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Suitability and use of the ^{15}N -isotope dilution method to estimate nitrogen fixation by actinorhizal shrubs

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Abstract

Nitrogen fixation rates were estimated by the ^{15}N -isotope dilution method for *Ceanothus velutinus* and *Purshia tridentata* in the understory of central Oregon ponderosa pine forests. Field rates were measured in small pole-, large pole-, and sawtimber-sized pine stands using two shrubs (*Arctostaphylos patula*, *Ribes cereum*) and one graminoid (*Carex rossii*) as non-fixing reference species. Shrub cover ranged from 23 to 59% for *Ceanothus* and from 9 to 15% for *Purshia*. Foliage samples were collected monthly following two applications of ^{15}N ammonium sulfate (5 kg N ha^{-1} at 10 atom% ^{15}N) to compare N uptake patterns and determine the fraction of N derived from fixation (Ndff). Several violations of the underlying assumption of the isotope dilution method that N fixing and reference plants have access to similar pools of soil ^{15}N were identified. These included non-uniform distribution of ^{15}N in the soil profile and dissimilarities in rooting volumes, N uptake patterns, and plant ages between the reference and N fixing plants. Despite these compromises, there were only minor differences in percentage of Ndff when calculated independently using each reference species. Low ^{15}N uptake by *Ceanothus* and *Purshia* evidently negated the requirement that N fixing and reference plants have comparable spatial and temporal access to soil ^{15}N . Both *Ceanothus* and *Purshia* were highly effective, obtaining more than 80% of their N from fixation regardless of pine stand, shrub cover, or shrub age. Annual N fixation by *Ceanothus* ranged from 4 to $15 \text{ kg ha}^{-1} \text{ year}^{-1}$. This rate is lower than previously reported for *Ceanothus* in other ecosystems in the western United States, yet is sufficient to replace N losses from disturbances such as prescribed fire. *Purshia* fixed considerably less N, ca. $1 \text{ kg ha}^{-1} \text{ year}^{-1}$, roughly equivalent to the rate of atmospheric N deposition in central Oregon. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

The lack of a simple and accurate method to quantify symbiotic N fixation limits our understanding of local, regional, and global N inputs. Several methods have been developed to estimate field rates of N fixation in terrestrial ecosystems, including ^{15}N isotope dilution (McAuliffe et al., 1958), ^{15}N natural

abundance (Shearer and Kohl, 1986), acetylene reduction (Hardy et al., 1968), N difference (Weaver and Danso, 1994), and N accretion (Knowles, 1980). Each method has a unique set of assumptions which are often violated in field studies and can lead to miscalculation and inaccuracy. As a result, annual N fixation rates for both individual species and whole ecosystems are, at best, rough approximations.

Stable isotope methods are generally considered the most accurate of the quantitative measures of symbiotic N fixation (Warembourg, 1993; Danso, 1995).

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Their strength lies in the ability to measure N fixation cumulatively during one or more growing seasons and in the high degree of precision of stable isotopes. In comparison, the acetylene reduction method, which is an accurate measure of nitrogenase activity (Vessey, 1994), has been criticized as a non-cumulative method that is often improperly used to determine annual N fixation rates (Danso, 1995). The N difference and N accretion methods are simple, cumulative measures of N fixation, yet are infrequently used outside of agro-nomic settings due to difficulties in accounting for the numerous transformations and translocations of fixed N in natural ecosystems. However, stable-isotope methods are not without drawbacks. They rely on non-fixing reference plants to determine the proportion of plant N derived from fixation (percentage of Ndff) versus soil N uptake. Finding a reference plant which mimics the growth characteristics of a N fixing plant, with the exception of how it obtains plant N, is practically impossible in the absence of a non-nodulating host. The isotope dilution method is further restricted by the high cost of ^{15}N fertilizer and plant analyses and the restrictive use of small sample plots.

Improvements to the isotope dilution method offer assistance to the problems associated with mismatched reference plants. Sequential sampling of both soil ^{15}N nitrate and foliage ^{15}N has been proposed as a means to completely eliminate the need for reference plants (Hamilton et al., 1992; Smith et al., 1992; Chalk et al., 1996). Alternatively, use of several reference species has been suggested for improving *in situ* estimates of N fixation (Boddey et al., 1990; Viera-Vargas et al., 1995). Utilizing reference plants with different growth and N uptake patterns produces a range of estimates of percentage of Ndff and provides an indication of the accuracy in estimating N fixation (Boddey et al., 1995).

Nitrogen fixation rates by actinorhizal shrubs in central Oregon ecosystems have not been well documented. Dalton and Zobel (1977) used the acetylene reduction method to estimate N fixation by *Purshia* in ponderosa pine (*Pinus ponderosa* Dougl.) understory, while Youngberg and Wollum (1976) estimated N fixation in pure *Ceanothus* stands using the N accretion method. Stable isotope methods have not been used to date. Providing an accurate estimate of N fixation by these two shrubs is especially warranted

given their wide geographical distribution and ecological importance in pine and mixed conifer forests. *Purshia*, for example, is found from southern British Columbia to New Mexico, with a distribution estimated at 138 million hectares of forest and range land (Hormay, 1943). Ecologically, it is a major browse species of mule deer, accounting for as much as 85% of their annual diet (Gay, 1998), and contributes to granivore food source (Vander Wall, 1994) and soil N accretion (Busse et al., 1996). *Ceanothus* is also distributed widely in the western United States (Conard et al., 1985) and is noted for its role in improving soil quality (Johnson, 1995) and for its rapid recolonization following site disturbance (Conard et al., 1985).

The objective of my study was to use the ^{15}N -isotope dilution method to estimate potential N fixation rates by *Ceanothus* and *Purshia* in ponderosa pine forests of differing stand age. To ensure accuracy and to verify the assumption that both N fixing and reference plants have access to similar soil ^{15}N pools, I compared the (1) uniformity of ^{15}N labeling throughout the rooting profile, (2) rooting patterns of the reference and N fixing plants, and (3) N uptake patterns of the reference and N fixing plants during the growing season.

2. Materials and methods

2.1. Study sites

Three study sites were selected along a 140 km, north-south transect on the Deschutes National Forest, located on the eastern slope of the central Oregon Cascades. This region has a dry continental climate with warm, dry summers and cold winters. Mean daily temperature is 17°C in July and -1°C in January. The soils are Cryandepts, with ash and pumice parent material derived predominantly from the eruption of Mt. Mazama ca. 7000 years ago. The sites included a young plantation (small-pole site), an intermediate-aged, second-growth stand (large-pole site), and a mature stand (sawtimber site). Site characteristics are listed in Table 1. In addition to providing a range of stand ages, my goal in site selection was to ensure 20% or greater ground cover of *Ceanothus*. Occupancy by *Purshia* was not a criterium in the design of

Table 1
Vegetation and soil characteristics at the ^{15}N -isotope dilution study sites

	Site		
	Small pole	Large pole	Sawtimber
<i>Ceanothus</i> cover (%)	59	23	31
<i>Purshia</i> cover (%)	0	15	9
Overstory age (year)	26	41–58	104–139
Mean DBH (cm)	12.2	20.8	44.5
Stand density (stems ha^{-1})	308	782	160
Soil pH	6.4	5.9	6.0
Soil C (g kg^{-1})	50.0	29.5	23.0
Soil N (g kg^{-1})			
0–15 cm	2.0	0.9	0.8
15–30 cm	1.4	0.7	0.4
30–45 cm	0.9	0.4	0.3

the experiment, however the species was included in the study given its presence at two of the three sites.

The small-pole site is ca. 60 km north of Bend, Oregon, on the eastern flank of the Mt. Jefferson Wilderness. This is the highest (1950 m elevation) and wettest (89 cm annual precipitation) of the three sites. Soil texture is loamy sand and the surface horizon is 30 cm deep overlying a cobbly, 90 cm thick buried soil of glacial till origin. The site was planted to ponderosa pine in 1963 following a stand replacement wildfire in 1954. Competing vegetation was controlled initially with (2,4-dichlorophenoxy) acetic acid to enhance stand establishment. The understory at the beginning of the study (1989) was dominated by *Ceanothus velutinus* and *Arctostaphylos patula*.

The large-pole site is ca. 18 km southwest of Bend, Oregon in an even-aged ponderosa pine forest that regenerated naturally following logging between 1920 and 1940. The elevation is 1520 m and the annual precipitation is 53 cm, primarily in the form of snow. Soil texture is loamy sand and the surface horizon is 12 cm deep overlying a poorly developed, 75 cm thick pumice layer (AC and C horizons). Understory vegetation included *Ceanothus velutinus*, *Purshia tridentata*, and *Arctostaphylos patula*, with scattered *Ribes cereum*, *Carex rossii*, *Stipa occidentalis*, *Epilobium angustifolium*, and *Fragaria virginiana*.

The sawtimber site is located at the base of Walker Mt. ca. 77 km south of Bend, Oregon. The elevation is 1470 m and the annual precipitation is 38 cm. Soil

texture is loamy sand and the surface horizon is 15 cm deep overlying coarse pumice. Ponderosa pine is the dominant overstory species, with scattered sugar pine (*Pinus lambertiana*) and white fir (*Abies concolor*). Understory shrubs, including *Ceanothus velutinus*, *Purshia tridentata*, *Arctostaphylos patula*, and *Castanopsis chrysophylla*, have resprouted or germinated from seed following a moderate-intensity prescribed fire in 1976.

2.2. ^{15}N plots

Four 5 m \times 5 m plots were established at each site in September 1989. Because *Purshia* and *Ceanothus* were not intermixed at the large-pole site, an additional four plots were added at the site to provide four *Ceanothus*-dominated and four *Purshia*-dominated plots. Plots were randomly located along a 1.5 km transect at the large-pole site, and were within 400 m of each other at the small-pole and sawtimber sites. Aqueous ^{15}N -ammonium sulfate (10% atom excess) was applied using a pressurized handsprayer to the surface of the mineral soil at a rate of 0.5 g m^{-2} in September 1989 and again in April 1990 immediately after snow melt. All forest floor material was removed immediately before fertilizer application and replaced on the mineral surface after 48 h. To aid in uniform fertilizer distribution and to avoid possible loss of ^{15}N by volatilization, plots were irrigated with 3.5 cm of water (875 l plot^{-1}) following each ^{15}N application using a pressurized sprayer.

Each plot within a site was considered a pseudo-replication for the purpose of analyzing the effects of stand age on N fixation. Consequently, treatment effects and means comparisons were not analyzed by inferential statistics. Instead, standard deviations are presented as a measure of the precision of the estimate of percentage of Ndff and total N fixation for each stand condition.

2.3. Suitability of the isotope dilution method

Testing of reference plants for their physiological 'match' with the N fixing shrubs was conducted at the large-pole site since it contained the greatest diversity of understory species. Two shrubs (*Arctostaphylos* and *Ribes*) and one graminoid (*Carex*) were selected on the basis of their diverse growth forms and frequency on

the plots. Current-year foliage was collected monthly during the 1990 growing season to compare temporal patterns of N concentration and ^{15}N uptake among species. Foliage from every reference and N fixing shrub within a plot was sampled and analyzed separately. *Carex* samples were composites of 10 randomly located, 0.15 m \times 0.15 m subplots per plot. All samples were placed in an oven within 3 h of collection and dried at 60°C for 72 h. Analysis of foliage N concentration and $^{15}\text{N}/^{14}\text{N}$ ratio was conducted by mass spectrometry on duplicate samples at the Stable Isotope Research Unit, Oregon State University. Temporal differences between plant species in N concentration were tested using repeated measures analysis (SAS Institute Inc., 1988). To test for potential sampling error resulting from isotopic discrimination, four randomly chosen plants of each shrub species were harvested at the end of the growing season and ^{15}N content of fine roots (<0.5 cm diameter), coarse roots (>1 cm diameter), large stems, and foliage was measured.

Uniform ^{15}N enrichment in the rooting profile is a theoretical prerequisite of the isotope dilution method, and is of particular importance when the 'match' between N fixing and reference plants is poor. This requirement ensures that each species has access to similarly enriched ^{15}N pools. Without it, poorly matched plants obtain different ^{15}N enrichments, introducing considerable error in the estimate of percentage of Ndff. Several cultural techniques have been used with limited success to improve soil ^{15}N uniformity. These include physical mixing (Boddey and Victoria, 1986), use of slow-release or organic ^{15}N -fertilizer (Witty, 1983; Viera-Vargas et al., 1995), and multiple applications of ^{15}N -fertilizer (Boddey et al., 1995). Since physical mixing or tilling was not an option in my study due to the excessive damage it causes to perennial shrubs, the spatial uniformity of ^{15}N following fertilizer applications depended on the unpredictable transport of ^{15}N in gravitational water. Split applications of ^{15}N fertilizer were applied in attempt to limit the decline in soil ^{15}N enrichment during the growing season.

Soil ^{15}N content was measured at the end of the 1990 growing season to determine the uniformity of ^{15}N within the soil profile. Composite samples were collected from each plot and site at 0–4; 4–8; 8–15; 15–30; and 30–45 cm depths. Eight subsamples, col-

lected systematically along two transect lines between opposite plot corners, were composited prior to air drying, sieving (2 mm), and analyzing for ^{15}N . Differences in soil ^{15}N with depth were tested using ANOVA. For comparative purposes, composite samples were collected from each of the five soil depths from outside the plots, where no ^{15}N had been applied, to determine soil ^{15}N natural abundance.

2.4. Determination of percentage of Ndff and N fixation rates

The percentage of N derived from atmospheric N for individual *Ceanothus* and *Purshia* plants was calculated by the equation:

$$\% \text{Ndff} = ([\delta^{15}\text{N}_r - \delta^{15}\text{N}_a]/\delta^{15}\text{N}_r) \times 100 \quad (1)$$

where $\delta^{15}\text{N}_r$ is the average per mil ^{15}N excess of the reference plant, *Arctostaphylos*, determined using October 1990 foliage samples; and $\delta^{15}\text{N}_a$ is the per mil ^{15}N excess of foliage from each N fixing plant sampled in October 1990.

Total N fixed (kg plant^{-1}) was calculated by the equation:

$$\text{Total N fixed} = (\% \text{Ndff}) \times (\text{biomass}) \times (\text{N conc.}) \quad (2)$$

Biomass (kg plant^{-1}) was determined by destructively sampling each plant for aboveground and belowground dry mass in late October 1990. Because of the extensive rooting system of *Ceanothus* and the amount of labor required to exhume each plant, only those roots >1 cm diameter were sampled. *Purshia* plants were generally smaller in size compared to *Ceanothus*, allowing for sampling of the entire root system. Nitrogen concentration was determined on whole plant samples. Six randomly selected plants per species were collected from each site (aboveground + belowground biomass) and analyzed for total N by mass spectrometry. Average N concentrations for each site were used in Eq. (2) to determine total N fixed. Total N fixed was calculated on an area basis as the sum of total N fixed by each plant within a plot.

Annual rate of N fixation was determined for individual plants by dividing total N fixed (from Eq. (2)) by the age of the plant. Shrub ages were measured by

counting annual rings present at the soil surface. This method provides a linear average for annual N fixation during the life of a plant, and presumably underestimates N fixation at its peak rate while overestimating N fixation during plant establishment and early growth. Annual N fixed was calculated on an area basis as the sum of annual N fixation by each plant within a plot.

3. Results and discussion

3.1. Soil ^{15}N enrichment

Spatial uniformity of soil ^{15}N was poor at each of the study sites. A large decline in ^{15}N was found with soil depth for the ^{15}N fertilized plots (Fig. 1), parti-

cularly at the sawtimber and large-pole sites. This is a likely outcome of the method of ^{15}N application (surface applied) and the low annual precipitation in central Oregon pine forests. In fact, the driest site (sawtimber stand) was not enriched above natural ^{15}N abundance at a soil depth greater than 15–30 cm. The wettest site (small-pole stand) showed the most consistency in ^{15}N enrichment, although significant differences in atom% ^{15}N excess were still detected with depth ($p < 0.05$). Of note, these data reflect the total soil ^{15}N pool and are not necessarily indicative of plant available ^{15}N . Whether plant available ^{15}N followed a similar pattern of rapid decline with soil depth can only be inferred.

3.2. Suitability of reference plants

Given the lack of uniformity in soil ^{15}N enrichment, identifying a reference plant(s) with a similar rooting profile as *Ceanothus* and *Purshia* was necessary to show that N fixing and control plants have similar spatial patterns of ^{15}N uptake. Roots were assessed visually for general growth characteristics and quantitatively for root : shoot ratios when excavated at the end of the growing season. As expected, the rooting pattern of the graminoid, *Carex*, was unique among the five species, with a prolific fine-root system in the upper 10–20 cm of mineral soil. *Purshia*, *Arctostaphylos*, and *Ribes* had similar maximum rooting depths and general patterns of structural and fine root distribution. *Ceanothus*, in contrast, occupied a much greater volume of soil than the other shrubs and had substantially greater coarse root biomass. The mean root : shoot ratio ($\pm\text{s.d.}$) was about twice as great for *Ceanothus* compared to the other shrubs (*Ceanothus* = 0.69 ± 0.13 ; *Purshia* = 0.23 ± 0.06 ; *Purshia*; *Arctostaphylos* = 0.36 ± 0.10 ; *Ribes* = 0.32 ± 0.05).

Differences in rooting profiles were further confounded by the range of shrub ages found at the three sites (Table 2). For example, the maximum age of *Purshia* at the large-pole site was about twice that of the other shrubs, implying greater root development and differential access to ^{15}N pools by mature *Purshia*. Other isotope dilution studies of perennial, N fixing species have avoided this problem by only comparing plants or seedlings of the same age (Diagne and Baker, 1994; Sanginga et al., 1996; Binkley, 1997). While this is a preferred option, it was not an alternative in

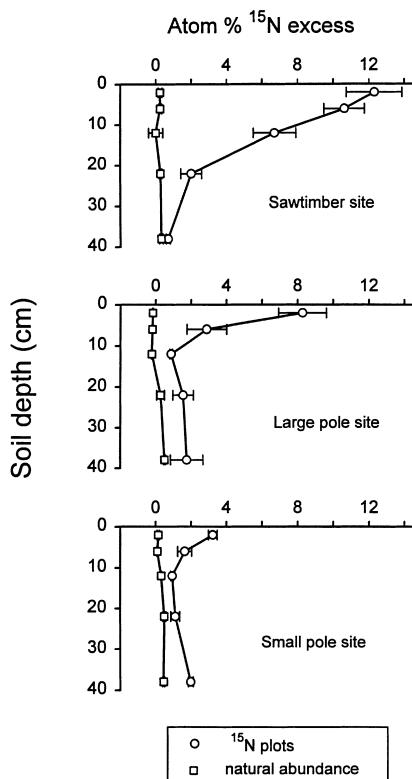


Fig. 1. Variation in ^{15}N with soil depth following two applications of 5 kg N ha^{-1} (10% atom ^{15}N excess). Soil from ^{15}N enriched plots and adjacent, non-enriched soil (natural abundance) were sampled at the end of the 1990 growing season. Error bars are ± 1 standard deviation.

Table 2

Age of N fixing and reference shrubs at the ^{15}N -isotope dilution study sites

Species	Small-pole site (year)			Large-pole site (year)			Sawtimber site (year)		
	n	Mean	Range	n	Mean	Range	n	Mean	Range
<i>Ceanothus velutinus</i>	17	14	5–23	31	14	9–32	22	12	5–14
<i>Purshia tridentata</i>	0	–	–	53	25	10–50	20	11	5–22
<i>Arctostaphylos patula</i>	7	16	7–28	14	24	10–29	15	11	7–14
<i>Ribes cereum</i>	0	–	–	6	17	15–21	0	–	–

my study given the diversity of ages of *Ceanothus* and *Purshia* found in natural pine stands of central Oregon. Failure to identify plants with similar rooting profiles and ages violates the main assumption of the isotope dilution method and suggests the introduction of considerable error in the measurement of percentage of Ndff by multiple-aged stands of *Ceanothus* and *Purshia*.

Additional error in estimating N fixation can result if the reference and N fixing plants have unique temporal patterns of N uptake. Mismatched timing of N uptake can result in an estimated 50% error in predicting percentage of Ndff due to changes in soil ^{15}N content during the growing season (Witty and Geller, 1991). Two indirect lines of evidence suggest that *Arctostaphylos* was the only reference species to match the N uptake pattern of *Ceanothus* and *Purshia*. First, the N concentration in current-year foliage of *Arctostaphylos* showed a similar monthly pattern as *Ceanothus* and *Purshia* (Fig. 2). There was significantly greater N concentration ($p < 0.05$) on July 20th than on any other sampling date for these three shrubs.

In contrast, foliage N concentration declined during the growing season for *Carex*, and was constant at all sampling dates for *Ribes*. However, these observations fall short of a direct measure of N uptake. Plant biomass accumulation must be measured in conjunction with N concentration in order to determine total N uptake pattern. Unfortunately, quantifying monthly biomass accumulation is impractical for slow-growing woody shrubs and was not determined in this study. Comparison of gross phenological characteristics was the second line of evidence pointing to *Arctostaphylos* as a well-matched reference species. Timing of leaf budding, flowering, peak annual production, and leaf senescence were similar for *Arctostaphylos* and the N fixing shrubs. Stem elongation and foliage growth were greatest during June and July, which matches the timing of maximum foliage N concentration (Fig. 2) and suggests a similar pattern of peak N assimilation among the three species. In comparison, *Ribes* broke dormancy and flowered nearly a month earlier than the other shrubs, and *Carex* became senescent by mid-September, well in advance of the shrub species.

There was no decline in foliar ^{15}N during the growing season for any of the shrub species (Fig. 3). This suggests that the enrichment of soil ^{15}N was constant throughout the sampling period and that any differences in timing of N uptake between plants were inconsequential to the accuracy of the isotope dilution method. Lack of ^{15}N depletion can be attributed to the short growing season, low plant coverage, and low N demands of vegetation in moisture-limited pine forests. This contrasts with results from high N demanding, agricultural systems in which depletion of ^{15}N is common for foliage (Sanginga et al., 1996) and soil (Witty, 1983; Boddey et al., 1995).

The three reference plants used in this study were selected *a priori* to help overcome the uncertainties of

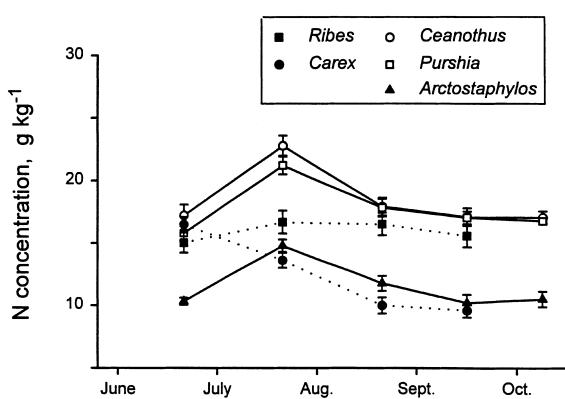


Fig. 2. Monthly foliage N concentration for N fixing and reference species at the large-pole site. Error bars are ± 1 standard deviation.

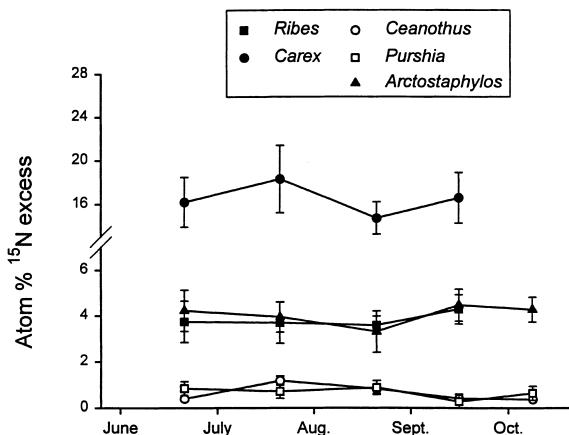


Fig. 3. Monthly foliage ^{15}N atom excess for N fixing and reference species at the large-pole site. Error bars are ± 1 standard deviation.

finding an appropriate match for N fixing plants. This conservative approach provided an upper and lower limit for the calculation of percentage of Ndff (Table 3). Accurate estimates result if the range of percentage of Ndff values, derived independently from a variety of reference plants, is narrow (Boddey et al., 1995). The results for *Ceanothus* and *Purshia* are a prime example. Both plants had high percentage of Ndff values regardless of reference species. Even the graminoid species (*Carex*), which had about 4-fold greater foliage ^{15}N concentration than the other two references due to its high proportion of fine roots in the ^{15}N enriched upper soil profile, produced only in a minor increase in percentage of Ndff for either *Ceanothus* or *Purshia*. Near identical estimates of percentage of Ndff when using *Ribes* or *Arctostaphylos* as the reference plant are also noteworthy given the differences in phenology and N accumulation between these

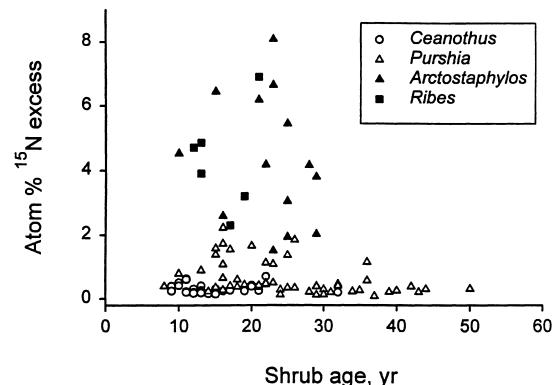


Fig. 4. Variation in foliation ^{15}N with shrub age for N fixing and reference species at the large-pole site.

two species. These results confirm that the isotope dilution method was accurate at estimating percentage of Ndff by *Ceanothus* and *Purshia*. Further, they support the claim that providing a true physiological match between reference and N fixing plants is not crucial for highly effective symbioses (Viera-Vargas et al., 1995).

Foliar ^{15}N concentrations of individual shrubs were measured to determine the relationship between plant age and percentage of Ndff. My objectives were to determine (1) whether percentage of Ndff varies with plant age, and (2) if differences in foliar ^{15}N were sufficiently large to warrant calculation of percentage of Ndff based on a series of age classes. *Ceanothus* and *Purshia* had low foliar ^{15}N relative to the reference plants for the entire range of shrub ages (Fig. 4). There was not a strong correlation between shrub age and atom% ^{15}N excess, however (*Ceanothus*: $r^2 = 0.16$; *Purshia*: $r^2 = 0.04$; *Arctostaphylos*: $r^2 = 0.06$; *Ribes*: $r^2 = 0.36$). As a result, percentage of Ndff calculations

Table 3

Atom% ^{15}N excess in foliage of N fixing and reference plants and percentage of Ndff for *Ceanothus* and *Purshia* calculated independently using each reference species^a

Plant species	n	Foliation ^{15}N atom% excess	Percentage of Ndff	
			<i>Ceanothus</i> (%)	<i>Purshia</i> (%)
<i>Ceanothus velutinus</i>	31	0.37 (0.03)	–	–
<i>Purshia tridentata</i>	53	0.64 (0.07)	–	–
<i>Arctostaphylos patula</i>	14	4.33 (0.54)	91.5 (3.6)	85.2 (12.1)
<i>Ribes cereum</i>	6	4.28 (0.64)	91.4 (3.7)	85.0 (12.2)
<i>Carex rossii</i>	5	16.58 (2.32)	97.8 (0.9)	96.1 (3.2)

^a Values in parentheses are standard deviations.

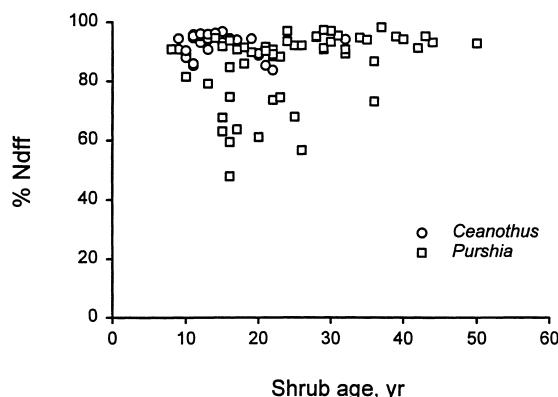


Fig. 5. Variation in percentage of Ndff with shrub age for *Ceanothus* and *Purshia* at the large-pole site.

were not stratified based on age class. Both *Ceanothus* and *Purshia* were highly effective, obtaining the majority of their N from fixation regardless of plant age (Fig. 5). The only uncertainty is for *Purshia* plants >30 years old due to the lack of comparably aged reference plants.

The high symbiotic efficiency of *Purshia* and *Ceanothus* compares favorably with other actinorhizal species. Previous ^{15}N studies have found percentage of Ndff values from 48 to 100% for a variety of temperate zone and tropical tree species (Cote and Camire, 1984; Gauthier et al., 1985; Comenach et al., 1989; Beaupied et al., 1990; Mead and Preston, 1992; Parrota et al., 1994; Binkley, 1997). No comparisons are available for actinorhizal shrubs at present. Symbiotic efficiency of actinorhizal plants can vary depending on several interactive properties, including plant N demand, *Frankia* effectiveness, soil N content, and environmental constraints on plant growth. The fact that both *Ceanothus* and *Purshia* obtained nearly

all of their N from N fixation likely reflects the low soil N content found at the three sites (Table 1).

3.3. Nitrogen fixation

Nitrogen fixing characteristics of *Ceanothus* and *Purshia* are presented in Table 4. *Arctostaphylos* was used as the reference species in calculating percentage of Ndff due to its commonality to all sites. Both *Ceanothus* and *Purshia* were highly effective at fixing N, deriving >80% of their N from N fixation. Their versatility as N fixers is evident by the variety of overstory stand conditions (from an open-grown plantation at the small-pole site to a dense, second-growth pine stand at the large-pole site) and shrub coverages found at the study sites. Consistently high levels of percentage of Ndff, regardless of stand conditions, indicate that N fixation is not a limiting process in the growth of either *Ceanothus* or *Purshia* in central Oregon pine forests.

Total N fixation varied substantially among sites as a result of differences in shrub cover and biomass. The highest level of N fixation by *Ceanothus* was measured at the small-pole site which had between 2- and 3-fold greater biomass than the other sites. *Purshia* fixed considerably less N than *Ceanothus* owing to its low biomass content and slower growth rate. Total N fixed for either species should be considered an underestimation, however, since loss of plant biomass N due to annual leaf fall or root mortality was not determined. This omission probably had only a minor impact on the estimate of N fixation. Annual leaf fall is not excessive for *Ceanothus*, which is an evergreen shrub that retains a large percentage of its annual foliage, or *Purshia*, which allocates only a small proportion of its biomass to foliage. Further, annual

Table 4

Nitrogen fixing characteristics of *Ceanothus velutinus* and *Purshia tridentata* at the ^{15}N -isotope dilution study sites^a

Site	<i>Ceanothus</i>				<i>Purshia</i>			
	Biomass (Mg ha ⁻¹)	Percentage of Ndff (%)	Total N fixed (kg ha ⁻¹)	Annual N fixed (kg ha ⁻¹ year ⁻¹)	Biomass (Mg ha ⁻¹)	Percentage of Ndff (%)	Total N fixed (kg ha ⁻¹)	Annual N fixed (kg ha ⁻¹ year ⁻¹)
Small pole	24.5 (2.8)	80 (7)	246 (26)	15.1 (1.8)	—	—	—	—
Large pole	7.8 (1.4)	91 (4)	83 (3)	4.0 (0.7)	2.4 (0.6)	85 (12)	31 (4)	1.5 (0.4)
Sawtimber	11.8 (2.1)	90 (3)	165 (20)	11.1 (3.1)	0.4 (0.4)	82 (10)	11 (5)	0.4 (0.3)
Average	14.7 (8.7)	87 (6)	165 (82)	10.1 (5.6)	1.4 (1.4)	83 (2)	21 (14)	0.9 (0.8)

^a Biomass values are the sum of aboveground plus belowground dry masses, excluding *Ceanothus* roots <1 cm in diameter. Values in parentheses are one standard deviation ($n = 4$ for each site).

root turnover is less prolific for shrubs than for other vegetative lifeforms (Aerts et al., 1992; Persson, 1979). Failure to harvest *Ceanothus* roots smaller than 1 cm in diameter also contributed to the slight underestimation of total N fixed.

The annual rate of N fixation by *Ceanothus* ($4\text{--}15 \text{ kg ha}^{-1} \text{ year}^{-1}$) is considerably lower than previously reported in studies conducted in western Oregon (Zavitkovski and Newton, 1968; Youngberg and Wollum, 1978; Binkley et al., 1982; McNabb and Cromack, 1983). With one exception (Zavitkovski and Newton, 1968), these studies found rates ranging from 80 to $100 \text{ kg ha}^{-1} \text{ year}^{-1}$. Regional differences in N fixation by *Ceanothus* are to be expected, and reflect the colder and drier climatic conditions of central Oregon. Also, the western Oregon studies were conducted in shrub fields at near-full site occupancy with *Ceanothus*. The objective of my study, in comparison, was to quantify the N contribution of *Ceanothus* as an understory species at low to moderate site occupancy in pine forests. Annual additions of $4\text{--}15 \text{ kg N ha}^{-1}$, although comparatively small, function as important inputs to the N-poor soils of central Oregon and are sufficient to replace N losses during disturbances such as prescribed fire (Busse, 2000). The estimated rate of N input from *Ceanothus* is sufficient to replace N volatilized during low- to moderate-intensity prescribed burning in central Oregon (estimated at 100 kg N ha^{-1} by S.A. Simon (Simon, 1990, unpublished report on file, Fremont National Forest, Lakeview, Oregon, USA)) within 6–25 years.

The average rate of N fixation for *Purshia* of $0.9 \text{ kg ha}^{-1} \text{ year}^{-1}$ is in agreement with the conclusion of Dalton and Zobel (1977) that *Purshia* is a minor contributor to the N economy in central Oregon pine stands, adding an equivalent amount of fixed N as atmospheric N deposition. They estimated an annual rate of 0.057 kg ha^{-1} for *Purshia* using the acetylene reduction method, and attributed the low rate, in part, to sparse root nodulation. My results differed in that (1) shrubs at the large-pole and sawtimber sites were well nodulated when sampled in late spring, and (2) all plants were found to actively fix N during the growing season (Fig. 5). Instead, N fixation appears to be limited by the slow growth rate and low N demand of *Purshia*. Whether this conclusion is valid in non-forested lands where the faster growing, erect form of *Purshia* is found is untested.

4. Conclusions

Ceanothus velutinus and *Purshia tridentata* are dominant understory shrubs in many coniferous forests in central Oregon and the western United States. Their contribution to ecosystem N pools has not been well documented, in part due to the lack of an accurate measure of N fixation. The isotope dilution method was tested for its suitability as a field measure of N fixation by these shrubs. Three reference species (*Arctostaphylos patula*, *Ribes cereum*, *Carex rossii*) with differing N uptake and growth patterns were used to develop a range of percentage of Ndff. Several constraints to the underlying requirement that N fixing and reference plants have access to similar pools of soil ^{15}N were encountered. These included dissimilar rooting profiles and foliar N concentrations between reference and N fixing plants, poor mixing of ^{15}N fertilizer in the soil profile, and a wide range of shrub ages. Such violations, however, did not compromise the suitability of the isotope dilution method. Instead, their importance was minimized by the superior N fixing ability of both *Ceanothus* and *Purshia*. Percentage of Ndff values were high regardless of which species was used as the reference, showing that the requirement of a well-matched reference plant is unnecessary for highly effective symbioses.

Three study sites were selected to provide a range of overstory stand conditions from a young, open-grown pine plantation to a mature sawtimber pine stand. Both *Ceanothus* and *Purshia* were highly efficient N fixers at each site, obtaining $>80\%$ of their N from fixation. Symbiotic efficiency was high for all ages of shrubs present in the stands (9–32 years old for *Ceanothus*; 8–50 years old for *Purshia*). Nitrogen fixation rates for *Ceanothus* ($4\text{--}15 \text{ kg ha}^{-1} \text{ year}^{-1}$) were lower than previously reported, yet not unexpected given the dry, cool climate of central Oregon. Importantly, they are sufficient to replace N losses from disturbances such as prescribed fire. *Purshia* fixed considerably less N ($1 \text{ kg ha}^{-1} \text{ year}^{-1}$) than *Ceanothus* roughly equivalent to the rate of atmospheric N deposition in central Oregon.

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