrogen-fixing members of the Enterobacteriaceae associated with Hericum abietis and Phellinus chrysoloma infectious in living White Fir trees. Total bacterial densities of 7×10^6 cells/ml were found in expressed sap; of which, 5 percent were nitrogen-fixers. The isolates were characterized as Enterobacter aerogenes, E. agglomerans (= Erwinia herbicola),

metabolically a typical Enterobacter species, and Klebsiella pneumonia. Nitrogen fixation measured by acetylene reduction has been found to occur in decaying Douglas fir logs both in the stream and terrestrial environment (B. Buckley, OSU, personal communication).

Nitrogen-fixing bacteria in the phyllosphere of Douglas fir (Jones, 1970 and Jones et. al., 1974) as well as other conifers (Jones, 1976) have been widely studied in England. Estimates of resultant nitrogen fixation by the unidentified bacteria is thought to significantly add to the forest nitrogen budget. In Washington, USA, Denison et. al. (1976) reported small rates of acetylene reduction associated with the micro-epiphytic communities on the surface of Douglas fir needles.

The coniferous forest canopy contains many species of cyanophilic (containing a blue-green algae as the primary or secondary phycobiont) lichens in addition to lichens with only a green algal phycobiont. In a review of nitrogen-fixing lichens, Millbank (1977) listed 45 species in 18 genera of which species are recorded by Pike et. al.(1975) as being present in the canopy or understory of the coniferous forest. The author has compiled a list of nitrogen-fixing lichens collected specifically from the canopies of old growth Douglas fir, designating those species found in the tree used in this study (Appendix 4). One species, Lobaria oregana, is by far the most abundant with an estimated biomass of 500-800 Kg/ha (W.C. Denison, OSU, personal communication).

TABLE 1. Selected references of microbial research in temperate forests.

Reference	System Studied	Bacteria[1]	Actino-Mycetes[1]	Fungi[1]	Microbial Activity[2]
Aho et. al., 1974	White Fir, Oregon	+			NF
Bernstein and Carroll, 1977	Douglas fir needles, Oregon			+	
Goodfellow et. al., 1966 Goodfellow, 1968	A_1 Chorizon, Pine forest soil, U.K.	+			BC
Hagedorn, 1976	Coniferous forest soils, Oregon		+		
Hisset and Gray, 1973 Gray et. al., 1974	Deciduous forest litter and soil, U.K.	+			BC
Holm and Jensen, 1972	Beech forest soil and phyllosphere, Denmark	+	+		
Jones, 1970 Jones, 1976 Jones et. al., 1974	Douglas fir phyllosphere and soil, conifer phyllosphere, U.K.				NF
Jurgensen and Davey, 1974	Pacific NW coniferous forest soil, Washington	+			NF
Minyard and Driver, 1972	Decomposing douglas fir needles, Oregon			+	
Niemela and Sundman, 1977	Northern Spruce forest soil, Finland				BC
Sherwood and Carroll, 1974	Douglas fir needles, Oregon			+	
Spaulding, 1977	Mixed coniferous litter, Oregon				ENZ
Wicklow et. al., 1974	Alder-conifer stand litter, Oregon			+	
Wright and Bollen, 1961	Douglas fir forest soil, Oregon	+	+	+	

[1] Indicates population studies

[2] Microbial activities: BC - biochemical activities (i.e., utilization of sugar, cellulose hydrolysis); ENZ - enzyme activities measured; NF - nitrogen fixation measured.

TABLE 2. Carbohydrates routinely associated with components of the Douglas-fir Canopy

	Bark/woody[1,2,3] Tissue	Foliage/[4] Litter	Lichens[5,6]	Mosses[7,8]
Arabinose	+	+		+
Rhamnose	+		+	+
Xylose	+	+	+	+
Fructose			+	+
Galactose	+	+	+	+
Glucose	+	+	+	+
Mannose	+		+	+
Cellobiose	d*	d	d	d
Sucrose	+	+		+
Trehalose			+	
Raffinose	+			
Adonitol (=Ribitol)	+		+	
Arabitol	+		+	
Dulcitol	+			
Inositol	+		+	
Mannitol	+		+	
Arabinogalactan	+		+	
Cellulose	+	+	+	+
Galactomannan	+			
Lichenin			+	
Pectin	+	+		+
Starch	+	+		
Xylan	+			

* as decomposition product of cellulose

[1]Chen, 1973

[2]Zerrudo, 1973

[3]Smith and Zavarin, 1960

[4]Sowden and Ivarson, 1962

[5,6]Culberson, 1969, 1970

[7]Magaris and Kalaitzakis, 1974

[8]Theander, 1954

TABLE 3. Carbohydrate utilization by bacterial isolates in forest soil studies

	Northern Spruce Forest Soil[1] (Finland)	Pine Forest Soil A_1 Horiz.[2] (U.K.)	Deciduous Forest Humus[3] (U.K)
	Percent of Isolates Utilizing		
Acid From:			
arabinose	94.3	29	19
rhamnose	53.5	26	10
xylose	84.1	41	30
galactose	NT[4]	40	24
glucose	93.1	(82)	52
mannose	94.9	42	28
cellobiose	54.8	60	18
sucrose	25.9	47	16
dulcitol	NT	22	2
Hydrolysis:			
cellulose	9.5	0.4	NT
chitin	4.8	7	2
laminarin	NT	4	NT
pectin	NT	2	NT
starch	11.8	47	28
xylan	NT	25	NT
Utilization of:			
p-hydroxy benzoic acid	NT	1	5
protocatechuic acid	82.0	NT	NT
vanillin	13.0	2	NT

[1] Niemala and Sundman, 1977

[2] Goodfellow, 1968, 1969

[3] Hissett and Gray, 1973

[4] Not tested

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3.1 INTRODUCTION

The canopy of an old-growth Douglas fir tree (Pseudotsuga menziesii (Mirb.) Franco) is a major subunit of the coniferous forest and is composed of diverse, interacting organisms (Denison, 1973). In addition to the tree components of bark, foliage, and wood, the tree surface supports a wide range of epiphytic macro- and microorganisms. Pike et al. (1975) reported that the macro-epiphytic component included 74 species of lichens, 32 species of bryophytes, and 1 fern species. Significant coverage of needle and twig surfaces by fungi, yeasts, and protococcoid algae has also been observed (Sherwood and Carroll, 1974; Bernstein and Carroll, 1977). Using a systematic sampling technique and a computer to expand measurements to whole tree estimates, Pike et al. (1977) determined the foliar biomass to be 198 kg and the total macro-epiphyte biomass to be 17.8 kg. Of the entire epiphyte biomass, 57% was represented by a single lichen, Lobaria oregana (Tuck.) Mull. Arg., which has a secondary blue-green algal phycobiont and is capable of nitrogen fixation.

In most biological systems, bacteria play a major role in decomposition processes and nutrient cycling. Bacterial research in the Pacific Northwest coniferous forest has been performed on various aspects of the soil and litter microflora; such as seasonal populations (Wright and Bollen, 1961), Streptomyces in acid soils (Hagedorn, 1976), microbial respiration (Phillips, 1975), nitrogen fixation (Jurgensen and Davey, 1971; Aho et al., 1974), and extracellular enzyme activities (Spalding, 1977). Nitrogen fixation by bacteria associated with the foliage of Douglas fir in England has been reported to provide a significant percentage of the annual nitrogen input (Jones et al., 1974).

This paper deals with a study of bacterial populations in the canopy of an old-growth Douglas fir tree located in the Cascade Mountains in western Oregon. The research consisted of monitoring populations from 6 substrates, seasonally, at 3 heights in the canopy, and included i) identification of the principal taxonomic groups of organisms present and their biochemical activities; ii) determination of any significant sites of nitrogen fixation; and iii) measurement of extracellular enzyme activities.

3.2 MATERIALS AND METHODS

The study was performed on a 70 m, 129 cm dbh old-growth Douglas fir, designated Galadriel, located at 560 m elevation in the H. J. Andrews Experimental Forest, Lane County, Oregon, U.S.A. The site is a typical old-growth stand in the Tsuga heterophylla zone (Dyrness et al., 1974). Access to the canopy was facilitated using the tree-climbing techniques described by Denison et al. (1972).

Aseptic samples (5-10 gm) of six major canopy substrates were collected from the low, middle, and top thirds of the tree on a seasonal basis, designated as: I: January 1977, II: April 1977, III: August 1977, IV: November 1977, V: January 1978, and VI: April 1978. Samplings I and II were taken during a severe drought. The samples selected were (1) bark from both wet and dry sides of the trunk; (2) green foliage; (3) moss represented by Antitrichia sp.; (4) lichens with a green phycobiont composite of Alectoria sarmentosa (Ach.) Ach., Hypogymnia imshaugii Krog, Sphaerophorus globosus (Huds.) Vain., Parmelia sp., and Platismatia sp.; (5) cyanophilic lichens (containing a blue-green algal phycobiont) represented by Lobaria oregana (Tuck.) Mull. Arg.; and (6) perched soil, a collection of debris in various stages of decomposition analogous to litter. Biomass and chemical characterization of these substrates are listed in Table 1.

Bacterial populations were determined by serial dilutions of the various substrate samples (in 0.1% peptone) onto duplicate plates of one-third strength Tryptic Soy Agar (Difco) for total plate counts; the fluorescent pseudomonad medium of Sands and Rovira (1970); and starch-casein agar for Streptomyces (Kuster and Williams, 1964). All media were adjusted to pH 5.0 to approximate the substrate pH and incubated at

20 C. Resultant colonies were counted and representative isolates were selected and purified based on morphology, size, and pigmentation. Populations of nitrogen-fixing bacteria were estimated using the three tube replicate 'most probable number' (MPN) technique for positive acetylene reduction. Anaerobes were cultured in Hino-Wilson medium (Campbell and Evans, 1969; substituting glucose for sucrose and supplemented with 0.01% yeast extract) while aerobes were grown on Dobereiner's malate medium without bromthymol blue (Dobereiner, 1974). Representative isolates that demonstrated acetylene reduction were purified and characterized by standard biochemical and morphological techniques (Lenette et al., 1974) while generic identification of the isolates was performed according to Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974).

Rates of nitrogen fixation were measured from 0.5 to 1.0 gm samples collected at three heights in the canopy at each sampling period for: the bacterial substrates, 1-2 cm diameter dead twigs, the mosses Dicranum and Isothecium, and the hepatics Porella and Scapania. The acetylene reduction assay (Hardy et al., 1973) was incorporated to determine presumptive nitrogen fixation. Samples were incubated in an atmosphere of 1:10, acetylene:air, gas phase at 20 C. After 24 hrs, gas samples were assayed for ethylene on a Gowmac 750 flame ionization detector gas chromatograph fitted with a Poropak column (Waters Associates). Species of lichens suspected of having a blue-green algal phycobiont and therefore capable of nitrogen fixation were assayed as above except that the incubation period was 4 hr at 20 C under 2000 lux illumination.

Cellulase and xylanase activities were measured for all six substrates during the last four sampling periods using the methodology of Spalding

(1978). The procedure was modified to utilize the higher extraction efficiency of citrate buffer and the reaction was performed at 20 C.

In addition to the biochemical tests, bacterial isolates were tested for utilization of 11 sugars, 5 polyols, 6 polysaccharides, and 3 aromatic compounds associated with the tree substrates. All tests were performed on a basal salts media composed of (gm/liter): $(NH_4)_2SO_4$ 2.0, K_2HPO_4 1.0, $MgSO_4$ 0.5, NaCl 0.1, $CaCl_2$ 50 mg, $FeSO_4$ 5 mg, $ZnSO_4$ 50 µg, $CuSO_4$ 50 µg, $NaMoO_4$ 10 µg, $MnSO_4$ 10 µg, and H_3BO_3 10 µg. The sugars and polyols were used at 0.5% (w/v), the aromatics at 0.05% (w/v) and the polysaccharides at 0.2% (w/v). The Streptomyces and fungal isolates were tested for polysaccharide utilization on the basal media of Kuster and Gottlieb (1948). Polysaccharide hydrolysis was confirmed by measuring release of reducing sugars with dinitrosalicylic acid (Bernfield, 1955). Arylsulfatase activity was determined by the release of chromogenic p-nitrophenol (Tabatabai and Bremner, 1970) in a media composed of (per liter): glucose 10 gm, $(NH_4)_2HPO_4$ 3 gm, NaCl 2 gm, $MgCl_2$ 1.4 gm, K_2HPO_4 2 gm, $Zn(C_2H_3O_2)_2$ 16 mg, $MnCl_2$ 8 mg, $FeCl_3$ 8 mg, $(NH_4)_6Mo_7O_{24}$ 8 mg, $CoCl_2$ 8 mg, EDTA 40 mg, $CaCl_2$ 8 mg. Additional buffering was provided by 100 ml/liter 0.2 M, pH 7.0 Na-phosphate buffer. The components of the media were sterilized separately, after which 0.1% filter-sterilized p-nitrophenol sulfate was added.

3.3 RESULTS

The seasonal, total bacterial populations, averaged for the lower, middle, and top third of the canopy, are presented in Figure 1. During the droughty winter and spring of 1977, the populations were much lower than during the subsequent three samplings when precipitation had returned to near normal levels. In the course of the study, populations ranged from: recovering only fungi in contrast to 2.4×10^5 bacterial colony-forming units (CFU)/gm on bark, 24 to 270×10^3 CFUs in foliage, 3.3 to 200×10^5 CFUs on the cyanophilic lichen Lobaria oregana, 13 to 270×10^5 CFUs on non-cyanophilic lichens, 23 to 390×10^5 CFUs in moss, and 19 to 610×10^5 CFUs on perched soil. There was no obvious vertical distribution of total bacterial counts during the first four sampling periods. In the last two samplings, all substrates yielded 1.4 to 2.6 times greater plate counts in the bottom third of the canopy as compared to the top third, while the mid-canopy valves were intermediate. The only other topographic variation was between the dry- and wet-side bark, which, during the first three sampling periods, yielded only fungi from the dry-side and both bacteria and fungi from the wet-side (Pike et al., 1975).

Fluorescent pseudomonads were recovered regularly only from moss and perched soil and represented 0.015 to 1.8% of the total bacterial plate count. Occurrence in the other substrates was sporadic through both vertical distribution and time. Likewise, streptomycetes were recovered from moss and perched soil at each sampling and sporadically from the other substrates. Colony counts ranged from 0.005 to 2.2% of the total plate counts.

Populations of nitrogen-fixing microorganisms, as assessed by positive acetylene reduction of MPN tubes, were extremely low and sporadic in occurrence. Moss, found only in the middle and bottom portions of the canopy, yielded no nitrogen-fixing bacteria in the summer (period IV) to a maximum of 4000 nitrogen-fixing bacteria/gm recovered in the wet spring (period V). During periods V and VI, smaller populations (maximum 700/gm) were recovered from perched soil in the lower canopy and once from Lobaria oregana.

No measurable acetylene reduction was observed from any of the substrates examined except for Lobaria oregana. The glucose amendment procedure recovered nitrogen-fixing bacteria only from 5-year-old needles during sampling periods II and VI and from Lobaria oregana during period VI. No nitrogen-fixing bacteria were recovered from bark or non-cyanophilic lichens. Single sample measurements of acetylene reduction rates by cyanophilic lichens collected in the canopy are listed in Table 2. Eleven species in six genera were found capable of reducing acetylene and therefore presumably of nitrogen fixation at rates ranging from 151 to 1420 nmoles C_2H_4/gm/hr.

Of the 562 selected bacterial isolates, the dominant groups were the aerobic, gram negative, yellow-pigmented rods Flavobacterium and Xanthomonas (35.2%); Arthrobacter-like pleomorphic rods (30%), and Bacillus sp. (7.8%). Members of the Alcaligenes-Achromobactar group, Aeromonas, Chromobacterium (C. lividum), Micrococcus (M. luteus and roseus), and Pseudomonas were less frequently isolated (Table 3). The only organisms producing a soluble fluorescent pigment on the appropriate medium was Pseudomonas fluorescens and, although two morphological types

were recovered (one smooth and one granular), both strains were biochemically identical. Only one type of streptomycete was isolated from any of the tree substrates. Morphologically, all were members of the genus Streptomyces, with white pigmented spores and simple flexus morphology of the aerial mycelium. Confirmed nitrogen-fixing bacteria from the MPN series and glucose enrichments were, most commonly, Enterobacter agglomerans, Bacillus polymyxa, and an unidentified gram negative rod which did not produce acid from carbohydrates. Citrobacter freundii was recovered from moss only during the last two samplings. Fungi were isolated occasionally from all substrates, but most commonly from bark, moss, and perched soil. Penicillium sp., Monascus sp., Trichoderma sp., and an unidentified imperfect were the most frequently isolated genera.

Extractable cellulase and xylanase activities from moss and perched soil were consistently measurable during the four periods assayed (III-VI). In Figure 2, the respective linear regressions are plotted. There were high correlations ($r = 0.94$ for moss and 0.81 for perched soil) between cellulase and xylanase activity, with peak activities occurring during period V, not coincident with peak bacterial populations (Figure 1b, c). Carbohydrase activities were also detected in both cyanophile and non-cyanophile lichen samples, but not during all periods. Lower activities in the lichens ranged from (micromoles reducing sugar released/gm/hr) 5.6 to 34.2 generated by cellulase and 2.8 to 23.2 from xylanase in the cyanophile and 4.7 to 32 for cellulase and 3.6 to 42.3 for xylanase in the non-cyanophiles. The regression coefficients were $r = 0.78$ and $r = 0.75$, respectively.

The carbohydrate utilization tests are presented in Table 4 and, in addition to these results, 61.9% of the isolates produced ammonia from

protein, 41.5% were proteolytic (gelatin liquification), 18.3% possessed an active nitrate reductase system, 40.2% possessed an arylsulfatase, 3% could produce hydrogen sulfide from cysteine, and 47.7% were lipolytic (Tween 80). The fluorescent pseudomonads could utilize all of the simple sugars and polyols except cellobiose and dulcitol; utilized only protocatechuic acid, and hydrolyzed none of the polysaccharides. This group could also ammonify, liquify gelatin, and hydrolyze lipids and arylsulfate esters. The Streptomyces and fungal isolates were generally capable of hydrolyzing a variety of plant and lichen related polysaccharides (Table 5). The hemicelluloses, arabinogalactan, galactomannan, and xylan were attacked by more isolates than the structural glucans cellulose (B-1,4) and lichenin (B-1,3, B-1,4). Arylsulfatase was produced by the Streptomyces isolate and a majority of the fungi.

3.4 DISCUSSION

The bacterial populations of the major substrates of the Douglas fir canopy are strongly affected by moisture and temperature. The droughty winter, spring, and summer of 1977 afforded a unique opportunity to examine the effects of moisture stress and the typically moist fall, winter, and spring of 1977-1978 provided data on more natural fluctuations. Populations in the wet winter and spring (Figure 1, samples IV, V, VI) were higher than the populations from the corresponding periods (I and II) during the dry winter and spring. The marked difference between populations from the dry and wet-side bark is another indication of the importance of moisture in bacterial growth. The dry-side, which is dry even in the rain, had a much lower population than the wet-side, which is wet during precipitation because of stem flow. Increasing temperatures from winter to spring were uniformly reflected in population increases during the wet sampling periods (IV, V, and VI). The same relationship occurred during the dry winter and spring (I and II) but not as consistently. Total plate counts from both cyanophilic and non-cyanophilic lichens declined from winter to spring. Summer (sample III) is usually warm and dry and this was reflected by lower populations in all cases except moss and wet-side bark. The occurrence of higher populations at the bottom of the canopy during the rainy winter and spring of 1978 is indicative either of leaching of nutrients downward or direct transport of entire microbial cells. If the latter is the case, then there may be a significant input of biologically active cells to the forest floor.

The overall magnitude of the various substrate populations is probably determined by a variety of factors such as carbohydrate availability, pH, nitrogen content, and the presence of possible antagonistic

substances. The lowest populations occurred on bark and foliage, which are direct surfaces of the tree, while the highest populations occurred on the perched soil, mosses, and lichens, which reside superficially on tree surfaces. The bark is acidic, very low in nitrogen (Table 1), and lacks large amounts of free carbohydrates as these are probably combined in cellulose and hemicelluloses. Foliage, conversely, has a higher pH, higher nitrogen content, and is a veritable factory of sugar compounds. However, the fir needle is covered by a waxy cuticle which may physically limit bacterial activity. The microbial populations of the superficial components are typically about two orders of magnitude greater than bark and foliage. Perched soil appears to be analogous to the litter on the forest floor in that it does undergo decomposition as evidenced by measureable cellulase and xylanase activity (Figure 2b). Hydrolysis of polysaccharides by these extracellular enzymes would provide simple carbohydrates. Mosses and lichens are active photosynthesizers and, without a protective cuticle, may produce exudates on which bacteria can feed. There was also detectable cellulase and xylanase activity associated with mosses and lichens. The cyanophile, Lobaria oregana, having the highest pH, the largest nitrogen content, and known to leach carbohydrates (Cooper, G. and G. C. Carroll, 1978) would seem the best bacterial substrate in the canopy; however, this is not the case. The bacterial plate counts indicated that both L. oregana and the non-cyanophilic lichens had lower populations than moss or litter. A possible explanation is that lichens are known to produce a variety of bacteriostatic compounds (Ashina and Shibata, 1954) that may inhibit microorganisms in situ.

The numbers of bacteria encountered in this study were comparable to two other forest microbial studies; <5 to 1.5×10^8 CFUs/gm in Douglas

fir litter (Wright and Bollen, 1961) and 0.25 to 3.9 x 10^7 bacteria/gm in the phyllosphere of a Danish beech forest (Holm and Jensen, 1972).

Since nitrogen is reported to be the major limiting nutrient in Pacific Northwest coniferous soils (Gessel et al., 1951), biological nitrogen fixation may provide a major input. No significant bacterial rates of nitrogen fixation (as measured by acetylene reduction) and only small and sporadic populations of nitrogen-fixing bacteria were found associated with any of the variety of canopy substrates tested. This is in sharp contrast to the work of Jones (1970, 1976; Jones et al. 1974) who reported high rates of nitrogen fixation by the foliage of Douglas fir and other trees in England. Denison et al. (1976) also reported nitrogen fixation (by acetylene reduction) associated with the micro-epiphytic coating on Douglas fir needles in Olympia, Washington (U.S.A.). It is probable that all significant nitrogen fixation in the canopy is through the agency of blue-green algae in certain lichens. Millbank (1977) listed 18 genera of nitrogen-fixing lichens, of which 6 were found in this study (Table 2). Lobaria oregana was the dominant canopy lichen and accounted for 55% of the cyanophile biomass and 51% of the total lichen biomass (Table 1). Annual nitrogen fixation rates by L. oregana have been estimated to be between 2 and 11 kg/ha/yr (Denison, 1973). One potential site of nitrogen fixation not investigated in this study was decaying heartwood in the tree trunk. Aho et al. (1974) found large populations of nitrogenfixing Enterobacter species associated with fungal decay in living white fir. Any significant nitrogen fixed at this site would probably be utilized by fungi and not directly available to canopy components.

The bacterial flora of the canopy substrates was similar to that routinely associated with soil systems. Arthrobacter and Bacillus were common genera in this study and in those reported for an English pine forest and mixed hardwood forest soils (Goodfellow, 1968; Hissett and Gray, 1973). Another dominant canopy group, the yellow-pigmented gram negative rods (usually classified as Flavobacterium or Xanthomonas) were also numerically dominant in a study concerned with beech leaves (Holm and Jensen, 1972). The authors also noted a virtual absence of Bacillus on the leaf surfaces. Lesser populations of Alcaligenes, Micrococcus, Streptomyces and Pseudomonas were described in the two English studies. The nitrogen-fixing bacteria are genera previously reported to be associated with forest soils or wood. Bacillus polymyxa has been reported from Douglas fir forest soils (Jurgensen and Davey, 1971), but in numbers probably too small to have any significant input. Members of the Enterobacteraceae, including Enterobacter agglomerans and Citrobacter freundii (in this study), have been isolated coincident with measureable acetylene reduction from various wood sources (Aho et al., 1974; Neilson and Sparell, 1976). No aerobic nitrogen-fixing bacteria were isolated from the fir canopy although in the tropical phyllosphere Azotobacteraceae are reported to provide major inputs of nitrogen (Ruinen, 1974).

Spalding (1977), in a study of enzymatic activities related to the decomposition of coniferous leaf litter, concluded that certain enzymes might be used to monitor decomposition processes. In examining 84 different mixed conifer litters, he found a sufficiently high regression coefficient ($r = 0.91$) between cellulase and xylanase activity to suggest that only these needed to be measured. Our data showed high regression coefficients for the activities of these two enzymes extracted from moss

and perched soil ($r = 0.94$, $r = 0.81$, respectively) but the slopes were different enough ($P<0.15$) to suggest that there might be substrate differences in these enzyme activities. The peak of observed enzymatic activities occurred in the winter of 1978 and subsequently declined in the spring, although the total plate counts increased during the same interval. This corresponded with the observation of Wright and Bollen (1961) who noted that, in changing from winter to spring, the peak fungal population distinctly preceeded those of the bacteria in the litter layer of a Douglas fir soil.

The canopy bacterial isolates were more frequently capable of utilizing simple carbohydrates than aromatics or polysaccharides (Table 1). Similar results have been noted in studies of isolates from Finnish Spruce forests (Niemela and Sundman, 1977) and English Pine forests (Goodfellow, 1968). The general inability of the bacterial isolates to utilize plant polysaccharides, except for starch (Table 4), and the ability of the Streptomyces and fungal isolates (Table 5) to use a variety of polysaccharides suggests that the latter are the major polysaccharide decomposers while the former primarily utilize simple sugars and polyols (whether free or as hydrolysis products). The combination of proteolysis and ammonification of protein, present respectively in 41.5% and 61.9% of the bacterial isolates, indicates a potential for rapid turnover of nitrogenous compounds in the canopy. Similarly, the ability to release sulfate from aromatic sulfate esters (40.2% of the bacterial isolates as well as all Streptomyces and most fungal isolates) indicates a potential for rapid mineralization of sulfur. The low incidence of hydrogen sulfide production (3%) suggests insignificant bacterial-mediated losses of this major nutrient.

This preliminary survey of the bacterial ecology of an old-growth Douglas fir canopy suggests the presence of taxonomically and biochemically diverse bacterial populations.

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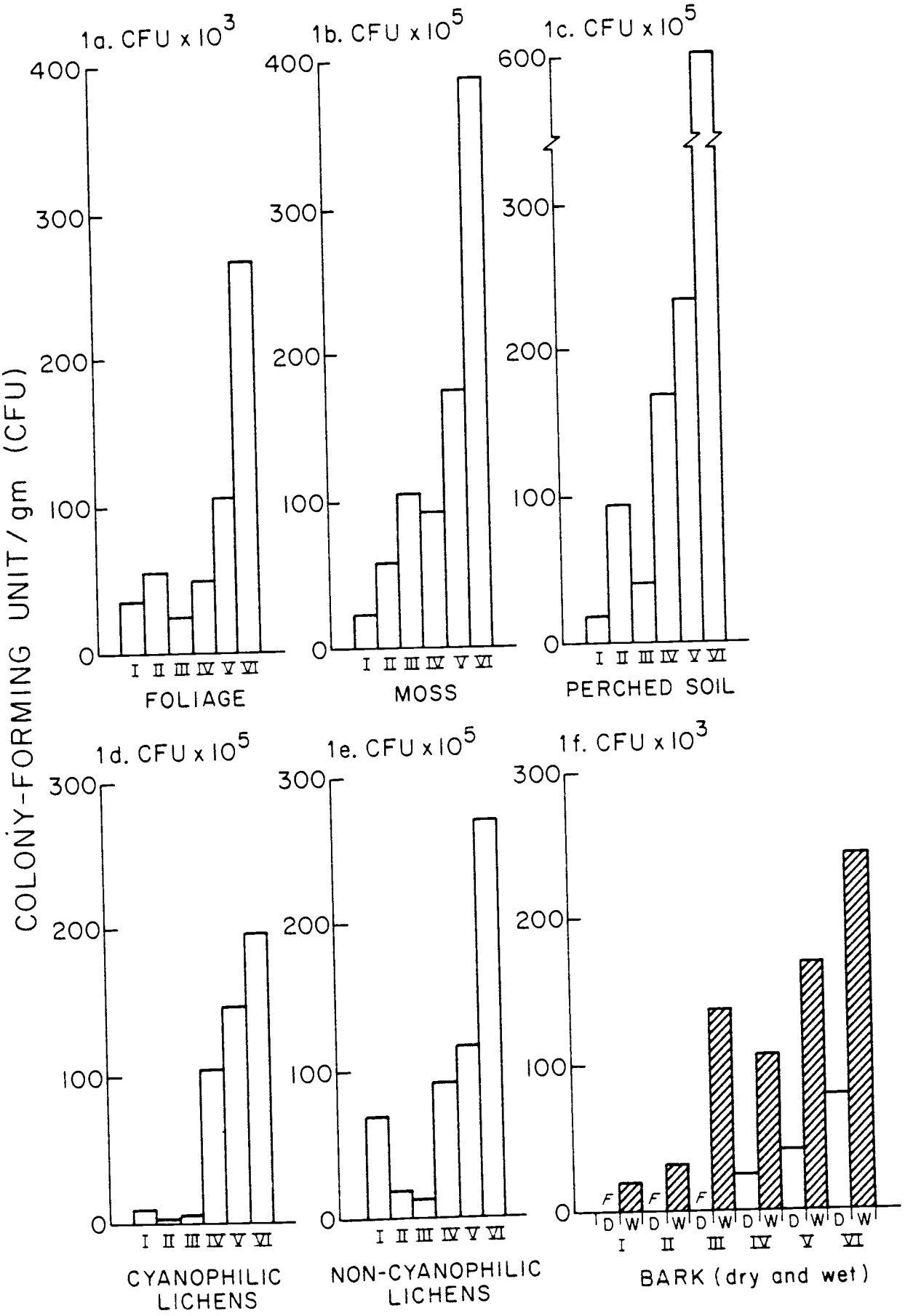
LEGEND

FIGURE 1. Average seasonal bacterial plate counts on six substrates from an old-growth Douglas fir canopy (averaged for the lower, middle, and upper regions of the canopy). Sampling periods: I: January 1977, II: April 1977, III: August 1977, IV: November 1977, V: January 1978, and VI: April 1978.

FIGURE 2. Extractable cellulase and xylanase activities of moss and perched soil substrates from the canopy of an old-growth Douglas fir. Sampling periods: III: August 1977, IV: November 1977, V: January 1978, and VI: April 1978.


1a. CFU x 10^3
1b. CFU x 10^5
1c. CFU x 10^5
COLONY-FORMING UNIT / gm (CFU)
400
300
200
100
0
600
I II III IV V VI
FOLIAGE
MOSS
PERCHED SOIL
1d. CFU x 10^5
1e. CFU x 10^5
1f. CFU x 10^3
CYANOPHILIC LICHENS
NON-CYANOPHILIC LICHENS
F
D W D W D W D W D W D W
BARK (dry and wet)

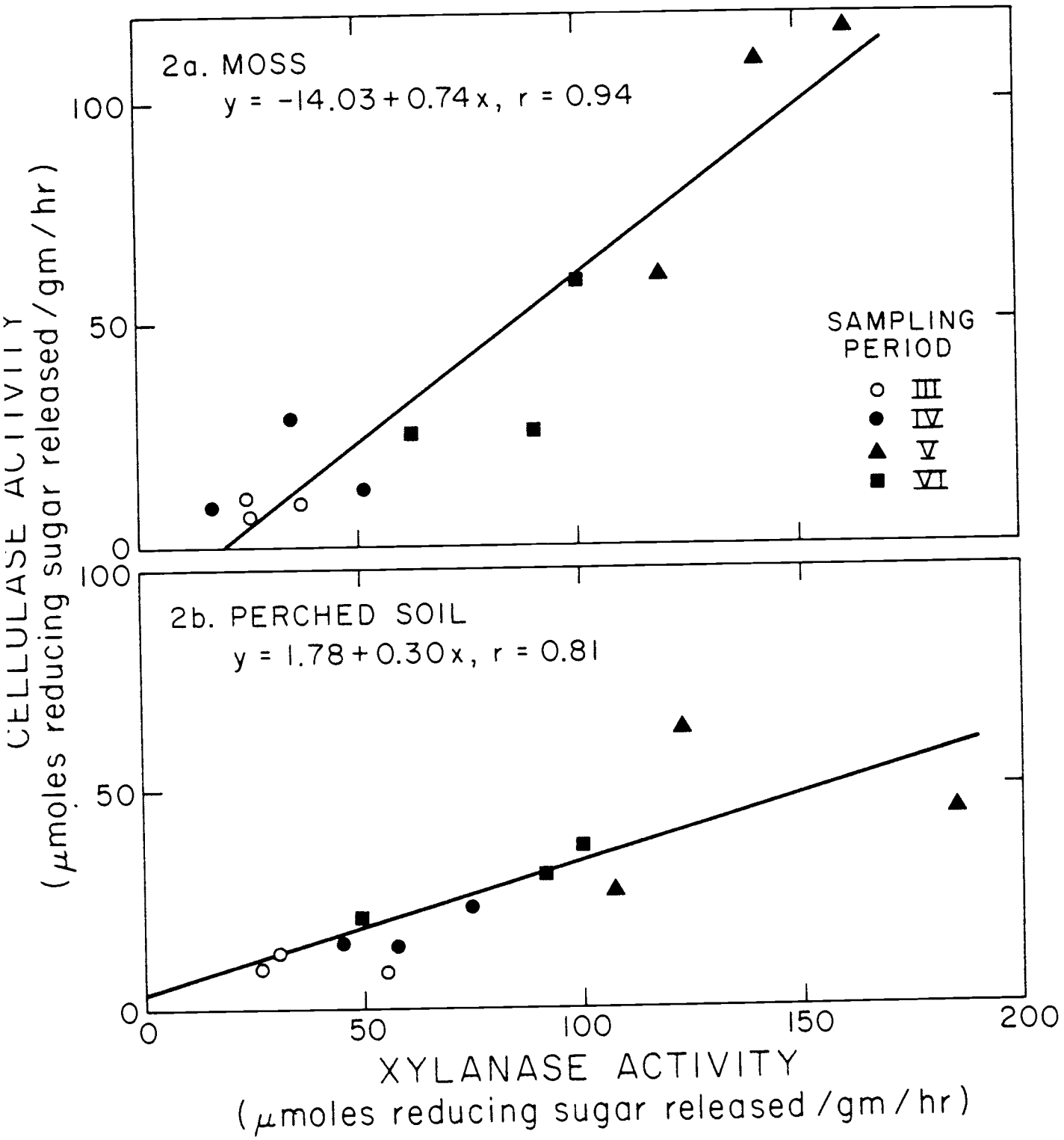

2a. MOSS
y = -14.03 + 0.74x, r = 0.94
SAMPLING PERIOD
III
IV
V
VI
2b. PERCHED SOIL
y = 1.78 + 0.30x, r = 0.81
CELLULASE ACTIVITY
(μmoles reducing sugar released/gm/hr)
XYLANASE ACTIVITY
(μmoles reducing sugar released/gm/hr)
0
50
100
150
200

Table 1. Composition of the canopy substrates sampled for bacterial populations.

Substrate	Biomass (kg)[1]	pH[2]	%N[3]
Bark	1820[4]	3.8	0.21
Foliage	186.4	4.8	1.00
Non-cyanophilic lichens	2.1	5.1	0.52
Lobaria oregana (total cyanophiles)	15.0 (27.4)	5.4	2.19
Moss (*Antitrichia* sp.)	2.1	4.5	0.78
Perched soil	0.748[5]	4.3	0.99

[1]Based on visual estimation of epiphyte surface area for each branch system (R. Rydell, unpublished data).

[2]1:2.5, substrate:distilled water.

[3]Kjeldahl nitrogen.

[4]Calculated from equation by Grier and Logan, 1977.

[5]Based on a biomass volume of 4987 cm^3 and a density of 0.15 gm/cm^3.

Table 2. Nitrogen-fixing lichens from the canopy of an old-growth Douglas fir.

Lichen species	Acetylene reduction[1] (nmoles C_2H_4/gm/hr)
Lobaria oregana (Tuck.) Mull. Arg.	225
Lobaria pulmonaria (L.) Hoffm.	544
Lobaria scrobiculata (Scop.) DC.	1200
Nephroma bellum (Spreng.) Tcuk.	151
Nephroma sp.[2]	347
Parmeliella saubinetti Zahlbr.	1420
Peltigera leucophlebia Gyeln.	842
Pseudocyphellaria anomola Magn.	971
Pseudocyphellaria anthraspis (Ach.) Magn.	269
Pseudocyphellaria rainierensis Imsh.	212
Sticta weigelii (Ach.) Vain.	713

[1]Samples incubated 4 hrs at 20 C, 2000 lux illumination.

[2]Previously undescribed species.

Table 3. Occurrence of major bacterial taxa on canopy substrates.[1]

Bacterial group	Bark	Foliage	Lichens		Moss	Perched soil
			Cyanophilic	Non-cyanophilic		
Gram negative, yellow pigmented rods	+	*	*	*	*	
Arthrobacter-like rods	+	*	+	+	*	*
Gram positive spore-forming rods	+	+	(+)	(+)	*	*
Gram negative aerobic rods	+	+	+	+	*	*
Chromobacterium sp.	-	(+)	(+)	(+)	+	+
Micrococcus sp.	-	(+)	-	-	+	+
Pseudomonas fluorescens	(+)	-	+	+	*	*
Streptomyces sp.	(+)	-	(+)	+	*	*
Nitrogen-fixing bacteria						
Enterobacter agglomerans	-	(+)	(+)	-	+	+
Bacillus polymyxa	-	-	-	-	+	+
Citrobacter freundi	-	-	-	-	+	-
Unidentified gram negative rod	-	-	-	-	+	+

[1]Key: [*] - isolated in all samples; [+] - not always isolated; [(+)] - single isolation; [-] - not recovered.

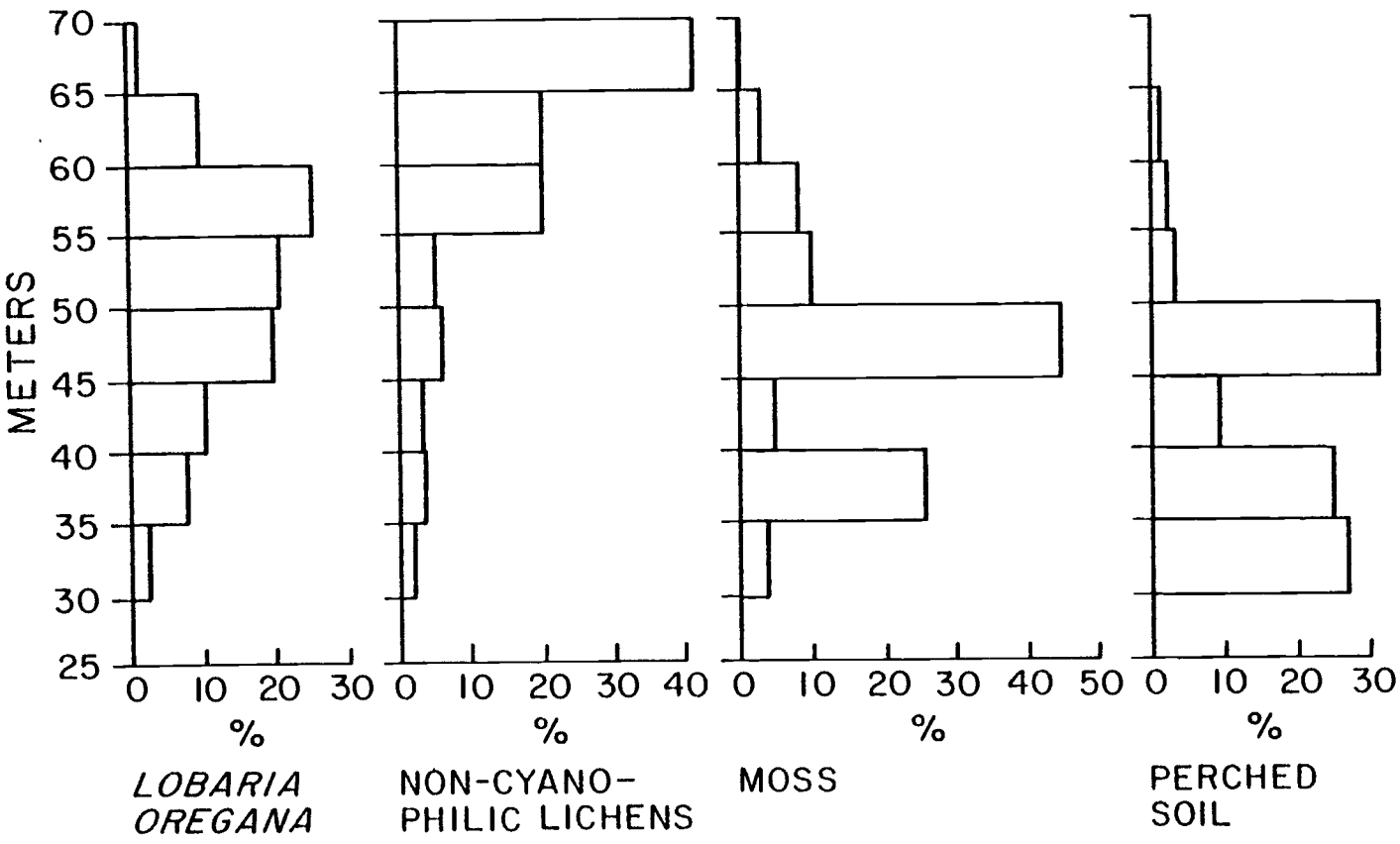
Table 4. Percentage positive results among the 562 isolates tested for carbohydrate utilization.

Test		% positive
Utilization of	Arabinose	87.4
	Rhamnose	56.8
	Xylose	67.1
	Fructose	83.8
	Galactose	96.8
	Glucose	98.0
	Mannose	96.8
	Cellobiose	53.6
	Sucrose	82.7
	Trehalose	64.1
	Raffinose	72.8
	Adonitol (= Ribitol)	29.9
	Arabitol	55.5
	Dulcitol	0
	Inositol	39.9
	Mannitol	69.0
Hydrolysis of	Arabinogalactan (Larch)	14.0
	Cellulose (carboxymethyl-)	0
	Galactomannan (locust bean)	5.3
	Lichenin	2.4
	Starch	52.5
	Xylan	12.7
Utilization of	p-hydroxy benzoic acid	28.7
	protocatechuic acid	27.8
	Vanillin	1.3

Table 5. Biochemical activities of canopy *Streptomyces* and fungal isolates.

Organism	Arylsulfatase	Hydrolysis					
		Arabinogalactan	Cellulose	Galactomannan	Lichenin	Starch	Xylan
Streptomyces sp.	+	+	+	+	+	+	+
Penicillium sp.	-	+	-	+	-	+	+
Monascus sp.	+	+	-	+	-	+	+
Trichoderma sp.	+	+	+	+	+	+	-
Unidentified fungus	+	+	-	+	-	+	-

APPENDIX 1. Distribution of substrate biomass in an old-growth Douglas fir canopy.



APPENDIX 2. Mean seasonal plate count (colony-forming units/gm)

SUBSTRATE	Sampling Periods I	II	III	IV	V	VI
BARK dry	F	F	F	25×10^3	41×10^3	78×10^3
wet	21×10^3	33×10^3	134×10^3	105×10^3	167×10^3	167×10^3
FOLIAGE	35×10^3	55×10^3	24×10^3	50×10^3	105×10^3	266×10^3
LICHEN with BLUE-GREEN ALGAE	11×10^5	3.3×10^5	4.6×10^5	104×10^5	145×10^5	200×10^5
LICHEN with GREEN ALGAE	69×10^5	19×10^5	13×10^5	90×10^5	115×10^5	270×10^5
MOSS	23×10^5	57×10^5	105×10^5	93×10^5	175×10^5	390×10^5
PERCHED SOIL	19×10^5	97×10^5	38×10^5	194×10^5	234×10^5	610×10^5

APPENDIX 3. Populations of fluorescent pseudomonads and *Streptomyces* as percentage of total plate count (Appendix 2)

SUBSTRATE		Sampling Period I	II	III	IV	V	VI
BARK	PsF[1]	-	-	-	-	-	0.15*
	Str[2]	-	-	-	-	-	0.39*
FOLIAGE	PsF	-	-	-	-	-	-
	Str	-	-	-	-	-	-
Lobaria oregana	PsF	0.75*	0.13*	-	1.4	0.83	0.45
	Str	-	-	-	-	-	0.25*
LICHENS with GREEN ALGAE	PsF	0.9*	-	-	1.1	0.63*	0.28*
	Str	-	2.1	0.75*	0.85	0.58*	0.12*
MOSS	PsF	1.1	0.27	0.67	1.8	1.0	1.0
	Str	0.74	0.48	0.35	0.39	0.5	0.75
PERCHED SOIL	PsF	1.2	0.36	1.5	0.8	0.63	0.015
	Str	1.1	2.2	0.065	0.13	0.004	0.005

* Organisms did not occur at all three heights in the canopy

1 Fluorescent Pseudomonads

2 *Streptomyces*

APPENDIX 4. Nitrogen-fixing lichens from the Douglas fir canopy.

Lichen Species	Acetylene Reduction[1] (Nmoles C_2H_4/gm/hr)	Present in GALADRIEL
Lobaria oregana (Tuck.) Mull. Arg.	225	+
Lobaria pulmonaria (L.) Hoffm.	544	+
Lobaria scrobiculata (Scop.) DC.	1200	+
Nephroma bellum (Spreng.) Tuck.	151	+
Nephroma helveticum var. sipeanum (Gyeln.) Wetm.	117	
Nephroma laevigatum Ach.	1290	
Nephroma parile (Ach.) Ach.	480	
Nephroma resupinatum (L.) Ach.	1110	
Nephroma sp.[2]	347	+
Parmeliella saubinetii Zahlbr.	1420	+
Peltigera leucophlebia Gyeln.	842	+
Pseudocyphellaria anomala Magn.	971	+
Pseudocyphellaria anthraspis (Ach.) Magn.	269	+
Pseudocyphellaria crocata (L.) Vain.	240	
Pseudocyphellaria rainierensis Imsh.	212	+
Sticta fuliginosa (Dicks.) Ach.	1120	
Sticta weigelii (Ach.) Vain.	713	+

[1]Samples incubated at 20°C; 2000 lux illumination

[2]Previously undescribed species

Carbohydrase activity (micro moles reducing sugar released/gm/hr)

Sampling Period	III		IV		V		VI	
	Cell[1]	Xyln[2]	Cell	Xyln	Cell	Xyln	Cell	Xyln
ML[3]	12.5	25.0	12.4	51.3	60.6	120	25.9	89.7
MM	9.9	37.3	8.4	17.5	117	162	59.4	100
MH	6.7	28.5	27.2	35.0	109	142	24.5	62.6
PL	12.5	30.5	23	74.5	64.2	123	36.7	99.7
PM	8.0	55.5	13.8	57.9	45	183	30.1	92.4
PH	9.2	27.2	14.7	44.8	26.4	107	20.3	49.8
L_bL	-	-	-	-	-	-	14.7	14.7
L_bM	10.6	18.9	-	-	-	-	34.2	23.2
L_bH	5.6	2.8	-	-	-	-	14.7	13.4
L_gL	4.7	5.3	-	-	18.3	27.0	4.8	21.7
L_gM	-	-	-	-	20.5	30.7	16.9	42.3
L_gH	5.3	3.6	-	-	32.0	36.7	14.1	12.4

[1] Cellulase activity against carboxy methylcellulose

[2] Xylanase activity against Larch xylan

[3] M - moss; P - perched soil; L_b - cyanophilic lichen; L_g - non-cyanophilic lichen; L, M, H - low, middle, high position in canopy.

Regression equations	Cellulase (Y), Xyanlase (X)	
Moss:	Y = -14.03 + 0.74X;	r = 0.938
Perched soil:	Y = 1.78 + 0.30X;	r = 0.812
Cyanophilic lichen:	Y = -0.16 + 1.10X;	r = 0.775
non-Cyanophilic lichens:	Y = 3.37 + 0.5X;	r = 0.748