

THE EFFECTS OF ACID RAIN ON NITROGEN FIXATION
IN WESTERN WASHINGTON CONIFEROUS FORESTS¹

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ABSTRACT

We investigated both the current status of nitrogen fixation in western Washington forests, and the potential effects of acid rain on this vital process.

Even the low concentrations of sulfur dioxide presently found in the Northwest are thought to have an adverse effect on nitrogen fixation by limiting the distribution of the epiphytic nitrogen-fixing lichen, *Lobaria pulmonaria*, which is found mainly in deciduous forests. A close relative, *L. oregana*, was found to be the major nitrogen fixer in old-growth coniferous forests. It fixes less nitrogen following exposure to sulfuric acid of pH 4 or less.

A more serious threat to nitrogen fixation than acid rain is the practice of deliberately suppressing red alder to keep it from competing with Douglas fir. Also, *L. oregana* is a late successional species and does not develop in forests where short cutting cycles are practiced.

INTRODUCTION

One possible effect of acid precipitation mentioned in the Report of the Swedish Preparatory Committee for the U.N. Conference on the Human Environment (Engstrom, 1971) was a depressing effect on nitrogen fixation. We set out to determine whether that was likely to be the case in western Washington coniferous forests.

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Since nitrogen is a major limiting factor in northwest coniferous forests (Gessel et al., 1951), a decrease in N_2 -fixation could seriously affect the forest ecosystem.

The effects of acid rain on nitrogen transformations other than fixation should also be considered in evaluating the effects of acid rain on the nitrogen economy of an ecosystem. Nitrification is inhibited by low pH (Alexander, 1967), which might result in conservation of nitrogen since nitrate is relatively soluble and more readily lost to streams or groundwater in some ecosystems (Likens, et al., 1970). Also, if oxides of nitrogen, rather than sulfur, are the source of the acid, then there will be an increased input of nitrate in rainwater. This is often the case in the northeastern U.S. (Likens, 1975). However, the nitrate ion is rare in rain in our area (Cole and Johnson, 1974). Our study was limited to nitrogen fixation by free-living microorganisms and by lichens.

SITE DESCRIPTIONS

QUINAULT

The Quinault site lies on the west side of the Olympic Peninsula at an elevation of about 100 m, in an old-growth spruce/hemlock forest with some remaining old-growth Douglas fir. The annual precipitation of about 4.5 m falls mostly in the form of rain and is largely free of man-made pollutants.

The litter and humus layers are well developed and extend to a depth of 20 cm. The soil is fine, dark, and free of rocks.

The mixed overstory includes *Picea sitchensis*, *Tsuga heterophylla*, *Thuja plicata*, *Pseudotsuga menziesii*, *Alnus rubra*, *Rhamnus purshiana*, and *Acer circinatum*. The understory consists of *Polystichum munitum*, *Blechnum spicant*, *Rubus spectabilis*, *Vaccinium parvifolium*, *Gaultheria shallon*, *Oxalis oregana*, *Trillium ovatum*, and mosses.

CENTRALIA

The Centralia site lies about 10 km northeast of the new coal-fired power plant near the town of Centralia, in a second-growth Douglas fir forest. The elevation is about 500 m and the topography is steep and dissected. The annual precipitation is considerably less than at Quinault and is presumably acidified by sulfur emissions from the power plant. This site was chosen to provide baseline data for evaluating the effects of the power plant, as it is unlikely that there have been major effects on the ecosystem to date.

The litter layer in this area is less than one centimeter deep. The soil is dark and rich in humus.

The overstory vegetation consists of *T. heterophylla*, *P. menziesii*, *Acer macrophyllum*, and *A. rubra*. The understory includes *T. ovatum*, *Montia* sp., *Smilacina racemosa*, *V. parvifolium*, *Disporum hookeri*, *R. spectabilis*, *Galium triflorum*, *Berberis aquifolium*, *Asarum caudatum*, *Dicentra formosa*, and saxifrage.

CEDAR RIVER

The Cedar River site lies in the foothills of the Cascade Mountains east of the metropolitan area of Seattle-Tacoma. The site is in an even-age Douglas fir forest on a level site within the University of Washington's Thompson Experimental Forest, at an elevation of 200 m.

The ground is extremely rocky and there is no definite litter layer. The soil is light in color and contains little organic matter.

The overstory contains *P. menziesii* and *A. circinatum*. The understory consists of *Holidiscus discolor*, *P. munitum*, *Pteridium aquilinum*, *Rubus ursinus*, *G. shallon*, *R. spectabilis*, *Linnea borealis*, and mosses.

RAIN, THROUGHFALL, AND SOIL PH

METHODS

Rain pH was monitored in Lacey (adjacent to Olympia) from November 5 to December 11, 1974. Samples were collected daily and frozen until shortly before the pH was measured. All pH measurement was by the glass electrode method.

Although it seldom rains in Washington during the summer, we put out polyethylene bottles with funnels at each of our study sites to collect rainfall and throughfall. Rainfall and throughfall, if any, were brought back to the laboratory for pH determination.

Measurement of soil and litter pH followed the method of C.B. Davey (1974). Twenty milliliters of soil are stirred for thirty minutes with enough distilled water to give "the consistency of heavy cream." pH is then measured using the glass electrode method.

RESULTS AND DISCUSSION

The frequency distribution of rain pH in Lacey, Washington weighted according to volume, is shown in Figure 1. While the rain during the

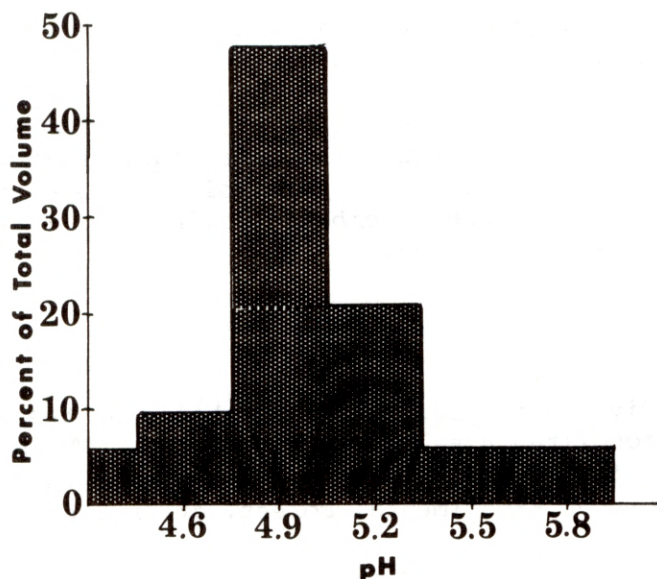


Figure 1. Frequency distribution of rain pH for Lacey, Washington, from November 5 to December 11, 1974.

sampling period was not startlingly acid, it appears that rain pH in this area is no longer under carbonic acid control.

Table I summarizes the pH values we found for rain, throughfall, litter, and soil at our three study sites.

TABLE I

pH values for rain, throughfall, litter, and soil.

SITE	SAMPLE	MEDIAN	MAX	MIN	N
Guinault	rain	5.76	5.47	6.30	4
	throughfall	5.10	4.20	6.24	11
	litter	4.24	3.93	4.34	4
	soil	4.70	4.13	5.76	6
Centralia	throughfall	4.66	4.58	5.62	5
	litter	4.64	4.22	5.15	3
	soil	6.02	5.84	6.04	3
Cedar River	rain	4.24	4.24	4.24	1
	throughfall	4.62	4.09	6.95	5
	soil	5.17	4.28	5.94	5

Although limited, our data are consistent with the hypothesis that the coniferous forest canopy buffers normal rain to a lower pH value, and acid rain to a higher pH value. Both rain and throughfall were less acid at Quinault than at the two second-growth sites, yet the litter and soil were more acid at Quinault.

PH BUFFERING STUDY

Acid rain does not ensure that soil microorganisms will be exposed to lowered pH, at least not immediately. pH buffering by the forest canopy has been reported by Eaton et al. (1973) and Cole and Johnson (1974). Additional buffering takes place in the litter and soil.

METHODS

We attempted to simulate the effects of acid rain on soil water in the laboratory, using apparatus patterned after that described by Tim Wood elsewhere in these Proceedings. Soil samples were placed in Buchner funnels on a turntable and rotated under a stream of "rain" adjusted with sulfuric acid to pH 3. Water passing through the soil or litter in the funnels was collected in beakers and pH measured by the glass electrode method. Rain was applied at the rate of about 0.5 cm/hr. The major difference between our methods and those of Wood is that we measured leachate pH at about ten minute intervals over the course of a several hour rain application, but only repeated the application once or twice for each soil sample.

RESULTS AND DISCUSSION

The results of this experiment were highly variable, but those of a typical application of simulated acid rain are shown in Figure 2. Over a period of four to eight hours the pH of water passing through our soil or litter samples dropped appreciably, in some cases nearly to the pH of the acid being applied.

However, the initial leachate pH of a second simulated acid rain application a day or two later was generally nearly as high as that for the first application. This is consistent with the findings of Wood and others that a great deal of acid can be applied to soil without permanently acidifying it. However, even the temporary acidification of soil water which we observed could be highly significant for soil microorganisms.

In an attempt to determine whether the pH recovery mechanism in soil and litter is biological in nature, we autoclaved some soil samples and repeated the acid treatment. Buffering was greatly

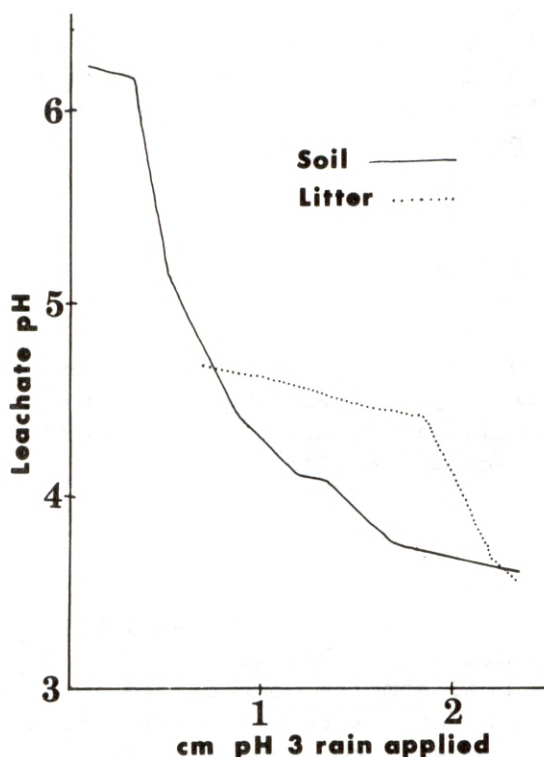


Figure 2. Leachate pH trends for soil and litter from the Centralia site. About 2.5 cm of simulated pH 3 "rain" was applied over a four hour period.

increased, to the extent that there was little decrease in leachate pH over several hours. Possibly lysis of cells occurred, releasing organic buffers.

C. O. Tamm (1975) suggests that rapid but temporary acidification of soil water can be explained by purely physical and chemical properties of the soil. If the soil is composed of large aggregates of particles, only a small fraction of the cation exchange sites are exposed to the acid applied in a given application. These sites are quickly leached, but a large number of unleached sites remain, which may in turn be exposed to cation exchange leaching if the soil is disturbed in any way between acid applications.

MEASUREMENTS OF NITROGEN FIXATION

GENERAL METHODS

Nitrogen fixation was measured by the acetylene reduction method (Hardy et al., 1968). This method is based on the fact that the nitrogenase enzyme is a powerful reducing catalyst. It will act on acetylene as a substrate and reduce it to ethylene. The rate of nitrogen fixation can be calculated from ethylene production.

Samples for which N_2 -fixation was to be determined were placed in 20 ml culture tubes stoppered with gas-tight rubber septums. The tubes were then evacuated by hand using a 50 cc syringe, and flushed with a

gas mixture of 10% acetylene, 20% oxygen, and 70% argon. Acetylene was generated by reacting calcium carbide with water and scrubbing the resulting gas with concentrated sulfuric acid. Oxygen and argon were industrial welding grade.

At the end of the incubation period, which varied from 30 minutes to 24 hours, ethylene production was measured by gas chromatography. A Gowmac model 750 gas chromatograph with flame ionization detector was used. A 2 m column packed with Poropak R was run at 50-60° C using helium as the carrier gas at a flow rate of 30 ml/minute. Typical retention times were 130 seconds for ethylene and 170 seconds for acetylene. Ethylene concentration was determined using acetylene as an internal standard, and ethylene production was calculated by subtracting the concentration of ethylene impurity in the acetylene. Calculated rate of nitrogen fixation was based on the theoretical ratio of 3 moles C_2H_4 produced per 1 mole N_2 fixed (Hardy et al., 1968).

Two modifications of our usual methods were used for some samples. First, for some of the epiphyte samples we added 0.05% CO_2 to the gas mixture. Second, for some of our later work, we simply injected 2 cc of acetylene into the stoppered tubes, rather than replacing the air in the tubes with gas mixture as above. The first modification gave slightly higher rates of ethylene production; the second gave slightly lower rates. Neither difference was statistically significant.

SOIL AND LITTER

Although buffering by the canopy may protect litter and soil from abnormally acid throughfall, we carried out a program of experimental acidification of soil and litter to see what effect it would have on N_2 -fixation.

Soil plots were isolated for experimental treatment by sinking plastic cylinders of about 30 cm diameter into the soil to a depth of 10-15 cm. One plot at each site was untreated and used as a control. The other four received 5.12 liters of either pH 6.0, pH 5.0, pH 4.0, or pH 3.0 water, applied with a sprinkling can, to simulate 5 cm of rain. The water was prepared with tap water adjusted to the correct pH with H_2SO_4 . Samples of litter and soil were collected for acetylene reduction analysis both immediately before and 30 minutes after the acid application. The above procedures were followed at each field site. Thus the acid treatment plots each received three applications of "acid throughfall" or a total of 15 cm. After rate of fixation was measured, samples were oven-dried and weighed.

There was no detectable N_2 -fixation in most of the soil samples. The rate of ethylene production prior to acid application, in moles C_2H_4 /g soil/hr, was 1.6×10^{-11} to 7.7×10^{-11} for Quinault, zero for Centralia, and zero to 1.4×10^{-11} for Cedar River. Fixation in

untreated litter was somewhat higher: 1.3×10^{-10} for Quinault, zero to 3.9×10^{-11} for Centralia, and zero to 1.9×10^{-10} for Cedar River.

Application of acid had little effect on the small amount of fixation taking place in the soil. Both soil and litter were very dry at Centralia and Cedar River, as is usual in western Washington in summer-time, and most of the water or acid applied was absorbed by the litter layer, leaving the soil dry. The effect on fixation in litter was more pronounced, and is shown in Figure 3. At the two second-growth sites,

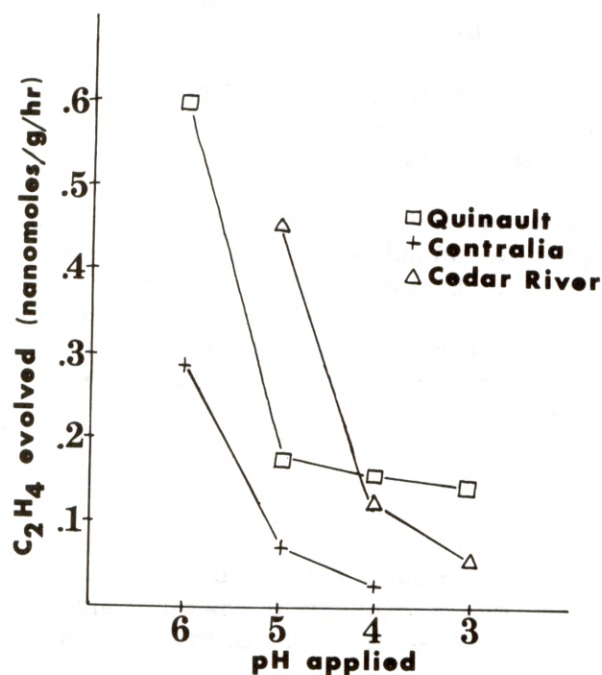


Figure 3. Rates of nitrogen fixation, expressed as ethylene production, for litter following application of 5 cm of water of the pH shown.

N_2 -fixation was actually stimulated by application of acid, but was stimulated more by dilute acid than by concentrated acid. At Quinault, where litter was more moist and initial rate of fixation greater than at the other two sites, there was a decrease in fixation after application of acid of pH 4 or lower. An extremely high rate of 3.7×10^{-9} moles C_2H_4 /g/hr, from the Centralia pH 3 plot, was omitted from the graph. This high rate does not indicate stimulation of fixation by acidity, since we found that the pH of the soil in that plot was actually higher than in the others, despite three applications of pH 3 "throughfall." Rather, this illustrates the heterogeneity of litter with respect to buffering and/or N_2 -fixing capacity.

Rates of nitrogen fixation were low in litter and even lower in soil. Moisture appears to be the major limiting factor, at least in summer, although pH is also important, fixation being inhibited by low pH. Application of large quantities of acid was not sufficient either to saturate the soil with water, or to effect a major change in pH.

NEEDLE SURFACE MICROORGANISMS

The surfaces of some conifer needles support a community of algae, fungi, bacteria, and micro-lichens. This community, collectively known as "scuzz" (Sherwood, 1971), is often present in quantities large enough to be plainly visible without magnification. We examined Douglas fir, *Pseudotsuga menziesii* needles by both light and scanning electron microscopy. Large bacteria were seen by light microscopy on slides prepared from scuzz. Under scanning electron microscopy the complex tangle of fungal hyphae and masses of algal cells could be seen. Sherwood and Carrol (1974) studied the fungal flora of needle surfaces. Jones (1970) found N₂-fixing bacteria on Douglas fir needles in Great Britain.

We found rates of acetylene reduction by scuzz of 2.1×10^{-9} moles/g needles/hr using our normal gas mixture, but only 1.7×10^{-10} moles/g/hr when oxygen was omitted. Rates of ethylene production by scuzz scraped from needles were from .87 to 1.3×10^{-8} moles/g scuzz/hr. These rates are substantially higher than those for litter.

EPIPHYTIC LICHENS

The epiphytic lichen *Lobaria pulmonaria* was reported by Millbank and Kershaw (1970) to fix nitrogen despite its lack of a blue-green phycobiont. They found that internal cephalodia in the lichen contain the N₂-fixing blue-green alga *Nostoc*. William Denison (1973) reported that the closely related species, *Lobaria oregana*, is abundant in the canopy of old-growth coniferous forests in Oregon, and makes a significant contribution to the nitrogen economy of these forests by fixing atmospheric nitrogen. He also describes a method of climbing old-growth trees with comparative ease and safety.

We climbed an old-growth *Picea sitchensis*, Sitka spruce, at the Quinault site and collected a supply of *L. oregana* for study purposes. The lichen is easy to maintain since it can be dried and kept indefinitely until ready to use. Fragments of lichen are simply moistened to restore metabolic activity, and were incubated in an environmental chamber under controlled conditions of temperature and photoperiod. The intensity of illumination used was 800 ft-candles.

To estimate the rate of fixation under winter conditions, we programmed the chamber for 9 hours of light at 10°C and 15 hours of dark at 5°C. For a 24 hour period, the weighted mean rate of ethylene production was 8.3×10^{-7} moles/g/hr. This is equivalent to 2.8×10^{-7} moles N_2 /g/hr, which is slightly more than the maximum rate reported by William Denison (1973), 2×10^{-7} . Thus, *L. oregana* fixes nitrogen at a rate roughly 1,000 times that of litter and 10,000 times that of soil.

We studied the effects of pH on N_2 -fixation by *L. oregana* and *L. pulmonaria*. Fourteen samples of each species were soaked in distilled water for one hour. The rate of fixation for each lichen fragment was then determined for a four hour incubation period at 20°C and 800 ft-candles. The lichens were then air dried overnight.

The next day, the same procedure was followed except that the lichens were soaked in water adjusted to pH 8, 6, 4, or 2 with NaOH or H_2SO_4 . The mean rate of fixation following this treatment, divided by the rate for distilled water soak, is shown in Figures 4 and 5. The graphs also show standard deviations. Rate of fixation appears to be lower at low pH. However, there is a great deal of unexplained

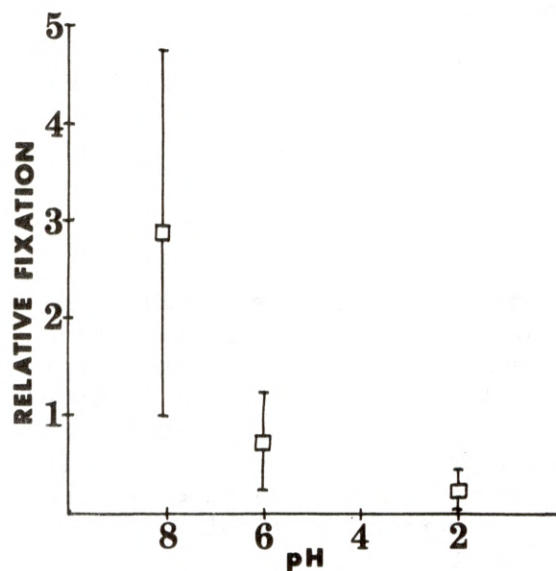


Figure 4. Short-term effects of pH on nitrogen fixation by *Lobaria oregana*.

variance.

After air drying the samples again, we repeated the distilled water soak to see whether the adverse effect of low pH lasted beyond the treatment itself. The results are shown in Figures 6 and 7. The rate of fixation after the second distilled water soak, divided by that for the first distilled water soak, is graphed as a function of the pH of the intermediate soak. The pH 2 treatment, at least, appears to have a severe and possibly permanent adverse effect on fixation.

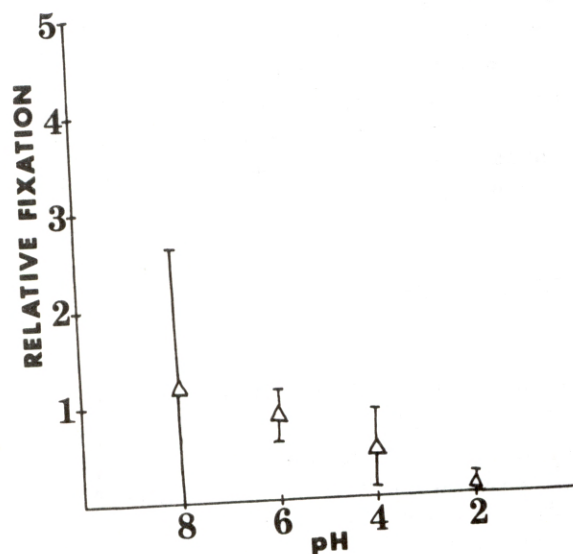


Figure 5. Short-term effects of pH on nitrogen fixation by *Lobaria pulmonaria*.

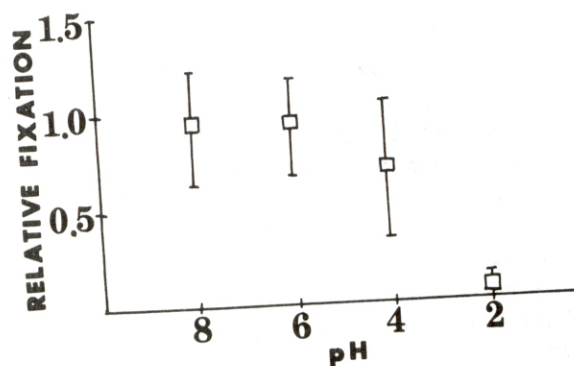


Figure 6. Lasting effects of pH on nitrogen fixation by *Lobaria oregana*.

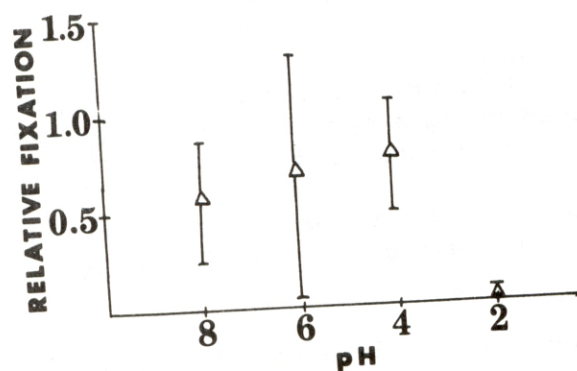


Figure 7. Lasting effects of pH on nitrogen fixation by *Lobaria pulmonaria*.

Other factors which might affect N_2 -fixation were also investigated. The optimum temperature for fixation seems to be between 20 and 30° C for both *Lobaria* species. The fixation rate is lower at low light intensities and zero when the lichens are dry. We believe that N_2 -fixation by these lichens is presently limited by temperature and possibly light in the winter, and by moisture in the summer. Acid rain could be important if it became more prevalent.

CULTURING OF NITROGEN-FIXING MICROORGANISMS

Despite low rates of nitrogen fixation in soil and litter, we attempted to culture the microorganisms involved, in order to identify them, study pH tolerance in pure culture, etc. Jurgensen and Davey (1971) found from zero to 3000 nitrogen-fixing bacteria per gram of soil in western Washington. We know of no reports of nitrogen-fixing blue-green algae from forest soils in this area.

BACTERIA

Soil samples were selected from the soil, humus, and litter layers at each site. 1.0 g of each sample was dissolved in 10 ml of distilled water. This solution was then further diluted 1:100, 1:1000, and 1:10000, with four plates being inoculated from each dilution. The following culture medium was used for bacteria:

900 ml	Basal salts medium (Aaronson, 1970)
1 ml	Micronutrient stock (Jurgensen and Davey, 1971)
100 ml	10% (w/v) dextrose (d-glucose) solution
10 ml	0.1% (w/v) $FeCl_3$ solution.

In addition, 1 ml of 1% (w/v) yeast extract was added to one-half of the plates. Some additional plates had biotin and thiamine added.

We incubated half the plates from each dilution aerobically and half anaerobically. We used a steel wool technique (Parker, 1955) to achieve anaerobiosis, which we confirmed using a methylene blue indicator. Both aerobic and anaerobic plates were incubated at 25 to 27°C.

After three days, small round mucoid colonies were observed on all of the plates. These were gram and spore stained and transferred to fresh plates and tubes.

When these cultures were tested by the acetylene reduction method for nitrogen fixation, no fixation was detected. Consequently, we did not attempt to identify the bacteria in the cultures. Davey (1974) points out that many bacteria will grow in "nitrogen-free" media, without the ability to fix nitrogen.

We did get measurable fixation by cultures of scuzz bacteria. From the appearance and rapid growth of the colonies, as well as the high rate of fixation, the decreased fixation when incubated in the absence of oxygen, and the large size of the bacteria, we speculate that a species of *Azotobacter* may be present in scuzz. The pH of throughfall and presumably of water flowing over the surfaces of conifer needles probably exceeds *Azotobacter's* tolerance for acid. However, perhaps among the masses of algae on the surfaces of some needles are sites where the micro-environment is more hospitable.

BLUE-GREEN ALGAE

Samples of soil, litter, and decaying wood were collected at each site and 0.5 g of each sample was swirled vigorously in a 50 ml sterile dilution blank for five minutes to distribute the organic matter. This solution was then used to inoculate 125 ml flasks with 0.75 ml aliquots and 50 ml serum jars with 0.25 ml aliquots.

A revised Chu #10 medium (Gerloff, et.al., 1950) was modified to exclude all sources of combined nitrogen by substituting CaCl_2 for the original $\text{Ca}(\text{NO}_3)_2$. The pH of one third of the media was left unadjusted at pH 7.5 to 9.0, while one third was adjusted with 0.1 N HCl to pH 7, and the final third was adjusted to pH 4.5, which was the approximate pH of the litter. One third of all the flasks contained a small amount of glass wool to serve as a physical substrate for algal growth (Bold, 1942).

All flasks were incubated at 23 to 25°C. The serum bottles were incubated in air-tight disiccators under a 1% CO_2 atmosphere, at 25°C.

Microscopic examination of the cultures revealed no algal growth after eight weeks. This supports the findings of M. A. Line (1971), who similarly failed to find nitrogen-fixing members of the phylum Cyanophyceae (blue-green algae) in acidic soils. The Cyanophyceae have been found to be viable in the limited pH range of 5.7 to 9.0 (Allison et. al., 1937). The pH values shown by the litter and soil layer in all three sites examined were considerably lower than that suggested for minimal growth. It is possible that some micro-habitats not examined could have pH values suitable for free-living blue-green algae, but it seems doubtful that significant amounts of nitrogen could be contributed thereby.

CONCLUSIONS

Rain in western Washington is slightly more acidic than one would expect under natural conditions. Increased urbanization and industrialization or relaxation of air quality standards could lead to increased precipitation acidity. Low pH can have an adverse effect on nitrogen fixation, but the severity of this effect depends on other factors as well, some of which are under man's control.

Let us consider in turn the various N_2 -fixers and the factors affecting each. It is important to keep in mind the relative quantities of nitrogen fixed by various ecosystem components. The amount of nitrogen contributed by an organism is the product of its rate of fixation and its abundance. Table II gives fixed nitrogen contributed to the

TABLE II

Nitrogen contributed by selected ecosystem components.

	Fixation rate (moles $C_2H_4/g/hr$)	Amount (kg/ha)	N_2 fixed (kg/ha/yr)
Centralia litter	3×10^{-11}	1×10^4	0.03
Cedar River soil	5×10^{-12}	1×10^6	0.4
Quinault litter	1×10^{-10}	1×10^5	0.8
Quinault soil	5×10^{-11}	1×10^6	4.1
<u>Lobaria oregana</u>	4×10^{-7}	4×10^2	12.0

three ecosystems studied, based on our estimates of mean annual fixation rate and quantity of soil, litter, and *Lobaria oregana*. The estimated amount of *L. oregana* is based on data published by William Denison (1973). For comparison, Zavitkovski and Newton (1968) estimated that *Alnus rubra*, red alder, contributes 100 kg N/ha/yr. We were unable to calculate a contribution for scuzz without knowing more about its abundance.

The low rate of N_2 -fixation in soil and litter is probably largely due to naturally acid conditions. *Azotobacter* and the blue-green algae are excluded by low pH. Moisture is also limiting in summer. Buffering by the canopy, litter, and soil may protect soil and litter microorganisms from the effects of acid rain, particularly if it is an infrequent occurrence.

Nitrogen fixation by *Lobaria oregana* would probably be decreased by acidic rain. In addition this species is highly sensitive to atmospheric sulfur dioxide. William Denison (1974) found that the closely related species *L. pulmonaria* was severely damaged by a five week exposure to 50 $\mu\text{g}/\text{m}^3$ of SO_2 . The main impact of man on this species, however, is destruction of its habitat, the old-growth forest. The cutting cycles currently practiced by the forest industry are not long enough to allow this species to develop. It might be possible to inoculate younger forests with the lichen to get some N_2 -fixation even with shorter cutting cycles.

Red alder also contributes large amounts of fixed nitrogen. It is frequently suppressed deliberately, because it competes with Douglas fir. Newton et al. (1968) discuss the coexistence of alder and Douglas fir. They also suggest longer cutting cycles. In this regard, the greater fixation in soil and litter at the old-growth site deserves mention. It is possible, however, that this is entirely due to higher rainfall in summer there than at the other two sites.

The significance of N_2 -fixation by needle surface microorganisms needs further investigation. We suspect that the amount of nitrogen fixed by these scuzz microorganisms is rather small. However, its ecological significance is increased by the possibility of direct foliar absorption. We have observed more scuzz near cities than in presumably less polluted areas. It is possible that some scuzz microorganisms use hydrocarbons from air pollution as an energy source. However, there is no evidence that N_2 -fixing microorganisms are so stimulated. Also, air pollution or acidic rain may damage cuticular wax which would otherwise limit the growth of scuzz.

Another interesting area for research is N_2 -fixation in recently logged areas. We found no detectable N_2 -fixation in a single investigation of soils in clearcut areas. However, some clearcuts have large numbers of N_2 -fixing members of the genera *Peltigera* and *Leptogium*. The ecology of these lichens is worthy of further study.

Lobaria pulmonaria is native to the deciduous forests of the Pacific Northwest. It is very abundant in the few remaining areas where it can tolerate the air pollution. Robert Denison (1975) reported that it appears to be limited to areas where the mean annual SO_2 concentration is less than 5 $\mu\text{g}/\text{m}^3$. Since its rate of fixation is about three times that of *L. oregana*, it could be an important input of fixed nitrogen if air quality were to improve dramatically.

It is clear that short cutting cycles, suppression of alder, and air pollution have resulted in a decrease in natural nitrogen fixation, with acid rain a possible threat in the future. It remains to be seen whether the practice of applying artificially fixed nitrogen to forests will be an adequate substitute. In any case, the high energy cost and pollution associated with artificial fixation should make preservation of natural nitrogen fixation a consideration in forest management and air

quality criteria.

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