Natural Abundance of Nitrogen-15 as a Tool for Tracing Alder-Fixed Nitrogen

DAN BINKLEY, PHILLIP SOLLINS, AND WILLIAM B. McGILL

ABSTRACT

Ratios of $^{15}$N to $^{14}$N often differ between pools within ecosystems, and measurement of these natural-abundance ratios might allow transfers among pools to be traced. We tested this approach for its ability to trace biologically-fixed N in conifer plantations. Ratios of $^{15}$N to $^{14}$N were measured in soil total-N, ammonium, and nitrate, and in foliage of Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] and red alder (Alnus rubra Bong.) at four sites. Two ecosystems were sampled at each site, one a pure conifer stand and the other a mixed alder-conifer stand. Isotope ratios differed significantly among stands, but no pattern was consistent across all locations. Soil NH$_4^+$ at all sites (and soil nitrate at one site) was significantly depleted in $^{15}$N relative to other N pools. Isotope discrimination clearly occurs during N transfers at these sites, but the $^{15}$N natural-abundance method does not provide a simple picture of N cycling at the ecosystem level.

Additional Index Words: stable isotopes, isotope discrimination, N fixation, N cycling, N mineralization.

NITROGEN FIXATION by red alder (Alnus rubra Bong.) typically accelerates N cycling in forest ecosystems even more than it increases N capital. For example, in mixed stands of red alder and Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco], N-capital increases of 30 to 50% have been accompanied by 3- to 10-fold increases in litterfall N (Tarrant et al., 1969; Cole et al., 1978). Tracing the fate of alder-fixed N in conifer plantations could be a major step toward better management of forest nutrition.

If the isotopic composition of alder-fixed N was sufficiently distinctive, it could be traced through soil N pools and into the tissues of non-N-fixers. Several researchers have noted significant differences between N recently fixed and "older" soil N (Kohl and Shearer, 1980; Kohl et al., 1981). But others have reported that variability in the isotope ratio within pools masks differences between pools (Broadbent et al., 1980, 1981). The magnitude of within-pool variation relative to be-
between-pool differences determines the usefulness of the $^{15}$N natural-abundance method (Black and Waring, 1977). In the present study, we undertook to trace alder-fixed N by measuring the natural abundance of $^{15}$N in soil and foliage from adjacent mixed alder/conifer and pure conifer stands at four locations in the Pacific Northwest.

### STUDY SITES

The four sites provided a factorial design with two levels of soil fertility and two stand ages (Table 1). Mixed Douglas-fir/alder stands at Mt. Benson (Vancouver Island, British Columbia) were located in portions of a 23-yr-old Douglas-fir plantation (site index without alder: 24 m at 50 yr) where red alder or Sitka alder (*Alnus sinuata* (Regel) Rydb.) had established naturally (Binkley, 1983; Binkley et al., 1984). The soil is an infertile gravelly clay loam (Typic Hapludults). The Douglas-fir/alder stand at the U.S. Forest Service Wind River Experimental Forest in southeastern Washington (site index 25 m) was located within a 20-m-wide fire break interplanted with red alder in 1930 (Tarrant and Miller, 1963; Miller and Murray, 1978; and Binkley and Greene, 1983). The soil is an infertile silty clay loam (Andic Hapludults).

At the fertile Skykomish site, near Sultan in northwestern Washington (site index 45 m), red alder had established naturally throughout the Douglas-fir plantation. The pure Douglas-fir stand consisted of an area from which red alder was removed in 1960, 2 yr after plantation establishment (Binkley, 1983). The soil is a Kitsap silty clay loam (Dystric Hapludult). The naturally established vegetation at the U.S. Forest Service Cascade Head Experimental Forest on the Oregon Coast (site index 40 m) is dominated by Douglas-fir and red alder, with about one-fifth of the conifer biomass (at age 53) in Sitka spruce (*Picea sitchensis* (Bong.) Carr) and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) The mixed conifer stand was a 0.2-ha plot from which alder was removed in 1930 (Franklin et al., 1968; Tarrant et al., 1969; Binkley and Greene, 1984). The soil is a fertile Astoria silty clay loam (Typic Hapludults).

### METHODS

The three stands at Mt. Benson were sampled in summer 1981. Soil was collected at a depth of 0 to 15 cm from 10 pits in each of the three stands, then air dried and stored for about 30 d before analysis. Mineralization potential was indexed as net production of ammonium and nitrate after 30 days aerobic incubation at field capacity and 20°C. For $^{15}$N/nitrogen determination of ammonium and nitrate, 0.3-kg unincubated subsamples were extracted with 700 mL of 2 M KCl and filtered. Samples of Douglas-fir foliage (10 per stand) consisted of needles of all ages from the upper crown; alder leaves (10 samples per stand) also came from upper crowns. The pairs of stands at Wind River, Skykomish, and Cascade Head were sampled in autumn 1982 by the same methods used at the Mt. Benson site except that foliage samples were obtained with a shotgun.

Ten red alder seedlings, grown in a N-free solution (Harper's + Co + Ni), provided data on the $^{15}$N/nitrogen ratio of symbiotically fixed N. Germinant roots were dipped in a solution containing *Frankia* isolates obtained from S. Rose, Dep. of Forest Science, Oregon State Univ., Corvallis, and the seedlings were transplanted to a tray filled with sterilized perlite. About 60 d later, when average seedling height was 15 cm, the greenhouse seedlings were harvested, oven-dried, and ground.

Plant and soil samples were digested by standard kjeldahl procedure (McKeague, 1976). Magnesium oxide was used to raise pH during distillation of non-digested soil extracts to minimize hydrolysis of organic N; NaOH was used with digestes. After distillation, ammonium was trapped in boric acid, further acidified with concentrated H$_2$SO$_4$, and evaporated to dryness in an oven. All glassware was prewashed with KOH/isopropyl alcohol, and 30 mL of ethyl alcohol was distilled between samples.

Isotopic ratios were determined on a V.G. Micromass 602C double-collecting mass spectrometer in the Soil Science Dep. at the Univ. of Alberta under the supervision of W. McGill. Readings were integrated for 20 s and replicated four times. Results are expressed in parts per thousand $^{15}$N excess ($\Delta^{15}$N, Hauck and Bremner, 1976) relative to atmospheric N (0.3663% $^{15}$N). Reproducibility was good, with standard deviations of 0.90 for the ammonium sulfate standard (mean $\Delta^{15}$N = +0.94, n = 18), 0.74 for the forest soil (n = 10), and 2.90 for the Douglas-fir foliage (n = 11). The standard deviation for blind replicates of a local agricultural soil was 1.56 (n = 12). Ammonium sulfate samples yielded the same $^{15}$N/nitrogen ratio before and after the digestion and distillation.

### Table 1. Site descriptions.

<table>
<thead>
<tr>
<th>Site</th>
<th>Soil total-N 0-60 cm</th>
<th>N mineralization index</th>
<th>Percent of total-N mineralized</th>
<th>Stocking</th>
<th>Aboveground net primary production</th>
<th>Aboveground biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g kg ha$^{-1}$</td>
<td>mg N kg$^{-1}$ soil</td>
<td></td>
<td>g stem ha$^{-1}$</td>
<td>Mg ha$^{-1}$ yr$^{-1}$</td>
<td>Mg ha$^{-1}$</td>
</tr>
<tr>
<td>Mt. Benson, Vancouver Is., B.C.†</td>
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<tr>
<td>Young stands, infertile soil, 510 m elevation, 250 cm yr$^{-1}$ precipitation</td>
<td>1 560</td>
<td>3.3</td>
<td>0</td>
<td>0.4</td>
<td>650</td>
<td>0</td>
</tr>
<tr>
<td>Douglas-fir</td>
<td>1 990</td>
<td>23.8</td>
<td>28.2</td>
<td>3.1</td>
<td>570</td>
<td>10 000</td>
</tr>
<tr>
<td>Douglas-fir and Sitka alder</td>
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<td>23.0</td>
<td>25.8</td>
<td>2.6</td>
<td>540</td>
<td>2 200</td>
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<td>Wind River, Washington‡</td>
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<td>0</td>
<td>0.2</td>
<td>380</td>
<td>2 200</td>
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<td>21.0</td>
<td>3.3</td>
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<td>280</td>
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<td>44.2</td>
<td>18.8</td>
<td>2.2</td>
<td>1 600</td>
<td>280</td>
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<td>55.9</td>
<td>12.3</td>
<td>2.2</td>
<td>1 860</td>
<td>290</td>
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<tr>
<td>Douglas-fir</td>
<td>5 565</td>
<td>44.2</td>
<td>18.8</td>
<td>2.2</td>
<td>1 600</td>
<td>280</td>
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<tr>
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<td>Trace</td>
<td>1.6</td>
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<td>12.5</td>
<td>1.8</td>
<td>115</td>
<td>530</td>
</tr>
<tr>
<td>Mixed conifers</td>
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<td>100.3</td>
<td>12.5</td>
<td>1.8</td>
<td>115</td>
<td>530</td>
</tr>
</tbody>
</table>

† From Binkley, 1983; Binkley et al., 1984.
‡ Binkley and Sollins, unpublished data.
indicating that there was no isotope discrimination during processing.
Sample distributions were heteroscedastic; means were compared with the Game and Howell method (Sokal and Rohlf, 1981) at \( p < 0.05 \).

**RESULTS**

Many means for pools at each site differed significantly, but no trend was consistent across all sites. At Mt. Benson, the presence of alder did not affect isotope ratios of soil total-N (Fig. 1A), which was not surprising as the ratio for soil total-N in the pure conifer stand did not differ significantly from that for the greenhouse-grown alder seedlings (\( \Delta^{15}N = -0.3, \text{SE} = 0.2 \)). Isotope ratios for soil ammonium of the pure conifer stand are not reported because samples were contaminated during drying. Soil ammonium was substantially depleted in \( ^{15}N \) relative to that in soil total-N in both Mt. Benson mixed Douglas-fir/alder stands; nitrate also appeared depleted in \( ^{15}N \) relative to soil total-N, but the difference was not significant. Douglas-fir foliage, alder foliage, soil ammonium and soil nitrate all yielded similar isotope ratios; alder-fixed N could therefore not be traced. The cause of \( ^{15}N \) depletion in the ammonium and nitrate pools is unknown, but discrimination during mineralization, immobilization, or uptake would account for the results.

At Wind River, the alder/conifer stand yielded a significantly lower isotope ratio for soil total-N (Fig. 1B). From the \( \Delta^{15}N \) of soil total-N at the pure conifer stand at Wind River and of greenhouse-grown alders, we calculated that the soil from the mixed conifer/alder stand was comprised of approximately 40% "older" soil N and 60% alder-fixed N. Surface soil (0-15 cm depth) from Wind River averaged 1.6 g N kg\(^{-1} \) without alder and 3.1 g N kg\(^{-1} \) with alder (Binkley and Sollins, unpublished data), which suggests that approximately 50% of the N in the alder soil was "older" and 50% was recently fixed by alder. These values are fairly similar to those based on \( ^{15}N \) natural abundance. As at Mt. Benson, the ammonium pool was significantly depleted of \( ^{15}N \) relative to the soil total-N pool. The \( ^{15}N:^{14}N \) ratio for the nitrate pool was also low but, as at Mt. Benson, not significantly different from any other pool. Foliage of both Douglas-fir and red alder appeared substantially enriched in \( ^{15}N \), but values differed significantly only from soil-ammonium \( ^{15}N \) from the red alder stand. Red alder foliage appeared less enriched than Douglas-fir foliage, which would be expected if alder foliage contains a

![Fig. 1. The \( ^{15}N:^{14}N \) ratios for nitrogen pools at four Douglas-fir dominated sites. Bars are one standard error (\( n = 10 \)). Letters identify pools whose ratios do not differ significantly (\( p < 0.05 \)).](image-url)
blend of native and alder-fixed N. Again, the difference was not significant.

Differences in isotope ratios were more pronounced at Skykomish than at the two infertile sites (Fig. 1C). Soil total-N at the Douglas-fir/red alder stand was enriched more than at the pure Douglas-fir stand, the reverse of the pattern that would be expected with the $\Delta^{15}N$ of alder-fixed N near zero. Soil ammonium was depleted of $^{15}N$ in both stands, and soil nitrate was significantly depleted relative to the ammonium pool. Foliation $^{15}N/^{14}N$ ratio matched that for soil ammonium but not for soil total-N or nitrate.

Soil total-N and ammonium yielded similar $^{15}N/^{14}N$ ratios at Cascade Head regardless of the presence of red alder (Fig. 1D). The soil nitrate pools appeared depleted relative to the ammonium pools, but the difference was significant only in the pure conifer stand. In the conifer/alder stand, Douglas-fir and red alder foliage and soil ammonium yielded similar ratios. The isotopic ratio for Douglas-fir foliage in the pure conifer stand matched that of no other pool.

**DISCUSSION**

The use of naturally occurring $^{15}N$ to trace alder-fixed N requires that the $^{15}N/^{14}N$ ratio differ significantly from that of other potential N sources ("older" soil N). The choice of parameter to index "older" soil N is problematic, since at our study sites, soil total-N and soil inorganic N yielded very different ratios. The value for soil total-N is unsuitable because mineralization must precede use by plants, but it provides opportunity for isotope discrimination. Values for inorganic N pools may be equally unsuitable if seasonal depletion and replenishment alter their isotopic composition. Kohl and Shearer (1980) recommended using the isotopic ratio of a non-N-fixer as the index of soil N, arguing that such a value is an index weighted over space and time. This seems reasonable but requires the assumption that N-fixers and non-N-fixers discriminate similarly against $^{15}N$ during uptake, transport, and assimilation. These assumptions may be met within N-fixing and non-N-fixing isolines of single species (Kohl et al., 1980), but we hesitate to presume a similar pattern for species so genetically dissimilar as red alder and Douglas-fir.

At Wind River, soil total-N was less enriched in $^{15}N$ in stands with alder than without, a result consistent with our proposed pattern. At Skykomish, however, the expected pattern was reversed; soil total-N was significantly more enriched in $^{15}N$ in stands with alder than without. Discrimination against $^{15}N$ during nitrification, coupled with nitrate leaching or denitrification, could cause $^{15}N$ enrichment of soil total-N (c.f. Blackmer and Bremmer, 1977). Such a phenomenon would preclude use of $^{15}N$ natural abundance for tracing alder-fixed N. Overall, the absence of a consistent pattern across locations prompts extreme caution in interpreting the data for any single location.

We conclude that the $^{15}N$ natural-abundance method does not provide a simple means for evaluating N dynamics at the forest sites we studied. However, we did find highly significant differences in isotopic composition of the various N pools within sites despite inconsistent patterns between sites. Soil inorganic N was markedly depleted of $^{15}N$ relative to many other pools at all sites. Isotope discrimination clearly occurs, but identifying mechanisms may be an arduous task given the substantial within-pool variability and the many interacting processes.

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**REFERENCES**


