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Natural Abundance of Nitrogen-15 as a Tool for Tracing Alder-Fixed Nitrogen¹

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ABSTRACT

Ratios of ¹⁵N to ¹⁴N 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 ¹⁵N to ¹⁴N 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 NH₄⁺ at all sites (and soil nitrate at one site) was significantly depleted in ¹⁵N relative to other N pools. Isotope discrimination clearly occurs during N transfers at these sites, but the ¹⁵N 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.

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N^{ITROGEN 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 Douglasfir [*Pseudotsuga menziesii* (Mirb.) Franco], N-capital increases of 30 to 50% have been accompanied by 3to 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-

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tween-pool differences determines the usefulness of the ¹⁵N natural-abundance method (Black and Waring, 1977). In the present study, we undertook to trace alder-fixed N by measuring the natural abundance of ¹⁵N 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 Douglasfir/alder stands at Mt. Benson (Vancouver Island, British Columbia) were located in portions of a 23- yr-old Douglasfir 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 Haplorthod unclassified to series). 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 Haplumbrept unclassified to series).

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 Xerochrepts). 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 Douglasfir 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 Dystrandepts).

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 ¹⁵N/¹⁴N 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.

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Ten red alder seedlings, grown in an N-free solution (Harper's + Co + Ni), provided data on the ¹⁵N/¹⁴N 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 digests. After distillation, ammonium was trapped in boric acid, further acidified with concentrated H_2SO_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.

Isotope 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 ¹⁵N excess ($\Delta^{15}N$, Hauck and Bremner, 1976) relative to atmospheric N (0.3663% ¹⁵N). 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 ¹⁵N/¹⁴N ratio before and after the digestion and distillation,

Table 1. Site descriptions.								
Site Stand type	Soil total-N 0-60 cm	N mineralization index		Percent of total-N	Stocking		Aboveground net primary	Aboveground
		Ammonium N	Nitrate N	mineralized	Douglas-fir	Alder	production	biomass
	kg ha-'	mg N kg ⁻¹ soil			stem ha-'		Mg ha-' yr-'	Mg ha''
Mt. Benson, Vancouver Is., B.C.†								
Young stands, infertile soil, 510 m								
elevation, 250 cm/yr precipitation								
Douglas-fir	1 560	3.3	0	0.4	650	0	6.9	57.9
Douglas-fir and Sitka alder	1 990	23.8	26.2	3.1	570	10 000	13.9	95.3
Douglas-fir and red alder	2 375	23.0	25.8	2.6	540	2 200	15.7	114.0
Wind River, Washington [‡]								
Old stands, infertile soil, 600 m								
elevation, 230 cm/yr precipitation								
Douglas-fir	3 470	3.8	0	0.2	380	0	8.0	142.2
Douglas-fir and red alder	4 0 2 0	82.4	21.0	3.3	250	280	25.0	244.4
Skykomish, Washington [†]								
Young stands, fertile soil, 35 m								
elevation, 130 cm/yr precipitation								
Douglas-fir	4 650	55.9	12.3	2.2	1 860	0	23.2	258.3
Douglas-fir and red alder	5 565	44.2	18.8	2.2	1 600	280	22.5	219.1
Cascade Head, Oregont			20					
Old stands, fertile soil, 180 m								
elevation, 250 cm/yr precipitation			10					
Mixed conifers	6 760	88.0	Trace	1.6	600	0	13.0	205.0
Conifers and red alder	6 810	100.3	12.5	1.8	115	530	13.0	250.0
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Table 1. Site descriptions

+ 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 ($\Delta^{15}N = -0.3$, 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 ¹⁵N relative to that in soil total-N in both Mt. Benson mixed Douglas-fir/alder stands; nitrate also appeared depleted in ¹⁵N 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 ¹⁵N 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 ¹⁵N natural abundance. As at Mt. Benson, the ammonium pool was significantly depleted of ¹⁵N relative to the soil total-N pool. The ¹⁵N:¹⁴N 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 ¹⁵N, but values differed significantly only from soilammonium $\Delta^{15}N$ 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).

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 Δ^{15} N of alder-fixed N near zero. Soil ammonium was depleted of ¹⁵N in both stands, and soil nitrate was significantly depleted relative to the ammonium pool. Foliage ¹⁵N/¹⁴N ratio matched that for soil ammonium but not for soil total-N or nitrate.

Soil total-N and ammonium yielded similar ¹⁵N/ ¹⁴N 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 isotope ratio for Douglas-fir foliage in the pure conifer stand matched that of no other pool.

DISCUSSION

The use of naturally occurring 15N to trace alderfixed N requires that the 15N/14N 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 isotope 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 ¹⁵N 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 ¹⁵N 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 15N in stands with alder than without. Discrimination against ¹⁵N during nitrification, coupled with nitrate leaching or denitrification, could cause 15N enrichment of soil total-N (c.f. Blackmer and Bremner, 1977). Such a phenomenon would preclude use of ¹⁵N 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 ¹⁵N 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 15N 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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