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D. H. McNabb and K. Cromack, Jr.

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Dinitrogen fixation by a mature *Ceanothus velutinus* (Dougl.) stand in the Western Oregon Cascades[1]

D. H. McNabb[2]
Forestry Intensified Research Program, Oregon State University, 1301 Maple Grove Drive, Medford, OR, U.S.A. 97501

AND

K. Cromack, Jr.
Department of Forest Science, Oregon State University, Corvallis, OR, U.S.A. 97331

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Dinitrogen fixation was measured in a 17-year-old snowbrush, *Ceanothus velutinus* var. *velutinus* (Dougl.), stand in the Western Oregon Cascades. Diurnal and seasonal rates of nitrogenase activity were measured in the field using the C_2H_2 reduction technique. Snowbrush had a total biomass, estimated with equations developed, of 42 680 kg · ha^{-1}, including 750 kg · ha^{-1} of nodule biomass. Snowbrush fixed N_2 for approximately 240 days annually. Except during precipitation events or periods of low xylem pressure potentials, C_2H_2 reduction rates in the summer and fall were significantly correlated with soil temperature (R^2 = 0.93**, n = 6). A diurnal variation in nitrogenase activity also was measured. The annual N_2 fixation rate was estimated at approximately 101 kg N · ha^{-1} (C_2H_2). Sustained periods of precipitation suppressed nitrogenase activity and reduced the estimate by about 19%. The annual N_2 fixation rate is higher than previously reported for other mature stands and primarily is attributed to the maintenance of a large nodule biomass.

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La fixation d'azote a été mesurée dans un peuplement arbustif âgé de 17 ans de *Ceanothus velutinus* var. *velutinus* (Dougl.) localisé dans le Western Oregon Cascades. Des taux diurnes et saisonniers de l'activité nitrogénase ont été mesurés au champ à l'aide des techniques de réduction de C_2H_2. Le peuplement, selon les équations développées, avait une biomasse totale estimée à 42 680 kg · ha^{-1}, incluant 750 kg · ha^{-1} de biomasse nodulaire. L'activité de fixation s'étend sur une période approximative de 240 jours par année. A l'exception des périodes de précipitation au cours desquelles le potentiel de pression dans le xylème est faible, les taux de réduction de C_2H_2 durant l'été et l'automne ont été significativement correlés à la température du sol (R^2 = 0,93**, n = 6). Une variation diurne de l'activité nitrogénase a aussi été mesurée. Le taux de fixation annuel de N_2 a été estimé à approximativement 101 kg N · ha^{-1} (C_2H_2). Des périodes prolongées de précipitation ont supprimé l'activité nitrogénase et réduit l'estimé d'environ 19%. Le taux de fixation annuel de N_2 est plus élevé que ce qui fut rapporté antérieurement pour d'autres peuplements à maturité et il est attribué principalement au maintien d'une grande biomasse nodulaire.

[Traduit par le journal]

Introduction

Snowbrush, *Ceanothus velutinus* var. *velutinus* (Dougl.), is a shiny, sticky leaved, evergreen shrub which becomes widely established in the Cascade Mountains of Oregon and Washington following fire. Early estimates of N_2 fixation by snowbrush of between 60 and 70 kg N · ha^{-1} · year^{-1} were based on either greenhouse data or limited field data (Wollum and Youngberg 1964; Delwiche *et al.* 1965; Russell and Evans 1966). Zavitkovski and Newton (1968) estimated N_2 fixation by measuring soil and snowbrush biomass N in stands of different ages and attributing changes occurring between stands with increasing age to snowbrush N_2 fixation. The N_2 fixation rate over a 15-year period was estimated at between 0 and 20 kg N · ha^{-1} · year^{-1}. Soil N was quite variable among stands and showed no consistent trends. Youngberg and Wollum (1976) established permanent plots to estimate snowbrush N_2 fixation as that accreting in the biomass and 0–23-cm soil depth over time. Their 10-year estimate of N_2 fixation was 750 and 1080 kg N · ha^{-1} for a site in Central Oregon and the Western Oregon Cascades, respectively. A remeasurement of the Western Cascades site found the N accretion to increase to 1261 kg N · ha^{-1} by the end of 15 years, an increase of only 181 kg N · ha^{-1} (Youngberg *et al.* 1979); their data indicate that snowbrush N_2 fixation rates can decrease substantially after the first decade.

In our study, we chose to estimate the N_2 fixation rate of a 15- to 20-year-old snowbrush stand. This age group

[1]Publication No. 1720 from the Forest Research Laboratory, Oregon State University, Corvallis, OR, U.S.A. 97331.
[2]Author to whom all correspondence should be addressed.

was selected because it has been at maximum leaf area for several years and is rapidly approaching a period of stand degradation (Zavitkovski and Newton 1968). Snowbrush stands of this age group are very common in the Cascade Mountains. We observed the effects of soil temperature, plant moisture stress, seasonal climatic changes, and leaf conductance on nitrogenase activity and determined whether a diurnal variation occurred. Biomass and leaf area equations also were used to project individual plant estimates of N_2 fixation to a stand basis.

Study area

Two experimental sites, one for determining diurnal and the other for seasonal changes in nitrogenase activity, were located on the H. J. Andrews Experimental Forest in the Western Cascades of Oregon. The area receives approximately 250 cm of precipitation annually with most of it occurring in winter. July and August are typically dry.

The site for estimating the annual N_2 fixation rate was in a *Tsuga heterophylla — Abies amabilis / Acer circinatum / Berberis nervosa* habitat type at an elevation of 1125 m (Dyrness *et al.* 1974). Most of the snowbrush became established from seed the spring following clear-cut harvesting and a fall broadcast burn; they were in their 17th growing season when sampled.

A nearly continuous stand of snowbrush within the harvest unit was selected for sampling. The sample site had a southeast exposure with slopes of between 35 and 45%. The soil, a medial, frigid Andic Dystrochept (Anonymous 1975), was poorly developed but was deep with a high available water storage capacity (R. B. Brown. 1975. M.S. thesis, Oregon State University, Corvallis).

Diurnal variation in nitrogenase activity was studied in a similar age snowbrush stand at 600 m elevation where small nodules were more plentiful.

Methods

Acetylene reduction assay

All C_2H_2 reduction assays were conducted in the field. The analytical methods have been reported previously (McNabb and Geist 1979). Excavated nodules were stored temporarily in glass canning jars in contact with freshly excavated mineral soil and protected from direct radiation to minimize changes in nodule temperature. The jars, ranging in size from 0.25 to 3.8 L, were chosen so that they were no more than half full of nodules. Within a few minutes of excision, the jars were closed with screw lids fitted with a rubber serum stopper. The jars were partially evacuated, ≈0.01 MPa (local pressure) C_2H_2 added, and the jars buried in the soil from which the nodules were removed. Soil temperature adjacent to the jar was recorded and the assay conducted for 1 h. The total time elapsed from excision to termination of the reaction did not exceed 1.5 h.

It would have been preferable to have used a much larger incubation vessel : nodule volume ratio to avoid possible problems caused by the differential solubilities of C_3H_2 and C_2H_4 in water and possible CO_2 inhibition of nitrogenase activity. However, the assay of large nodule samples seemed possible because of low nitrogenase activity. Less than 4% of the C_2H_2 was reduced during a reaction under optimum conditions. Thus, given the range of CO_2:C_2H_2 reduction ratios reported for actinorhizal species (Tjepkema and Winship 1980), the CO_2 levels should not exceed those expected to occur in mineral soil (Russell 1973).

Nodule biomass was determined on an oven-dry basis. Oven-dry weight (OD wt) of nodules was estimated from the ashing characteristics of oven-dried (70°C) soil, cleaned nodules, and partially cleaned nodules. Nodule clumps were broken into small pieces, partially cleaned of soil, coarse fragments, and organic debris with water, and oven-dried. Partially cleaned nodule pieces were then weighed, coarsely chopped in a blender, and subsampled for ashing. Duplicate subsamples were ashed at 550°C for 4 h. The weight of the nodule–soil mixture which was clean nodules was determined by solving simultaneous equations:

[1] Nodule OD wt + Soil OD wt = Subsample OD wt

[2] 0.055 (Nodule OD wt) + 0.787 (Soil OD wt) = Subsample ash wt

The ash weight – oven-dry weight ratios of clean nodules and soil were 0.055 and 0.787, respectively. The proportion of the partially cleaned nodule subsample which was nodule was calculated from the above equations and multiplied by the total, partially cleaned nodule weight to obtain the nodule oven-dry weight of each sample. These weights were used to determine C_2H_2 reduction rates on an oven-dry basis and to estimate plant and stand nodule biomass.

Diurnal C_2H_2 reduction rate

The diurnal fluctuation in nitrogenase activity was based on periodically conducting the C_2H_2 reduction assay on the same nodule while attached to the plant. Only attached nodules found on plants with most of their root system intact and major roots through the nodule undamaged were considered for analysis. These nodules were tagged for identification, reburied, and the soil surface shaded from direct radiation. The first diurnal test was conducted after 10 days and the test repeated 3 weeks later.

The reaction vessels were polyvinyl chloride (PVC) pipes with one end sealed that were placed over the nodule and sealed by a rubber stopper which remained in place around the root. The reaction vessels were attached to roots at 2- or 4-h intervals over a period of 25 h. Each C_2H_2 incubation lasted for 1 h. The reaction vessels containing a nodule were buried during the assay and the nodules reburied without the vessel when tests were not being conducted. The soil surface was shaded and covered with moist burlap bags at all times.

The xylem pressure potential of each snowbrush plant was measured with a pressure chamber each time the C_2H_2 reduction assay was conducted (Waring and Cleary 1967). Leaf conductance was measured on dry foliage during daylight hours with a diffusion porometer (Turner and Parlange 1970).

Estimation of N_2 annual fixation rate

Acetylene reduction assays were conducted at 3-week intervals beginning in late June and continuing through late November when the winter snowpack began to form. Additional measurements were made the following spring 2 to 3 weeks after the snowpack melted and again in early June.

TABLE 1. Biomass and leaf area equations for 17-year-old *Ceanothus velutinus* collected from a midelevation site in the Western Oregon Cascades

Eq. No.	Equation	n	R^2	MSE
[3]	ln weight, kg[a] = 0.380 + 0.328(length, m) − 0.205(diameter, cm/2)^2 + 3.909(diameter, cm) (aboveground)	43	0.96	0.06[illegible]
[4]	ln(leaf area, cm^2) = 1.234 − 1.116(diameter, cm) + 6.894(diameter, cm)$^{1/2}$	43	0.85	0.211
[5]	Root weight, kg = 0.318 + 0.199(plant weight, kg[b])	24	0.84	0.271
[6]	Nodule weight, kg = 0.0141(plant weight, kg) + 0.00054(plant weight, kg)^2 (plant weight <20 kg)	36	0.90	0.006
[7]	Nodule weight, kg = Eq. 5/2 + 0.023(plant weight, kg/2) (plant weight >20 kg)	—	—	—

[a]Logarithmic equations corrected for bias (Baskerville 1972).
[b]Plant weight is the sum of the individual stem weights estimated with the aboveground equation.

During the summer, six to eight whole plants per sampling period were excavated. These plants also were used to develop biomass equations. During the fall and early spring, three to nine plants per sampling period were excavated until one or two large nodule samples (>1 L) were obtained. During whole-plant excavation, all roots >3 mm were checked for nodules unless they were about 70 cm below the surface. Except for one plant with a very large nodule biomass, all nodules were assayed. The C_2H_2 reduction rate reported for each sampling period was the weighted mean of all the nodules collected.

An annual estimate of the N_2 fixation rate was based on integration of the average daily C_2H_2 reduction rate obtained during the periodic assay of nodules for a year and the nodule biomass of the snowbrush stand estimated by the equations we developed. A 3:1 conversion of C_2H_2 reduction data to elemental nitrogen was used to estimate the fixation rate by snowbrush (Hardy *et al.* 1973).

Biomass and leaf area equations

Forty-three stems, only 1 per plant, were collected for developing nondestructive biomass and leaf area equations. Most stems were taken from plants to be excavated for nodule biomass and C_2H_2 reduction assay. Stem diameters and length were immediately measured. A leaf subsample from each stem was then taken for determination of specific blade area (two sides). Leaf samples were stored at 4°C until analyzed. The remaining parts of each stem were oven-dried at 70°C, stripped of leaves, redried, and stem and leaf components weighed. The root collar and all excavated roots were weighed in the field. Field weight was converted to an oven-dry weight based on the moisture content of six oven-dried root systems. Petioles were removed from the leaves of the specific blade area samples and the projected blade area determined photoelectrically.

Regression equations were developed for predicting individual stem biomass and leaf area, and nodule and root biomass of whole plants. The biomass of whole plants was estimated using the equation for the individual stem biomass. Whole plant biomass was then used for developing root and nodule equations. Logarithmic equations were corrected for skewness (Baskerville 1972).

Estimation of stand biomass

Three 0.01-ha plots were established in an undisturbed portion of the snowbrush stand adjacent to the area where plants were excavated. The diameter and length of all snowbrush stems originating within the plot were measured. Stem, root, and nodule biomass and specific leaf area of snowbrush on each plot was estimated with the equations we developed.

Results and discussion

Biomass and leaf area equations

The biomass and leaf area equations developed for snowbrush are based on plants collected from only one site (Table 1). While the precision of the stem and leaf area equations are similar to those of other shrubs in the Pacific Northwest (Gholz *et al.* 1979), these equations have not been tested to determine if they are site specific. This caution is necessary because equations are based on the range of sizes and forms typical of a 17-year-old, closed stand. Biomass of younger or more open-grown plants are least apt to be represented by these equations. The aboveground equation is limited to stems less than 7.0 cm in basal diameter because of the negative squared radius term it contains.

The leaf area equation was developed from plants supporting only the current year's foliage. Snowbrush has been reported to retain the previous season's foliage until late July or August (Zavitkovski and Newton 1968). Our plants had dropped their old leaves by July. We initially attributed the premature loss of leaves to the droughty winter; however, leaves also were observed to drop early a 2nd year without a drought. Thus, early leaf fall in this older, fully stocked snowbrush stand may occur as a means of regulating seasonal water use (Kozlowski 1976).

The root biomass equation indicated that snowbrush has a shoot:root ratio of approximately 4:1. Although the root collar was included as part of the root biomass, it did not include all the roots. Roots less than approximately 3 mm or penetrating to depths >70 cm generally were not sampled unless they were removed as part of the normal excavation process to locate nodules.

Two equations were used for estimating nodule biomass (Table 1). Equation 6 was applicable to 95% of the plants in the stand, but the exponential increase in nodule biomass for a few large plants appeared unrealistic. Thus, equation 7 was developed as a simple modification of equation 6.

While the first three equations may be applied to similar snowbrush stands with some confidence, the nodule biomass equations should be site specific. Snowbrush stands do not all become nodulated or nodulate at the same rate (Zavitkovski and Newton 1968; Youngberg and Wollum 1976). Nodulation may be a function of how long snowbrush has been absent from the site (Wollum *et al.* 1968). Sites where the forest stand age exceeded 200 years often do not begin to nodulate until snowbrush is at least 3 years old. Nodulation of snowbrush is most rapid on soils that are relatively high in exchangeable calcium and percentage base saturation (W. Scott, 1973. Ph.D. thesis, Oregon State University, Corvallis). Once snowbrush is nodulated, unusually cold winters without adequate snowpack to cover the plants have been observed to kill their tops, and sometimes nodules.

Stand biomass and leaf area

Nondestructive estimates of snowbrush biomass and leaf area are given in Table 2. Individual plants averaged 4.5 stems per plant, ranging from 1 to 17 stems per plant. The model number of stems per plant was 3. Seventy percent of the plants had five or fewer stems. Plant density was well below that found by Zavitkovski and Newton (1968) for several snowbrush stands between 12 and 15 years of age elsewhere in the Cascades, but their plots were selected to maximize snowbrush density. Stem biomass is also near the median of those reported by Zavitkovski and Newton (1968) but well below that reported by Youngberg and Wollum (1976) for a 10-year-old stand, approximately 25 km north of ours.

The nodule biomass of this snowbrush ($750\,kg \cdot ha^{-1}$) ranks among the highest reported for any nodulated plant. *Lupinus arboreus* is capable of producing $2100\,kg \cdot ha^{-1}$ of fresh nodule biomass, which equates to approximately $630\,kg \cdot ha^{-1}$ oven-dry weight; however, nodule biomass may go as low as $400\,kg \cdot ha^{-1}$ fresh weight at other times during the growing season (Sprent and Silvester 1973). *Alnus glutinosa* may attain a nodule biomass of $440\,kg \cdot ha^{-1}$ when it is 20 years old (Akkermans and Van Dijk 1975). An 11-year-old snowbrush stand in northeast Oregon had a nodule

TABLE 2. Nondestructive estimates of stand characteristics from three 0.01-ha plots in a 17-year-old *Ceanothus velutinus* stand in the Western Oregon Cascades

	Mean ± SE
No. of plants, ha^{-1}	4 170 ± 1 080
No. of stems, ha^{-1}	18 770 ± 3 160
Aboveground biomass, $kg \cdot ha^{-1}$	33 890 ± 11 830
Root biomass, $kg \cdot ha^{-1}$	8 040 ± 2 280
Nodule biomass, $kg \cdot ha^{-1}$	750 ± 360
Leaf area (two-sided) $m^2 \cdot m^{-2}$	5.86 ± 1.56

biomass of $146\,kg \cdot ha^{-1}$; however, the plants were relatively small, stems never exceeding 2.0 m in length, and snowbrush crown cover was only 64% (D. H. McNabb, unpublished data).

The percent of the plants nodulated was over 98% ($n = 70$); however, the nodules were different from those observed on other sites. The nodules often were exceptionally large; many nodules were between 12 and 18 cm across. Nearly all of the nodules on an individual plant were found on a root or roots in the same area from 1 to 2 m from the base of the plant, instead of scattered among several roots as has been observed in other stands. On a few plants, individual nodules were so entwined with one another and other plant roots that they formed inseparable masses up to 60 cm across.

Our overall impression of the snowbrush stand is that it is slightly overmature but is more vigorous than many 17-year-old stands. The breaking of stems at the root collar as a result of snow creep which often signals the decline of snowbrush stands on steeper slopes generally was absent (Zavitkovski and Newton 1968).

Diurnal N_2 fixation rates

The attempt to measure diurnal N_2 fixation rate of snowbrush was only partially successful. Nodules were isolated on five different plants, but only one nodule remained active after the second sampling date. This nodule was on a small root (<2.0 mm) within 20 cm of the root collar. The other nodules were either farther away from the root collar or on large roots which had been damaged during excavation.

Snowbrush has a distinct diurnal cycle of nitrogenase activity which is very slow to respond to rapid changes in leaf conductance or xylem pressure potential (Fig. 1A). The steady increases in C_2H_2 reductions throughout the day until late afternoon, accompanied by a slower decline until after midnight when the decline is more precipitous, is similar to that observed in *Alnus glutinosa* (Wheeler 1969). Snowbrush does not show the distinct response to radiation levels observed in

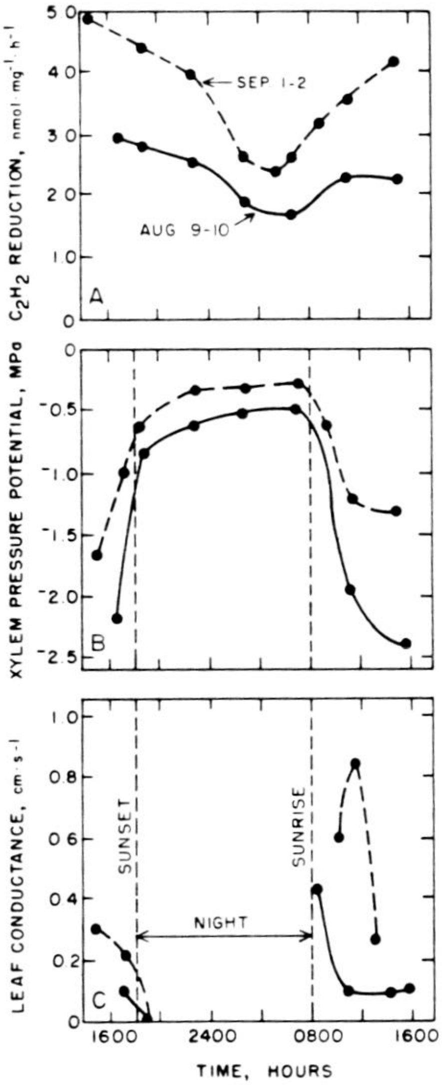

FIG. 1. Diurnal variation in C_2H_2 reduction rates of a single nodule (A), concurrent plant moisture stress (B), and leaf conductance (C) in a *Ceanothus velutinus* shrub in the Western Oregon Cascades.

soybeans (Hardy *et al.* 1968; Sloger *et al.* 1975), although a high overcast between 1200 and 1600 h on September 2 relative to clear skies on September 1 may account for the slightly lower C_2H_2 reduction rate the 2nd day.

Acetylene reduction rates increased in September in association with less negative xylem pressure potential (Fig. 1A, 1B). Leaf conductance also increased in association with the less negative xylem pressure potential (Fig. 1C). The improvement in the moisture status of the plant was attributed to over 8 cm of precipitation falling the week before the second sampling period, partially recharging the relatively dry soil profile (precipitation reporting station was 3 km from the site). Soil temperature changes between the two sampling periods were small, decreasing approximately 2°C, from a mean of 19°C to 17°C, by the second period.

Seasonal C_2H_2 reduction rates

Acetylene reduction rates ranged from 0.19 to 5.49 nm $C_2H_2 \cdot mg^{-1} \cdot h^{-1}$ (Table 3). Maximum rates were lower than expected. They were about a third of those measured in an 11-year-old snowbrush stand in northeastern Oregon (D. H. McNabb, unpublished data). We have additional data on 3-year-old plants about 20 km north of our site which also had a maximum rate about three times higher (unpublished data).

The lower C_2H_2 reduction rates for snowbrush at this site reflect the age and size of the nodules. The interior portions of large nodules were woody and some portions were starting to die and decompose. New nodules generally were absent, although most nodules produced new lobes beginning in early April. New lobes have been identified as the principal location of nitrogenase activity in the perennial nodules of *Myrica gale* L. (Schwintzer *et al.* 1982). We also measured higher C_2H_2 reduction rates on current exterior nodule lobes than on older, interior pieces of nodule.

Maximum C_2H_2 reduction rates occur in late July when soil temperatures are highest and before snowbrush xylem pressure potentials decreased appreciably (Table 3). Acetylene reduction rates decreased significantly ($P < 0.01$) in August from the previous sampling date, while soil temperature did not change significantly (Table 3). The decrease is attributed to a significant change ($P < 0.01$) in the xylem pressure potentials to more negative values. The plants were responding to a depletion of soil moisture without recharge from summer precipitation, a phenomenon common to the region (Johnsgard 1963). A decrease in C_2H_2 reduction rates of snowbrush has been reported previously to be significantly related to more negative predawn xylem pressure potentials (D. H. McNabb and J. M. Geist. 1977. Agronomy Abstracts, American Society of Agronomy, Madison, WI. pp. 127–128). Dalton and Zobel (1977) found moisture stress to affect C_2H_2 reduction rates of *Purshia tridentata* in central Oregon.

Acetylene reduction rates for the dates between June and November, but excluding August 16–18 and September 29, were correlated significantly with the incubation soil temperatures:

$$[8]\quad C_2H_2 \text{ reduction rate} = -0.82 + 0.35(\text{temperature})\ (R^2 = 0.93^{**},\ n = 6)$$

The August sampling date was excluded because high plant moisture stress suppressed nitrogenase activity

TABLE 3. Plant moisture stress, soil temperatures, and acetylene reduction rates for *Ceanothus velutinus* collected from a 17-year-old stand in the Western Oregon Cascades

Date	No. storms/total days[a]	No. of plants sampled	Xylem pressure potential $\bar{x} \pm$ SE (MPa)	Soil temperature $\bar{x} \pm$ SE (°C)	C_2H_2 reduction $\bar{x} \pm$ SE (nmol $C_2H_2 \cdot$ mg dry weight$^{-1} \cdot h^{-1}$)
1977					
7–9 July		8	−0.35±0.02	13.9±0.5	4.62±0.50
	0/0				
25–26 July		6	−0.35±0.03	17.8±0.9	5.49±0.31
	0/0				
16–18 August		8	−1.10±0.08	18.8±0.5	3.15±0.47
	2/5				
7–9 September		6	−0.40±0.02	15.7±1.2	3.96±0.58
	3/10				
28 September		3	ND[b]	10.7±0.3	3.32±0.83
29 September		7	ND	7.6±0.2	0.19±0.06
	1/1				
19–20 October		7	ND	11.3±0.7	3.13±0.82
	3/11				
8 November		9	ND	4.2±0.2	0.42±0.09
1978					
20 March					
	2/8				
12 April		9	ND	11.5±0.6	1.27±0.22
	6/16				
6 June		6	ND	16.4±0.5	3.26±0.69
	2/2				
7 July					

[a]Nitrogenase activity is assumed to be zero after the 1st day of storm events in which precipitation exceeds 0.2 cm. The data list the number of storm events and the number of days when nitrogenase is assumed to be zero between each sampling period.

[b]ND, not determined.

(Table 3). The September 29 data was excluded because it was collected during a sustained storm event to be discussed later. The correlation is linear; a similar relationship has been reported for *Elaeagnus umbellata* and *M. gale* over a comparable range of temperatures (Hensley and Carpenter 1979; Schwintzer 1979). Measured soil temperatures were below the 23–26°C temperatures found to be optimum for snowbrush nodule formation and growth under greenhouse conditions (Wollum and Youngberg 1969).

The rapid decline in C_2H_2 rates in late fall (October 19–20 versus November 8) is associated with a precipitous drop in soil temperature (Table 3). The drop in temperature is primarily attributed to the melt of an intermittent snowpack. The snowpack melting produced cold meltwater which rapidly cooled the soil profile as it percolated through it. The melting of an intermittent snowpack is a major factor contributing to the termination of nitrogenase activity in the fall.

The spring C_2H_2 reduction data were not included in the soil temperature correlation because the rates were as little as one-half of those measured in the summer and fall at equivalent temperatures. The reasons for the difference are most likely due to the interaction of soil temperature, nodule phenology, production of carbohydrates by photosynthesis, and competition between nodules and other plant parts for photosynthate (Schwintzer *et al.* 1982).

During a period of sustained precipitation, lasting more than 3 days, C_2H_2 reduction rates were observed to decrease the 3rd day of the storm event (Table 3, September 28 and 29). Although no C_2H_2 reduction assays were made the 1st day of the storm, soil temperatures suggest the C_2H_2 reduction rates remained near normal through the first 2 days, decreasing the 3rd day to less than 6% of the 2nd day of storm average. Precipitation amounts were 0.96, 1.09, and 0.20 cm $\cdot$ day^{-1}, respectively, at the H. J. Andrews meteorological reporting station about 12 km from our site. The precipitation originated from a dense cloud mass which was often less than 100 m above the stand. Thus, radiation levels in the stand were substantially reduced.

This observation suggests that snowbrush N_2 fixation is slow to respond to variations that radiation levels cause in photosynthate production. Soluble carbohydrate reserves and sucrose, possibly stored in the large nodule biomass as well as the plant, supply energy in the interim until it is depleted after 2 days (Wheeler 1971). Wheeler demonstrated that 1-year-old *Alnus glutinosa* placed in a darkened incubator is capable of N_2 fixation for 1 day before it stops. The return of snowbrush C_2H_2 reduction rates to prestorm levels apparently is quite rapid. An ealier, 3-day storm of over 5 cm of precipitation had ended only 3 days prior to the 1st day of sampling (September 28), but C_2H_2 reduction rates had already returned to near normal.

Annual N_2 fixation rate

An estimate of the annual N_2 fixation rate was based on an integration of the seasonal C_2H_2 reduction data (Table 3), a nodule biomass of $750\,kg \cdot ha^{-1}$, and the theoretical conversion ratio of C_2H_2 to N_2 fixation of 3:1. The C_2H_2 reduction rate was assumed to be zero between November 10, 1977 and March 20, 1978 because a winter snowpack covered the stand and soil temperatures were near 0°C. We assumed that the N_2 fixation rate on March 20, 1978, equaled that occurring on November 8, 1977, and increased linearly until April 12 (Table 3).

Field samples were collected between 0730 and 1400 h near the middle of the diurnal variation (Fig. 1A). Therefore, we assumed that no negligible correction was needed for diurnal variation in making summary estimates of C_2H_2 reduction rates for each 24-h time period.

To account for the effects that prolonged storms had on C_2H_2 reduction rates, all days when precipitation exceeded $0.2\,cm \cdot day^{-1}$, excluding the 1st day of each storm, were assumed to have a daily C_2H_2 reduction rate of zero. The data indicated C_2H_2 reduction rates are not affected until the 3rd day of a storm, so this assumption is conservative; however, this assumption also allows for radiation-limiting cloud cover to form before appreciable precipitation falls. The number of storms and number of days that C_2H_2 reduction rates are assumed to be zero, according to this assumption are summarized by sample interval (Table 3). Of the 240-day N_2 fixation season, 53 days are assumed to have a negligible N_2 fixation rate. Most of these days occur in the spring or fall when nitrogenase activity is low.

The adjusted annual N_2 fixation rate was 101 kg $N \cdot ha^{-1}$ (C_2H_2). Correcting for prolonged storm events reduced the N_2 fixation rate by 19%. Because the annual rate is affected by duration of snowpack, number of prolonged storms, and duration of the late summer high plant moisture stress, the annual rate can deviate from this estimate during other years. Climatic conditions were relatively normal for the year sampled. Therefore, the N_2 fixation rate is typical for the stand and site conditions.

A high N_2 fixation rate for a mature snowbrush stand is predominantly controlled by the accumulation and maintenance of a large nodule biomass, particularly when nitrogenase activity is low. The interaction of climatic factors is less important. Thus, the greatest error in estimating an annual N_2 fixation rate is the precision by which nodule biomass is determined.

The annual N_2 fixation rate of 101 kg $N \cdot ha^{-1}(C_2H_2)$ for a 17-year-old stand of snowbrush in the Western Cascades is higher than that measured by Youngberg *et al.* (1979). Similar N_2 fixation rates have been reported for 10- to 12-year-old stands by nitrogen accretion methods for three other sites in the Western Cascades (Binkley *et al.* 1982; Youngberg and Wollum 1976). The N_2 fixation rate of one of the stands between 10 and 15 years of age, however, was estimated at 36 kg $N \cdot ha^{-1} \cdot year^{-1}$ (Youngberg *et al.* 1979). Because snowbrush is sometimes slow to nodulate (Youngberg and Wollum 1976), and consequently nodule biomass slow to develop, juvenile snowbrush N_2 fixation rates can be low.

In the case of our 17-year-old stand, the potential to sustain a high N_2 fixation rate for nearly two decades substantially strengthens its role as an actinorhizal species in forest communities of the Pacific Northwest. Capitalizing on this capability would enhance the management of forests where N is often the most limiting nutrient element (Atkinson and Morison 1975).

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AKKERMANS, A. D. L., and D. VAN DIJK. 1975. The formation of nitrogen fixing activity of the root nodules of *Alnus glutinosa* under field conditions. *In* Symbiotic nitrogen fixation in plants. International Biology Program. Vol. 7. *Edited by* P. S. Putman. Cambridge University Press, Great Britain. pp. 511–520.

ANONYMOUS. 1975. Soil taxonomy. Agricultural Handbook No. 436. Soil Conservation Service, Washington, DC.

ATKINSON, W. A., and I. G. MORISON. 1975. The Pacific Northwest regional fertilization project, an integrated approach to forest nutrition research. *In* Forest soils and forest land management. *Edited by* B. Bernier and C. H. Winget. Les Presses de L'Université Laval, Quebec. pp. 477–484.

BASKERVILLE, G. L. 1972. Use of logarithmic regression in the estimation of plant biomass. Can. J. For. Res. **2**: 49–53.

BINKLEY, D., K. CROMACK, JR., and R. L. FREDRIKSEN. 1982. Nitrogen accretion and availability in some snowbrush ecosystems. For. Sci. **28**: 720–724.

DALTON, D. A., and D. B. ZOBEL. 1977. Ecological aspects of nitrogen fixation by *Purshia tridentata*. Plant Soil, **48**: 57–80.

DELWICHE, C. C., P. T. ZINKE, and C. M. JOHNSON. 1965. Nitrogen fixation by *Ceanothus*. Plant Physiol. **40**: 1047–1054.

DYRNESS, C. T., J. F. FRANKLIN, and W. H. MOIR. 1974. A preliminary classification of forest communities in the central portion of the Western Cascades of Oregon. College of Forest Resources, University of Washington, Seattle. Coniferous Forest Biome Bulletin No. 4.

GHOLZ, H. L., C. C. GRIER, A. G. CAMPBELL, and A. T. BROWN. 1979. Equations and their use for estimating biomass and leaf area of plants in the Pacific Northwest. Res. Pap. Oreg. State Univ. For. Res. Lab. No. 41.

HARDY, R. W. F., R. C. BURNS, and R. D. HOLSTEN. 1973. Applications of the acetylene–ethylene assay for measurement of nitrogen fixation. Soil Biol. Biochem. **5**: 47–81.

HARDY, R. W. F., R. D. HOLSTEN, E. K. JACKSON, and R. C. BURNS. 1968. The acetylene–ethylene assay for nitrogen fixation: laboratory and field evaluation. Plant Physiol. **43**: 1185–1207.

HENSLEY, D. L., and P. L. CARPENTER. 1979. The effect of temperature on N_2 fixation (C_2H_2 reduction) by nodules of legumes and actinomycete-nodulated woody species. Bot. Gaz. (Chicago), **140**: S58–S64.

JOHNSGARD, G. A. 1963. Temperature and water balance for Oregon weather stations. Oreg. Agric. Exp. Stn. Spec. Rep. No. 150.

KOZLOWSKI, T. T. 1976. Water deficits and plant growth. Vol. IV. Soil water measurement, plant responses, and breeding for drought resistance. Academic Press, New York.

McNABB, D. H., and J. M. GEIST. 1979. Acetylene reduction assay of symbiotic N_2 fixation under field conditions. Ecology, **60**: 1070–1072.

RUSSELL, E. W. 1973. Soil conditions and plant growth. Longman, London.

RUSSELL, S. A., and H. J. EVANS. 1966. The nitrogen fixation capacity of *Ceanothus velutinus*. For. Sci. **12**: 164–169.

SCHWINTZER, C. R. 1979. Nitrogen fixation by *Myrica gale* root nodules in a Massachusetts wetland. Oecologia, **43**: 283–294.

SCHWINTZER, C. R., A. M. BERRY, and L. D. DISNEY. 1982. Seasonal patterns of root nodule growth, endophyte morphology, nitrogenase activity, and shoot development in *Myrica gale*. Can. J. Bot. **60**: 746–757.

SLOGER, C., D. BEZDICEK, R. MILBERG, and N. BOONKERD. 1975. Seasonal and diurnal variations in $N_2[C_2H_2]$ fixing activity in field soybeans. *In* Nitrogen fixation by free-living micro-organisms. International Biology Program. Vol. 6. *Edited by* W. D. P. Stewart. Cambridge University Press, Great Britain. pp. 271–284.

SPRENT, J. I., and W. B. SILVESTER. 1973. Nitrogen fixation by *Lupinus arboreus* grown in the open and under different aged stands of *Pinus radiata*. New Phytol. **72**: 991–1003.

TJEPKEMA, J. D., and L. J. WINSHIP. 1980. Energy requirement for nitrogen fixation in actinorhizal and legume root nodules. Science (Washington, D.C.), **209**: 279–281.

TURNER, N. C., and J. Y. PARLANGE. 1970. Analysis of operation and calibration of a ventilated diffusion porometer. Plant Physiol. **46**: 175–177.

WARING, R. H., and B. D. CLEARY. 1967. Plant moisture stress: evaluation by pressure bomb. Science (Washington, D.C.), **155**: 1248–1254.

WHEELER, C. T. 1969. The diurnal fluctuation in nitrogen fixation in the nodules of *Alnus glutinosa* and *Myrica gale*. New Phytol. **68**: 675–682.

——— 1971. The causation of the diurnal fluctuation in nitrogen fixation in *Alnus glutinosa*. New Phytol. **70**: 487–495.

WOLLUM, A. G., II, and C. T. YOUNGBERG. 1964. The influence of nitrogen fixation by nonleguminous woody plants on the growth of pine seedlings. J. For. **62**: 316–321.

——— 1969. Effect of soil temperature on nodulation of *Ceanothus velutinus* Dougl. Soil Sci. Sco. Am. Proc. **33**: 801–803.

WOLLUM, A. G., II, C. T. YOUNGBERG, and F. W. CHICHESTER. 1968. Relation of previous timber stand age to nodulation of *Ceanothus velutinus*. For. Sci. **14**: 114–118.

YOUNGBERG, C. T., and A. G. WOLLUM, II. 1976. Nitrogen accretion in developing *Ceanothus velutinus* stands. Soil Sci. Soc. Am. J. **40**: 109–112.

YOUNGBERG, C. T., A. G. WOLLUM, and W. SCOTT. 1979. *Ceanothus* in Douglas-fir clear-cuts: nitrogen accretion and impact on regeneration. *In* Symbiotic nitrogen fixation in the management of temperate forests. *Edited by* J. C. Gordon, C. T. Wheeler, and D. A. Perry. Forest Research Laboratory, Oregon State University, Corvallis, OR. pp. 224–233.

ZAVITKOVSKI, J., and M. NEWTON. 1968. Ecological importance of snowbrush, *Ceanothus velutinus*, in the Oregon Cascades. Ecology, **49**: 1134–1145.