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High asymbiotic N_2 fixation rates in woody roots after six years of decomposition: controls and implications

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Abstract

The rates and controls of asymbiotic N_2 fixation associated with organic detritus such as decomposing woody roots have rarely been evaluated in forests. We measured acetylene reduction (AR) rates in woody roots after six years of decomposition in three sites of coniferous forest in Oregon, U.S.A., calibrated with measurements of $^{15}N_2$ fixation. Incubation site and root size significantly affected the AR rates of decomposing roots. Decomposing roots at the H.J. Andrews site had the highest AR rates, followed by a wetter site, Cascade Head, and a drier site, Pringle Falls. The mean AR rates of dead roots increased with increasing root size, while mass loss decreased with increasing root size. However, root species did not significantly affect AR with Douglas-fir, western hemlock, and ponderosa pine having mean rates of 13.7, 12.0, and 16.0 nmol/g/day, respectively. Mean N_2 fixation rates in decomposing roots are at least four times higher than rates reported for other asymbiotic sources of N_2 fixation such as dead wood and litter. The average conversion ratio for AR data measured by $^{15}N_2$ fixation in dead roots was 4.5:1. A preliminarily estimate of potential annual N_2 fixation by dead roots after a stand-replacing disturbance of an old-growth Douglas-fir forest at the H.J. Andrews site was 6.3 kg N/ha/year with a range of 2.1 to 10.4 kg N/ha/year, suggesting that dead roots have potentials to provide a significant N source to these N-limited forests.

In Wäldern wurden die Raten und Kontrollen der asymbiotischen N_2-Fixierung, die mit organischem Detritus wie sich zersetzenden holzigen Wurzeln verbunden ist, selten bewertet. Wir maßen die Acetylenreduktionsraten (AR) in holzigen Wurzeln nach sechs Jahren der Zersetzung auf drei Probeflächen in Nadelwäldern in Oregon, USA, die durch Messungen der $^{15}N_2$-Fixierung kalibriert wurden. Die Probefläche der Inkubation und die Wurzelgröße beeinflussten die AR-Raten der sich zersetzenden Wurzeln signifikant. Sich zersetzende Wurzeln der H.J. Andrews Probefläche hatten die höchsten AR-Raten, gefolgt von einer feuchteren Probefläche, Cascade Head, und einer trockeneren Probefläche, Pringle Falls. Die mittleren AR-Raten abgestorbener Wurzeln nahmen mit der Wurzelgröße zu, während der Massenverlust mit zunehmender Wurzelgröße abnahm. Die Art der Wurzel beeinflusste die AR jedoch nicht signifikant, da die mittleren Raten von Douglas-Fichte, westlicher Hemlock-Tanne und Ponderosa-Kiefer bei 13.7, 12.0 und 16.0 nmol/g/Tag lagen. Die mittleren N_2-Fixierungsraten in sich zersetzenden Wurzeln sind mindestens viermal so groß wie die Raten, die für andere asymbiotische Quellen der N_2-Fixierung wie Totholz und Falllaub gelten. Die mittlere Übertragunsrate für AR-Daten, die über N_2-Fixierung in abgestorbenen Wurzeln gemessen wurde, betrug 4.5:1. Eine vorläufige Schätzung der potenziellen jährlichen N_2-Fixierung durch abgestorbene Wurzeln nach Störung eines Altbestandes von Douglas-Fichten durch Bestandsersetzung in der H.J. Andrews Probefläche ergab 6.3 kg N/ha/Jahr mit einer Variation zwischen 2.1 und 10.4 kg N/ha/Jahr. Dies läßt vermuten, dass abgestorbene Wurzeln das Potenzial haben, eine signifikante N-Quelle für diese N-limitierten Wälder zur Verfügung zu stellen.

Key words: decomposing woody roots – acetylene reduction – incubation site – root size – root species – mass loss – AR: $^{15}N_2$ conversion ratio – annual N_2 fixation rate – old-growth Douglas-fir forests – Pacific Northwest

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Introduction

Nitrogen (N) is widely limiting to tree and fungal growth in many forests (Cowling & Merril 1966, Bormann et al. 1994, Binkley et al. 1995, Vitousek et al. 1997). The net primary production of these forests can be increased substantially by additions of N (Weetman et al. 1998). Unlike phosphorus and most other elements that are derived from weathering of primary minerals in the initial stages of soil development, N is largely gained through N_2 fixation and atmospheric deposition (Vitousek 1994, Thompson & Vitousek 1997, Vitousek & Hobbie 2000). The importance of symbiotic N_2 fixation in forest ecosystems in the Pacific Northwest has been highlighted in several studies (e.g., Sollins et al. 1980, Jurgensen et al. 1992). Early-successional, symbiotic N_2 fixers, especially red alder (*Alnus rubra* Bong.) and snowbrush (*Ceanothus velutinus* Dougl.), can fix 100 kg N/ha/year (Jurgensen et al. 1992), but they do not occur in later successional stages of forests. Where these symbiotic N_2 fixers are absent, asymbiotic N_2 fixers have been viewed as potential long-term sources of N to old-growth coniferous forests (Heath et al. 1988, Wei & Kimmins 1998).

Asymbiotic N_2 fixation is often observed in conjunction with the decomposition of organic detritus including logs (Silvester et al. 1982, Sollins et al. 1987, Jurgensen et al. 1989, Hendrickson 1991, Wei & Kimmins 1998, Hicks et al. 2002b), leaf litter (Heath et al. 1988, Silvester 1989, Vitousek 1994, Thompson & Vitousek 1997, Vitousek & Hobbie 2000), soil organic matter (Hendrickson 1990, Jurgensen et al. 1992), and woody roots (Wei & Kimmins 1998). The rates of asymbiotic N_2 fixation in logs, litter, and soil have been measured repeatedly in the Pacific Northwest and surrounding regions (for review, see Son 2001). However, rates of asymbiotic N_2 fixation associated with decomposing woody roots have rarely been estimated (Wei & Kimmins 1998) despite the fact that woody roots compose a large fraction of woody detritus in coniferous forests of the Pacific Northwest (Chen et al. 2001). Experimental studies testing factors that influence asymbiotic N_2 fixation in decomposing woody roots have not been reported so far (Jurgensen et al. 1997, Son 2001). Thus, the rates and controls on asymbiotic N_2 fixation in dead roots in coniferous forests in the Pacific Northwest are largely unknown.

A 10-year long-term woody root decomposition experiment established in 1995 in three old-growth coniferous forests in the Pacific Northwest (Chen et al. 2002) provides us a unique opportunity to evaluate the asymbiotic N_2 fixation activity associated with dead roots and the factors influencing this process in these forest ecosystems. The objectives of this study were two fold:

1. Examine how root species, size, and incubation site influence N_2 fixation rates in dead roots;
2. Compare the N_2 fixation rates of dead roots with other asymbiotic N_2 fixation sources;

Materials and methods

Sites and root collection

The long-term root decomposition experiment is being conducted in Sitka spruce (*Picea sitchensis* (Bong) Carr), Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), and ponderosa pine (*Pinus ponderosa* Laws.) dominated forests at Cascade Head Experimental Forest (Cascade Head), H. J. Andrews Experimental Forest (H. J. Andrews), and Pringle Falls Experimental Forest (Pringle Falls) in Oregon, U.S.A., respectively. These three sites form a climatic gradient from warm and wet at the coastal Cascade Head to cool and dry at Pringle Falls. Cascade Head is located on the Pacific coast near the town Otis, Oregon. The climate is maritime, with a mean annual temperature of 10 °C and a mean annual precipitation of 3420 mm. H. J. Andrews is located 80 km east of the city of Eugene, Oregon on the west slope of the Cascade Range. The climate is also maritime, with wet, relatively mild winters and dry, cool summers. Mean annual temperature is 8.5 °C and mean annual precipitation is 2300 mm. Pringle Falls is located 57 km southwest of the city of Bend, Oregon; east of the Cascade Range. The climate is modified continental, with a mean annual temperature of 5.7 °C and mean annual precipitation of 525 mm. A detailed description of these sites is provided in Chen et al. (2001).

At each site, four plots were established in 1995 with different species and sizes of roots buried at a soil depth of 20 cm in litterbags. For further details about the experimental design of this root decomposition experiment refer to Chen et al. (2002). For this study, we measured N_2 fixation of woody roots (Douglas-fir, western hemlock (*Tsuga heterophylla* (Raf.) Sarg), and ponderosa pine) collected from the three sites after six years of decomposition. The root size classes included fine roots (<2 mm diameter), small roots (2–10 mm), and large roots (10–50 mm). We collected litterbags of woody roots from three plots at each site in June 2001. During harvest each root litterbag was sealed in a plastic bag to prevent moisture loss. In total, we had 81 litterbags (3 sites × 3 species × 3 sizes × 3 plots) of decomposing roots. After recovery, root samples were returned to the laboratory and stored at 3 °C overnight.

Asymbiotic N_2 fixation rates and mass loss of dead roots

Collected root samples were used both for measurements of asymbiotic N_2 fixation rates and mass loss. The litterbags of roots were processed within 36 h of collection. Each root sample was carefully removed and separated from ingrowing roots and other debris, then weighed. For small and large roots, matchstick-sized pieces (approximately 5 mm × 5 mm × 70 mm) of each root sample were cut from the woody roots, weighed, placed in a screw-topped culture tube, and stoppered with a serum bottle cap. In general, the wet weight of each sample was 2–3 g. For each fine root sample, we simply sampled 2–3 g wet weight for N_2 fixation measurement. After N_2 fixation was measured, the root samples in culture tubes were dried to a constant mass at 65 °C and weighed.

Measures of nitrogenase activity were used as an index of N_2 fixation based on the acetylene reduction (AR) assay (Hardy et al. 1973). This method takes advantage of the fact that the enzyme responsible for N_2 fixation, nitrogenase, also catalyzes the reduction of acetylene (C_2H_2) to ethylene (C_2H_4), which is easily detected using a gas chromatograph. In general, we followed the methods of Griffiths et al. (1993) when measuring AR. Tube headspace was purged with argon; then a portion of the headspace was removed and replaced with lab air and high-grade C_2H_2. The final C_2H_2 concentration was 10% in all samples except the controls with roots that did not receive C_2H_2. Headspace oxygen concentration was adjusted to an optimal 4% (Griffiths et al., 1993, Hicks & Harmon 2002). This is also similar to the oxygen concentration of soils at the three sites (Kermit Cromack, Jr. personal communication). Samples were incubated at 22 °C for 24 h. Ethylene was measured on a Hewlett Packard model 5830 gas chromatograph fitted with a flame ionization detector. The gas chromatograph integrator was calibrated with external Scott® C_2H_4 gas standards. Control tubes without C_2H_2 were used to correct for endogenous C_2H_4 production. No endogenous C_2H_4 production during incubations was found in these control tubes. We did not account for endogenous ethylene production with C_2H_2 and a CO blocker as suggested by Nohrstedt (1983) for soil and Hendrickson (1988) for dead wood, because CO has low solubility in comparison to C_2H_2 resulting in an inability to distinguish between acetylene reduced by nitrogenase and endogenously produced ethylene. Background C_2H_4 contamination present in the C_2H_2 was accounted for with control tubes containing C_2H_2 but not dead roots. Griffiths et al. (1993) tested this method to check for effects from sample preparation time, oxygen concentration, incubation time, and air exposure. From these tests, they concluded the method did not introduce significant experimental error. The rate of AR was calculated as nanomoles of C_2H_4 produced per gram dry mass per day.

To convert AR data to the actual amount of nitrogen fixed, we directly measured nitrogen fixation with $^{15}N_2$ on a subset of root samples (n = 5 with 2 reps for 10 samples) and calculated the AR: $^{15}N_2$ ratio. Acetylene reduction rates, using the above methods, were first measured on five different root substrates at 22 °C. After measuring AR on the samples, the headspace was purged and oxygen was added to produce a concentration of 4%. Finally, 100 atom percent $^{15}N_2$ was added to produce a headspace with approximately 12.5 atom percent $^{15}N_2$ except in the control root samples that received no $^{15}N_2$. Wood samples were incubated for two days then removed, ground, and analyzed with a mass spectrometer to get the absolute and relative amounts of the nitrogen isotopes in the samples. We assumed activity rates were constant during the $^{15}N_2$ incubation periods. Headspace samples were also taken for nitrogen isotope ratio measurement to determine $^{15}N_2$ headspace concentrations and leakage rates.

The mass loss percentage of each root sample was calculated by dividing the difference between the original dry weight and the dry weight after six years of decomposition by the original dry weight. Similarly, moisture content was calculated by dividing the difference between sample weight before and after drying by the oven dry weight.

We estimated the total annual N_2 fixed by dead roots six years after a stand-replacing disturbance of an old-growth Douglas-fir forest at the H.J. Andrews. We used mean N_2 fixation rates for the three size classes of Douglas-fir and western hemlock roots from the H.J. Andrews based on AR values and the mean AR: $^{15}N_2$ conversion ratio of 4.5. To estimate a likely range for the annual N_2 fixation rate associated with dead roots at the stand level, we used the mean N_2 fixation rates plus or minus one standard error. An average of 10, 30, and 150 Mg/ha of dead biomass of fine, small, and large roots, respectively, is created in old-growth Douglas-fir forests after such a disturbance (Vogt et al. 1986, Harmon et al. 2003). We assumed that Douglas-fir and western hemlock comprised 60% and 40%, respectively, of these roots. We used the mass loss data of roots of these two species to calculate the mass remaining of dead roots after six years of decomposition. We calculated N_2 fixation rates on a monthly time-step and adjusted the rates according to mean monthly soil temperatures measured at the H.J. Andrews (Chen et al. 2002). A Q_{10} type curve (modified to allow Q_{10} to vary with temperature) developed for woody debris from the H.J. Andrews was used to adjust average N_2 fixation rates according to mean monthly temperatures for the H.J. Andrews (Hicks et al. 2002b).

Statistical analysis

AR rates had long-tailed distributions and required a natural log transformation prior to analysis. Analysis of variance (ANOVA) was performed to test the effects of site, species, size, and their interactions upon nitrogen fixation rates. We used the GLM procedure of SAS (1998) to perform ANOVA. When reporting results in these cases, means of the log transformed values were backtransformed for ease of interpretation. We performed pair-wise comparisons between sites, species, and root sizes using T tests. Statistical tests were considered significant if $P \leq 0.01$.

Results and discussion

Factors influencing AR rates

Incubation site and root size significantly affected the AR rates of decomposing roots (Table 1 and 2). However, species did not significantly affect the AR rates of decomposing roots. No significant interactive effects among sites, species, and root sizes occurred except for a weak interaction between site and root size ($P = 0.0507$). Decomposing woody roots at the H.J. Andrews site had the highest AR rates, followed by the coastal Cascade Head and dry Pringle Falls site (Table 2). The mean AR values of dead roots increased with increasing root size regardless of site (Table 2). The mean AR rate of large roots was 6, 9, and 3 times greater than the rates for fine roots at Cascade Head, H.J. Andrews, and Pringle Falls, respectively. However, the AR values of small roots and large roots were similar at these sites. The mean AR values of Douglas-fir, western hemlock, and ponderosa pine roots were 13.7, 12.0, and 16.0 nmol/g/day, respectively (Table 2).

As asymbiotic N_2 fixation rates are greatly affected by moisture and temperature (Hicks et al. 2002a), the significant site differences in the average of AR rates of decomposing woody roots partially reflects the differences in the moisture content of these roots given that they were incubated under the same laboratory temperature (22 °C, Table 2 and Fig. 1). *In situ* soil temperature may have some impact on the population and composition of the bacteria responsible for asymbiotic N_2 fixation, however, the mean soil temperature for 20 cm depth in June at Cascade Head, H. J. Andrews, and Pringle Falls were very similar: 11.5, 11.0, and 10.7 °C, respectively (Chen et al. 2002). Roots with low moisture contents generally had lower AR rates (Fig. 1). The low AR rates of dead roots at the Pringle

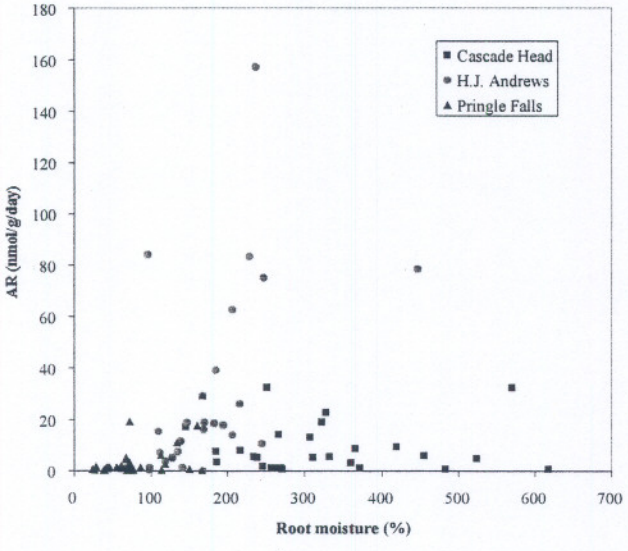

Fig. 1. Relationship between root moisture content and AR rate.

Table 1. ANOVA results for the AR rates of decomposing roots.

Source	F value	df	P value
Site	**20.92**	**2**	**< 0.0001**
Species	2.58	2	0.0851
Root size	**14.97**	**2**	**< 0.0001**
Site * Species	0.84	4	0.5034
Site * Root size	2.53	4	0.0507
Species * Root size	0.19	4	0.9438
Site * Species * Size	0.94	8	0.4903

Note: statistically significant sources of variation ($P \leq 0.01$) are bold.

Table 2. The mean AR rates of decomposing roots.

Types	Mean ± 1 SE (nmol/g/day)
Species	
Douglas-fir	13.7 ± 3.9
Western hemlock	12.0 ± 5.8
Ponderosa pine	16.0 ± 4.7
Incubation sites	
Cascade Head	8.6 ± 1.8
H.J. Andrews	29.9 ± 7.2
Pringle Falls	3.2 ± 1.0
Root sizes	
Fine roots	2.8 ± 0.7
Small roots	18.3 ± 4.7
Large roots	20.5 ± 6.4

Note: 1. each mean AR rate is the mean of 27 samples.
2. significant differences (T test $P < 0.05$) among incubation sites and root sizes are indicated if superscripts within a given type differ.

Falls site were probably due in part to their low mean moisture content (76%), whereas higher mean root moisture contents at the H.J. Andrews (172%) and coastal Cascade Head sites (325%) were accompanied by higher rates. Our results were consistent with other studies that examined the relationship of moisture content and AR rate. Jurgensen et al. (1992), Wei & Kimmins (1998), and Hicks et al. (2002a) all observed AR rates in woody debris to increase with increasing wood moisture content. However, AR rates were significantly lower at Cascade Head than those at the H.J. Andrews (Table 2). The extremely high average root moisture content at Cascade Head (Fig. 1) may have indirectly led to the lower rates. These moist roots could produce *in situ* anaerobic conditions and favor anaerobic N_2 fixer populations, whose activity is not accounted for in the current investigation, over the microaerophillic N_2 fixers commonly found in wood (Silvester et al. 1982).

The significant effect of root size on AR rates at all three sites (Table 1) may be due to the influence of root size class on root decomposition dynamics and the differences in substrate availability that accompany decomposition. In our study, fine roots lost about 60% of their initial dry weight after six years of decomposition in comparison with 45% for small roots and 25% for large roots (Fig. 2). Like most organic matter decomposition, the labile carbon and organic compounds of small molecular weight were leached and/or decomposed in the early stages of root decomposition (Chen et al. 2002). The materials remaining in later stages of decomposition generally were recalcitrant organic matter such as lignin, which is a poor energy source for N_2-fixing bacteria (Chan 1986). In contrast, larger roots decomposed much slower than fine roots. The material remaining in these woody roots after six years of decomposition is probably a more favorable substrate for N_2-fixing microorganisms given that the initial lignin concentration of these different sized woody roots was similar (Chen et al. 2001, 2002). This partially explains why the AR value of decomposing fine roots was lower than those of larger sized roots (Fig. 2).

Table 3. A comparison of asymbiotic nitrogen fixation rates among several substrates from the western Oregon of Pacific Northwest.

Substrate	N_2 fixed (nmol/g/day)[1]	n	Conversion ratio	Reference
Dead Roots	4.4 (1.01)	54	4.5:1	This study[2]
Dead Wood	1.3 (0.18)	93	4.4:1	Hicks et al. 2002b[3]
Soil Wood	1.1 (0.15)	40	4:1	Jurgensen et al. 1992[4]
Litter	0.8 (0.16)	40	4:1	Jurgensen et al. 1992
Soil	0.2 (0.04)	40	4:1	Jurgensen et al. 1992

[1] Mean value is presented with standard error in parenthesis. Incubation temperatures varied between studies. We standardized all N_2 fixation rates to an incubation temperature of 22 °C using a Q_{10} of 2.

[2] Two sites, H. J. Andrews and Cascade Head, from western Oregon were used for this comparison.

[3] Three sites from western Oregon were examined. Three species were sampled: *Pseudotsuga menziesii*, *Tsuga heterophylla*, and *Picea sitchensis*.

[4] One site from northern Idaho was examined where *Tsuga heterophylla*, *Thuja plicata* (Donn ex D. Don) Lindl.), and *Pseudotsuga menziesii* are dominant. Annual precipitation and mean annual temperature for this site are similar to western Oregon.

AR: $^{15}N_2$ conversion ratio

We directly measured nitrogen fixation with $^{15}N_2$ on a subset of samples (n = 5 with 2 reps for each sample) and calculated the AR: $^{15}N_2$ ratio. The average of this ratio was 4.5 ± 0.56 (± 1 SE). This ratio was close to a 4:1 theoretical conversion ratio of AR to nitrogen fixed (Simpson & Burris 1984, Bergersen 1991). Moreover, this ratio is similar to other measurements of the AR: $^{15}N_2$-fixation ratio (Silvester et al. 1982, Vitousek & Hobbie 2000, Hicks et al. 2002b). Vitousek & Hobbie (2000) found an average ratio of 3.9 (± 0.2) for decomposing leaf litter. Hicks et al. (2002b) found an average ratio of 4.4 for woody debris in western Oregon, USA.

Comparison of asymbiotic N fixation rates

Mean N_2 fixation rates in decomposing roots are clearly much higher than rates reported for other asymbiotic sources of N_2 fixation such as dead wood, litter, and soil (Table 3). Our study indicated the mean N_2 fixation of dead roots from two sites in western Oregon was 4.4 nmol/g/day. At these sites, Hicks et al. (2002b) found that the N_2 fixation rates of dead wood were much lower with a mean value of 1.3 nmol/g/day. Jurgensen et al. (1992) indicated that the mean N_2 fix-

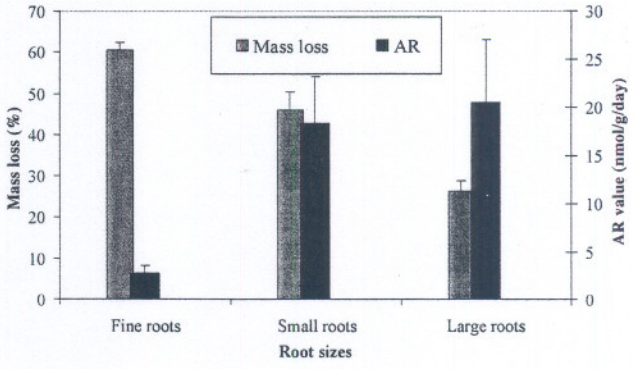

Fig. 2. Relationship between mass loss, root size, and AR rate of decomposing roots. Error bars represent 1 SE.

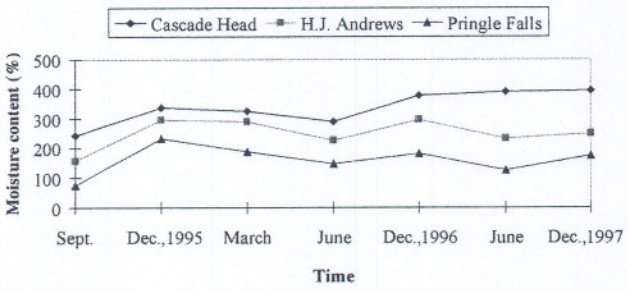

Fig. 3. Moisture dynamics of decomposing fine roots at the three sites.

ation rate of soil, litter, and soil wood (mainly buried logs) was 0.2, 0.8, and 1.1 nmol/g/day, respectively, at similar forest types.

It is still not very clear which factors contribute to the high asymbiotic N_2 fixation rates of dead roots. Both environmental and substrate effects could contribute to these high rates. The fluctuations in soil temperature at these sites were considerably less than those in air temperature for aboveground logs and litter (Chen et al. 2002), which is favorable to N_2 fixation. Moreover, the root moisture at these sites usually ranged from 100 to 300% (Fig. 3), a level that is favorable to N_2 fixation (Hicks et al. 2002a). The temperature and moisture conditions associated with decomposing roots are simply more favorable for N_2 fixation in comparison with moisture and temperature conditions of aboveground logs or litter (Heath et al. 1988, Harmon & Sexton 1995, Wei & Kimmins 1998, Chen et al. 2002). In addition, decomposing roots have more favorable substrates for the N_2-fixing bacteria and fungi than mineral soil (Wei & Kimmins 1998) and possibly dead wood and litter. The species of N_2-fixing bacteria may also be playing a role, perhaps having a higher N_2 fixation capacity compared to species found in woody debris, litter, and soil. Obviously, more studies are needed to explore what exactly cause the higher asymbiotic N_2 fixation rates in decomposing roots compared with other substrates such as dead wood, litter, and soil.

The high N_2 fixation rates in decomposing roots probably regulate root decomposition rates to some degree. Nitrogen-fixing bacteria are known to increase wood decay and mycelial growth by fungi (Blanchette & Shaw 1978). In addition, wood-inhabiting N_2-fixing bacteria have been observed to grow in close proximity to fungal hyphae (Blanchette & Shaw 1978). Presumably, N_2-fixing bacteria and fungi have a mutualistic association in which fungi gain N and vitamins while bacteria can scavenge products of the fungal degradation of cellulose and lignin.

Implications for N input to forests

Nitrogen fixation in dead woody roots could be an important N input to the coniferous forests in the Pacific Northwest, given the high asymbiotic N_2 fixation rates associated with dead woody roots (Table 2 and 3). Tree root systems store large amounts of organic matter and nutrients in these forest ecosystems. About 200 Mg/ha of dead woody roots are created when catastrophic disturbances such as forest fire, windfall, or a clear-cut harvest replace old-growth Douglas-fir forests in the Pacific Northwest (Vogt et al. 1986, Harmon et al. 2003).

We estimated the annual amount of N_2 fixed by these dead roots six years after a stand-replacing disturbance of an old-growth Douglas-fir forest at the H.J. Andrews. We used the mean N_2 fixation rates of decomposing woody roots from this site combined with the decomposition data for these roots. We used the average conversion ratio of 4.5:1 determined in this study to convert AR values to nitrogen fixed.

Three assumptions are made in this estimate. The first assumption is that nitrogenase activities vary mainly with soil temperature. To account for the first assumption, we calculated N_2 fixation rates on a monthly time-step and adjusted the rates according to mean monthly soil temperatures measured at the H.J. Andrews (Chen et al. 2002). A Q_{10} type curve (modified to allow Q_{10} to vary with temperature) developed for woody debris from the H.J. Andrews was used to adjust average N_2 fixation rates according to mean monthly temperatures for the H.J. Andrews (Hicks et al. 2002b). The second assumption is that *in situ* root moisture does not inhibit nitrogen fixation at this site. Over a two-year period from 1995–1997, root moisture at the H.J. Andrews site ranged from 150 to 300% (Fig. 3), a level that is favorable for N_2 fixation (Hicks et al. 2002a). The third assumption is that laboratory conditions, particularly O_2 concentrations, reasonably reflected those in the field. In our study, the headspace oxygen concentration was adjusted to an optimal 4% (Griffiths et al. 1993, Hicks & Harmon 2002). This oxygen level is similar to the oxygen concentration of soils at the three sites (Kermit Cromack, Jr. personal communication). Under these assumptions, decomposing woody roots fixed an average of 6.3kg N/ha/yr with a range of 2.1 to 10.4 kg N/ha/yr. Although our estimate is very preliminary, this annual N_2-fixation rate represents a considerable amount of N compared with other N inputs to these forests. For example, N input as precipitation and dust for this stand was 2.0 kg/ha/yr; cyanophycophilous lichens in the canopy fixed N_2 at a rate of 2.8 kg/ha/yr; and 14.1 kg N/ha/yr was returned to the forest floor by

aboveground litterfall (Sollins et al. 1980). Moreover, decomposition of woody roots, especially the large ones, generally take decades or even centuries to complete in the coniferous forests of the Pacific Northwest (Chen et al. 2001, 2002). Thus, the cumulative N gains by the decomposing woody roots over time would be appreciable.

Nitrogen fixation from decomposing dead roots may be important for replacement of N losses on sites where old-growth forests are clear-cut or burned. Such disturbances generally lead to significant N loss for forest stands in the Pacific Northwest. For example, the clear-cut of a Douglas-fir old-growth forest in watershed 10 at the H.J. Andrews site resulted in the loss of nearly 400 kg N/ha in wood boles and 1.2 kg N/ha/year in stream export during the first 1.5 years after cutting (Sollins & McCorison 1981). Severe forest fires led to a loss of 600 kg N/ha from the soil for similar stands in this region (Little & Ohmann 1988). Nitrogen gains from precipitation and dust (2.0 kg N/ha/year, Sollins et al. 1980) and from asymbiotic N_2 fixation in litter and logs (1.0 kg N/ha/year, Hicks et al. 2002b) would take 140–200 years to replace these losses. However, the length of time to replace these N loss would decrease to 78–118 years if we assume the lowest annual N fixation rate of decomposing roots at this stand (2.1 kg N/ha) would remain steady.

Future research work

Very few studies have measured N_2 fixation rates in dead roots. The high asymbiotic N_2 fixation rates in dead roots observed in this study show the potential importance of dead roots in N dynamics in the coniferous forests of the Pacific Northwest. More studies are needed to further constrain the uncertainties in asymbiotic N_2 fixation measurements of dead roots. These uncertainties include 1) how do the N_2 fixation rates of decomposing woody roots change over time? and 2) how do soil temperature, moisture, and oxygen concentration influence N_2 fixation rates? Thus, to better measure asymbiotic N_2 fixation rates at the root and stand levels and understand factors influencing this process, more studies are needed. In particular: 1) More systematic measurements of N_2 fixation rates associated with decomposing woody roots over time using different size classes, species, and incubation sites; 2) Development of response curves relating N_2 fixation rates to root temperature, moisture, and oxygen concentration; 3) Better estimates of woody root biomass and its distribution among species and size classes; and 4) Determination of appropriate conversion ratios for relating AR to N_2 fixed using ^{15}N approach on more decomposing root samples.

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