

Relationships between salmon abundance and tree-ring $\delta^{15}\text{N}$: three objective tests

D.C. Drake, Paul J. Sheppard, and Robert J. Naiman

Abstract: Quantification of a relationship between salmon escapement in rivers and riparian tree-ring $\delta^{15}\text{N}$ could allow reconstruction of prehistorical salmon abundance. Unfortunately, attempts to quantify this link have met with little success. We examined the feasibility of the approach using natural abundance of $\delta^{15}\text{N}$ in riparian tree rings formed before and after extirpation of salmon and ^{15}N tracer studies in a river and riparian soils. We concluded that (i) extractable (sap) N must be removed for interpretation of tree-ring $\delta^{15}\text{N}$ because it contains up to 78% of the N in wood, is mobile, and differs from structural N in isotopic composition, (ii) no significant change in structural tree-ring $\delta^{15}\text{N}$ was associated with salmon extirpation in a natural system, (iii) 500‰ $^{15}\text{NH}_4^+$ added to a stream was detected in riparian tree rings spanning at least 8 years, demonstrating interring movement of N that confounds detection of an annual signal, and (iv) addition of 28 000‰ $^{15}\text{NH}_4^+$ to riparian soils at a rate equaling 7.25 kg salmon·50 m⁻² resulted in maximum tree-ring $\delta^{15}\text{N}$ of ~100‰–600‰. Thus, the calculated maximum signal possible from salmon was 0.08‰–0.43‰, which is within the range of natural variation. Evidence suggested that neither total nor structural tree-ring $\delta^{15}\text{N}$ was useful for reconstructing salmon abundance.

Résumé : La quantification d'une relation entre les échappées de saumon dans les rivières et le $\delta^{15}\text{N}$ des cernes annuels de croissance d'arbres riverains pourrait permettre la reconstruction des patrons préhistoriques d'abondance du saumon. Malheureusement, les tentatives de quantification de cette relation ont eu peu de succès. Nous avons examiné la faisabilité de cette approche en utilisant l'abondance naturelle de $\delta^{15}\text{N}$ dans les cernes annuels d'arbres riverains formés avant et après les échappées de saumon et des études avec le traceur ^{15}N dans une rivière et les sols riverains. Nous avons conclu que (i) le N extractible (sève) doit être retiré pour l'interprétation du $\delta^{15}\text{N}$ des cernes parce qu'il contient jusqu'à 78 % du N du bois, qu'il est mobile et que sa composition isotopique diffère de celle du N structurel, (ii) aucun changement significatif dans le $\delta^{15}\text{N}$ des cernes n'était associé à l'échappée du saumon dans un système naturel, (iii) l'ajout de 500 ‰ de $^{15}\text{NH}_4^+$ dans un ruisseau fut détecté dans des cernes des arbres riverains qui s'étaient formés sur au moins huit ans, démontrant que la migration de N entre les cernes brouille la détection d'un signal annuel et (iv) l'addition de 28 000 ‰ de $^{15}\text{NH}_4^+$ aux sols riverains à un taux équivalent à 7.25 kg de saumon·50 m⁻² produisait un $\delta^{15}\text{N}$ maximum de ~100 ‰ – 600 ‰ dans les cernes des arbres. Ainsi, le signal maximum possible calculé pour le saumon variait de 0.08 ‰ – 0.43 ‰, ce qui demeure dans les limites de variabilité naturelle. De toute évidence, ni le $\delta^{15}\text{N}$ total ni le $\delta^{15}\text{N}$ structurel ne sont utiles pour reconstituer l'abondance du saumon.

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Introduction

Predictable differences in the isotopic composition of marine and terrestrial/riparian biota, especially N and C, have allowed broad inference about the fate of marine-derived nutrients carried to upstream environments by Pacific salmon (*Oncorhynchus* spp.). The famous migrations of Pacific salmon from the ocean to rivers result in the delivery of a pulse of N and other nutrients entering riparian forests each year, with the size of the pulse depending on the size of the salmon run (see Naiman et al. 2009). Salmon tissues contain all of the nutrients essential to plant growth, but analyses frequently center on N. This is because adult salmon $\delta^{15}\text{N}$ is usually in the range of 12‰–15‰ (Bilby et al. 2001; Hicks

et al. 2005), which is enriched relative to average regional plant and soil values of approximately –4‰ to +5‰ (Amundson et al. 2003). Nitrogen is also usually deficient in Pacific Northwest soils (Cole and Gessel 1992). These characteristics have fostered attention on the role of N in salmon–stream–riparian interactions (reviewed by Naiman et al. 2009). Elevated $\delta^{15}\text{N}$ of many stream ecosystem components has been attributed to contributions of marine-derived N from salmon, including aquatic and terrestrial insects (Kline et al. 1993; Hocking and Reimchen 2002; Minikawa et al. 2002), groundwater (O'Keefe and Edwards 2003), resident salmonids (Bilby et al. 2001), algae (Kline et al. 1993; Chaloner et al. 2002), and terrestrial carnivores (Ben-David et al. 1997;

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Hilderbrand et al. 1999). Nitrogen isotope studies have provided strong evidence that salmon-borne nutrients are also taken up and used by riparian vegetation (Ben-David et al. 1998; Reimchen et al. 2002; Bilby et al. 2003).

Mechanistic studies show that salmon can provide up to 27% of the N taken up by riparian trees in a particular year (Drake et al. 2005, 2006). The overarching question examined here is whether salmon abundance could be reflected in the N isotope composition of annually resolved tree-ring measurements. Tree-ring $\delta^{15}\text{N}$ has attracted attention because it potentially offers an intuitively appealing, stream-specific, and widely applicable method for reconstructing prehistoric salmon abundance. But despite widespread interest and investment in this approach, no clear quantitative relationship between salmon abundance and tree-ring $\delta^{15}\text{N}$ over time has been published. Reimchen et al. (2002), however, reported a gradient of decreasing xylem $\delta^{15}\text{N}$ in trees with distance from a salmon river.

Interpretation of tree-ring N isotope results remains hampered by a lack of understanding of soil N dynamics and tree-related physiological processes that move N between tissues and rings (e.g., Millard and Proe 1992). Movement of N between tree rings is thought by some to preclude the use of $\delta^{15}\text{N}$ as an indicator of past N availability (Handley and Scrimgeour 1997). Hart and Classen (2003), however, suggested that it is possible to quantify interring translocation noise (account for interring N movement), allowing the separation of environmental and physiological signals. Tree-ring $\delta^{15}\text{N}$ is also likely affected by the presence of mobile (e.g., sap) N. The extractable fraction of wood N is referred to here as “sap” but may include other N compounds not tightly bound in cell walls. Sap is N rich, moves between rings in the sapwood (frequently 50+ years), and leaves nitrogenous residue in heartwood (Bollard 1958; Sheppard and Thompson 2000). Structural N in the form of proteins comprises ~0.05% of pine xylem by mass (Bao et al. 1992) and is more likely than sap to reflect N taken up during the year of growth. The removal of nonstructural N, leaving structural N, therefore may yield more temporally precise results. We will use the notations $\delta^{15}\text{N}_w$ for unextracted wood, $\delta^{15}\text{N}_{ns}$ for non-structural N, and $\delta^{15}\text{N}_s$ for structural N remaining in wood after extraction.

This paper contains results from three independent studies that each test whether an annual signal in tree-ring $\delta^{15}\text{N}_w$ or $\delta^{15}\text{N}_s$ reflects the isotopic composition of N available from environmental sources in a given year. The three studies were conducted for different purposes but all provide relevant data for testing the overarching question. First, we examine changes in ponderosa pine (*Pinus ponderosa* Douglas ex P. Lawson & C. Lawson) tree-ring $\delta^{15}\text{N}$ associated with an unambiguous, large-scale change in salmon N supply, the extirpation of salmon from the Metolius River, Oregon. Second, we measure tree-ring $\delta^{15}\text{N}_w$ and $\delta^{15}\text{N}_s$ and root and foliage $\delta^{15}\text{N}$ 5 years after an in-stream 500‰ $^{15}\text{NH}_4$ tracer addition in Mack Creek, Oregon (Ashkenas et al. 2004), and quantify N tracer movement between tree rings of western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) and sap N contribution to tree rings. Third, we use results from a ^{15}N tracer study (where 28 000‰ $^{15}\text{NH}_4^+$ was added to riparian soils surrounding riparian western redcedar (*Thuja plicata* Donn ex D. Don) to simulate salmon decay (Drake et al. 2006)) to es-

timate enrichment of tree-ring $\delta^{15}\text{N}_s$ that could naturally result from salmon N contributions. The tracer data are used to develop a model designed to calculate the maximum possible annual isotopic signal attributable to salmon abundance.

Analyses from the three studies are organized around four questions. (i) Does sap affect tree-ring $\delta^{15}\text{N}_w$ (i.e., does the removal of sap aid in the identification of an isotopic N signal attributable to salmon)? (ii) Does $\delta^{15}\text{N}_s$ in tree rings reflect a large-scale change in salmon nutrient loading at a specific site (i.e., extirpation of salmon from the Metolius River)? (iii) Does tree-ring $\delta^{15}\text{N}_s$ reflect the isotopic composition of N that was taken up during the year of growth? (iv) What is estimated tree-ring $^{15}\text{N}_s$ enrichment from salmon based on ^{15}N tracer enrichment rates?

Methods

Study sites and experiments

Experiment 1: Natural abundance $\delta^{15}\text{N}$: salmon extirpation, Metolius River, Oregon

The Metolius River is a spring-fed tributary of the Deschutes River in central Oregon that historically supported spring Chinook (*Oncorhynchus tshawytscha*) and sockeye salmon (*Oncorhynchus nerka*). Spawning was observed as far upstream as the headwaters. The most reliable accounts suggest that several thousand salmon naturally spawned in the upper Metolius each year (Nehlsen et al. 1991; Nehlsen 1995), but by the 1920s, only a few tens or hundreds of fish returned annually. On average, only 52 Chinook spawned in the river between 1951 and 1966, and they were completely extirpated by 1967 after construction of the Round Butte Dam (Nehlsen et al. 1991; Nehlsen 1995). The unambiguous change in salmon N supply to the Metolius provides a clear before versus after test of the relationship between tree-ring $\delta^{15}\text{N}$ and salmon. Tree-ring $\delta^{15}\text{N}$ at a reference site on the Deschutes River (upstream of a waterfall that completely blocks salmon passage) was determined over the same period to account for potentially confounding regional-scale changes in tree-ring $\delta^{15}\text{N}_s$ over time. If salmon were contributing substantially to wood N, we would expect a reduction in tree-ring $\delta^{15}\text{N}$ after 1920 at the Metolius site relative to the Deschutes reference site. Although the Metolius and Deschutes sites are ~50 km apart, the sites are similar in terms of elevation, plant assemblage, and climate, and both support mature ponderosa pine forest. Ponderosa pine is recommended for dendrochemical studies (Cutter and Guyette 1993) due to its relative extent of sapwood, formation of secondary compounds, low radial permeability, and low moisture content.

Twenty cores were collected for cross-dating and other analyses at each of the Metolius and Deschutes sites, and the $\delta^{15}\text{N}$ analyses described here were a pilot using extra materials from the original study (unpublished). Tree-ring $\delta^{15}\text{N}_s$ before and after 1920 in two trees at the Metolius site was compared with the equivalent in two trees at the Deschutes reference site. One core from each tree was used for the analyses here. At least eight segments from each increment core (four prior to 1920 and four after 1920) containing wood from at least 5 years were N extracted and analyzed for $\delta^{15}\text{N}_s$.

Experiment 2: Stream water $^{15}\text{NH}_4^+$ tracer study, Mack Creek, Oregon

Mack Creek is a third-order stream on the west slope of the Cascade Mountains draining a mature Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) forest in the H.J. Andrews Experimental Forest. The small catchment has a mean annual air temperature of 8.5 °C and receives ~2300 mm of precipitation per year. Mack Creek mean annual discharge rate is ~0.5 m³·s⁻¹. The riparian forest supports western hemlock, vine maple (*Acer circinatum* Pursh), Douglas maple (*Acer glabrum* Torr.), Pacific yew (*Taxus brevifolia* Nutt.), and western redcedar. Mack Creek never supported salmon.

We piggybacked this study on a $^{15}\text{NH}_4^+$ tracer addition conducted as part of the LINX (Lotic Intersite Nitrogen eXperiment) study, a continental-scale comparison of stream N dynamics in North America (Ashkenas et al. 2004). Labeled N (0.03 g·L⁻¹ $^{15}\text{N-NH}_4\text{Cl}^{-1}$) was continuously added to Mack Creek from 21 July to 1 September 1998. The addition increased $\delta^{15}\text{N}$ of dissolved NH_4^+ to ~500‰ over baseline. Thus, the tracer N isotope signal was detectable at concentrations ~100 times lower than salmon N (~15‰ over baseline). We used the ^{15}N addition to Mack Creek as a proxy for N supplied by salmon as metabolic and decay products dissolved in stream water. Salmon metabolize a large portion of their tissue during upstream migration and spawning, and metabolized N is excreted through the gills as NH_3 , so delivery of N dissolved in stream water is potentially an important source of salmon N to streamside trees. Although trees were not examined in the original study, forbs growing within 2 m of Mack Creek contained the tracer N after the experiment ($\delta^{15}\text{N}$ was 20‰–50‰ over baseline; Ashkenas et al. 2004), suggesting that riparian trees also had access to the tracer.

We collected single, 12 mm diameter increment cores from four trees on Mack Creek in February 2003 for initial analysis of $\delta^{15}\text{N}$ in individual tree rings. This sample collection was expanded in July 2003 when 5.2 mm diameter cores, roots, and foliage from an additional five trees above the ^{15}N addition point (the reference site) and five trees below the ^{15}N addition point were collected (Table 1). Rings were dated by counting back from the bark. Foliage was aged based on leaf scars and changes in color on the branches. In total, we compared $\delta^{15}\text{N}$ of extractable and structural N in tree rings and other tissues in 10 trees growing downstream (<150 m) of the ^{15}N addition point with five reference trees growing upstream (<45 m) of the ^{15}N addition point (Table 1). Although *Tsuga* species are not recommended for dendrochemical studies based on high heartwood permeability and a high number of rings in the sapwood (Cutter and Guyette 1993), western hemlock is an extremely common and important riparian species in the Pacific salmon range and was the only abundant streamside tree at Mack Creek.

Experiment 3: Riparian soil $^{15}\text{NH}_4^+$ tracer study, Kennedy Creek, Washington

Kennedy Creek is ~12 km northwest of Olympia, Washington. Mean annual precipitation is ~2500 mm. Kennedy Creek is relatively small with a discharge rate of ~1–5 m³·s⁻¹ during the rainy (salmon spawning) season, but it supports a large chum salmon (*Oncorhynchus keta*) population. Escapement averaged 41 000 adults from 1992 through 2001, and the entire population spawns within a 5.2 km reach

(Washington Department of Fish and Wildlife, unpublished data). Upstream migration and spawning occur from mid-October through December. Western redcedar is an abundant, shade-tolerant riparian tree in the Pacific salmon range.

A pulse of ^{15}N -labeled NH_4^+ ($\delta^{15}\text{N}$ ~28 000‰ over baseline) was added to soils surrounding four mature (~70 years old) western redcedars in November 2003 to simulate salmon decay (Drake et al. 2006) (Fig. 1). This tracer N signal is detectable at concentrations more than 1000 times lower than salmon N. Two 5.2 mm diameter increment cores were collected from each tree prior to ^{15}N addition and approximately 1 year after the addition for measurement of structural and sap tree-ring $\delta^{15}\text{N}$. Rings were narrow and difficult to separate, so 10 year segments of the increment cores were composited for $\delta^{15}\text{N}$ analysis. Rings were dated by counting back from the bark.

Dendrochemistry methods

All core segments were ground using a No. 20 mesh in a Thomas Wiley mill. Each sample was then divided and half was analyzed with no treatment and sap was extracted from the second half (after Sheppard and Topa 2002). We used an oxidative extraction technique to remove mobile (sap) N from wood because N removal efficiency is higher than with solvent-based extractions. Determination of the forms of wood N removed by oxidative extraction (e.g., amino acids, ureides) is technically difficult (e.g., Barnes 1963) and was not performed. Each ground wood sample was sealed in a labeled porous glass fiber pouch by melting at the edges with a heat sealer. The samples were autoclaved (121 °C and 15 psi) with deionized water for approximately 1 h and then microwaved in batches of 10 on high power for approximately 2 min. A representative sample of processed wood was then examined under a microscope to ensure that cells were broken open for removal of cytoplasm and related constituents. The samples were then soaked in an ~3% hydrogen peroxide solution overnight on a heat plate set to low and covered with a watchglass. Finally, samples were soaked in deionized water for at least 2 h and water was wrung out by hand. This rinsing process was repeated at least five times. The processed samples were then removed from the glass fiber pouches and dried thoroughly by allowing them to stand in a desiccator for at least 2 days. Sample masses were determined to 10⁻⁶ g. A minimum of 20 mg of wood and 2 mg of other tissues was packaged in tin capsules for $\delta^{15}\text{N}$ determination.

Metolius and Deschutes River increment cores were dissected into 10 year increments (spanning 1800–2001). The 10 year segments were ground, oxidatively extracted, and $\delta^{15}\text{N}$ determined at the Cornell-Boyce Stable Isotope Laboratory, Pennsylvania. For the tracer studies, 12 mm diameter cores were dissected into individual years, and the 5.2 mm cores were dissected into 10 year segments with a scalpel. Foliage and roots were dried and then pulverized using a Wig-l-bug amalgamator (Dentsply Rinn Elgin, IL). Mass spectrometry was performed at the University of California - Davis Stable Isotope Laboratory.

Quantitative methods

Paired *t* tests (Excel for Windows 2000) were used to com-

Table 1. Characteristics of riparian western hemlock (*Tsuga heterophylla*) from Mack Creek, Oregon, used in the study.

	Comparison	Tree ID	Vertical distance above channel (m)	Horizontal distance to active channel (m)	Diameter at breast height (cm)	Distance downstream of ¹⁵ N addition (m)
¹⁵ N-labeled trees (downstream of ¹⁵ N addition)	Annual ring δ ¹⁵ N (collected February 2003)	Tracer 1	0.2	3.5	19	150
		Tracer 4	2	0.1	27	125
		Tracer 8	1.5	14	64	45
		Tracer 9	2.5	0.5	87	50
		Tracer 10	1	1	128	190
	Foliage, root, xylem, sap, and phloem δ ¹⁵ N (collected July 2003)	Tracer 1	0.2	3.5	19	150
		Tracer 20	0.75	1	41	50
		Tracer 21	1.5	1	42	60
		Tracer 22	0.5	1.2	35	85
		Tracer 23	1	1.5	47	100
Reference trees (upstream of ¹⁵ N addition)	Foliage, root, xylem, sap, and phloem δ ¹⁵ N (collected July 2003)	Reference 1	0.25	0.5	22	-15
		Reference 2	1.5	2.5	38.5	-45
		Reference 3	1.2	4	94	-40
		Reference 4	0.25	2.4	5	-30
		Reference 5	0.25	2.3	5	-30

pare sample means of extracted and unextracted wood, and unpaired *t* tests (assuming unequal variance) were used to compare ¹⁵N-labeled wood samples (downstream of the ¹⁵N addition) with reference samples (collected upstream of the ¹⁵N addition) for the Mack Creek tracer study and ^δ¹⁵N_w values at the Metolius and Deschutes sites. ^δ¹⁵N_{ns} was calculated using the equation

$$[1] \quad \delta^{15}N_w \times \text{mass}N_w = \delta^{15}N_{ns} \times \text{mass}N_{ns} + \delta^{15}N_{ns} \times \text{mass}N_{ns}$$

where total (unextracted) wood ^δ¹⁵N (^δ¹⁵N_w) equals extracted (structural) wood ^δ¹⁵N (^δ¹⁵N_s) plus nonstructural ^δ¹⁵N (^δ¹⁵N_{ns}) corrected for mass.

To determine whether it is theoretically possible to detect an isotopic signal from salmon, we used data from the Kennedy Creek 2003 tracer N addition and used assumptions that would maximize a potential ^δ¹⁵N signal. We assumed that the enrichment over baseline measured in the 10 year segment was from one tree ring only (2004). We also assumed that salmon N was enriched by 20‰ over N from other sources in this system. Maximum possible enrichment of Kennedy Creek tree rings formed in 2004, assuming no transport of tracer to earlier rings, was calculated from the ^δ¹⁵N of 10 year segments using the equation

$$[2] \quad \delta^{15}N_{95-04} \times N \text{ mass}_{95-04} = \delta^{15}N_{04} \times N \text{ mass}_{04} + \delta^{15}N_{95-03} \times N \text{ mass}_{95-03}$$

and a direct proportional conversion of tracer ^δ¹⁵N of 28 000‰ over baseline to salmon ^δ¹⁵N of 20‰ over baseline. Mean ^δ¹⁵N_s (in cores collected prior to the ¹⁵N addition) was 0.49‰.

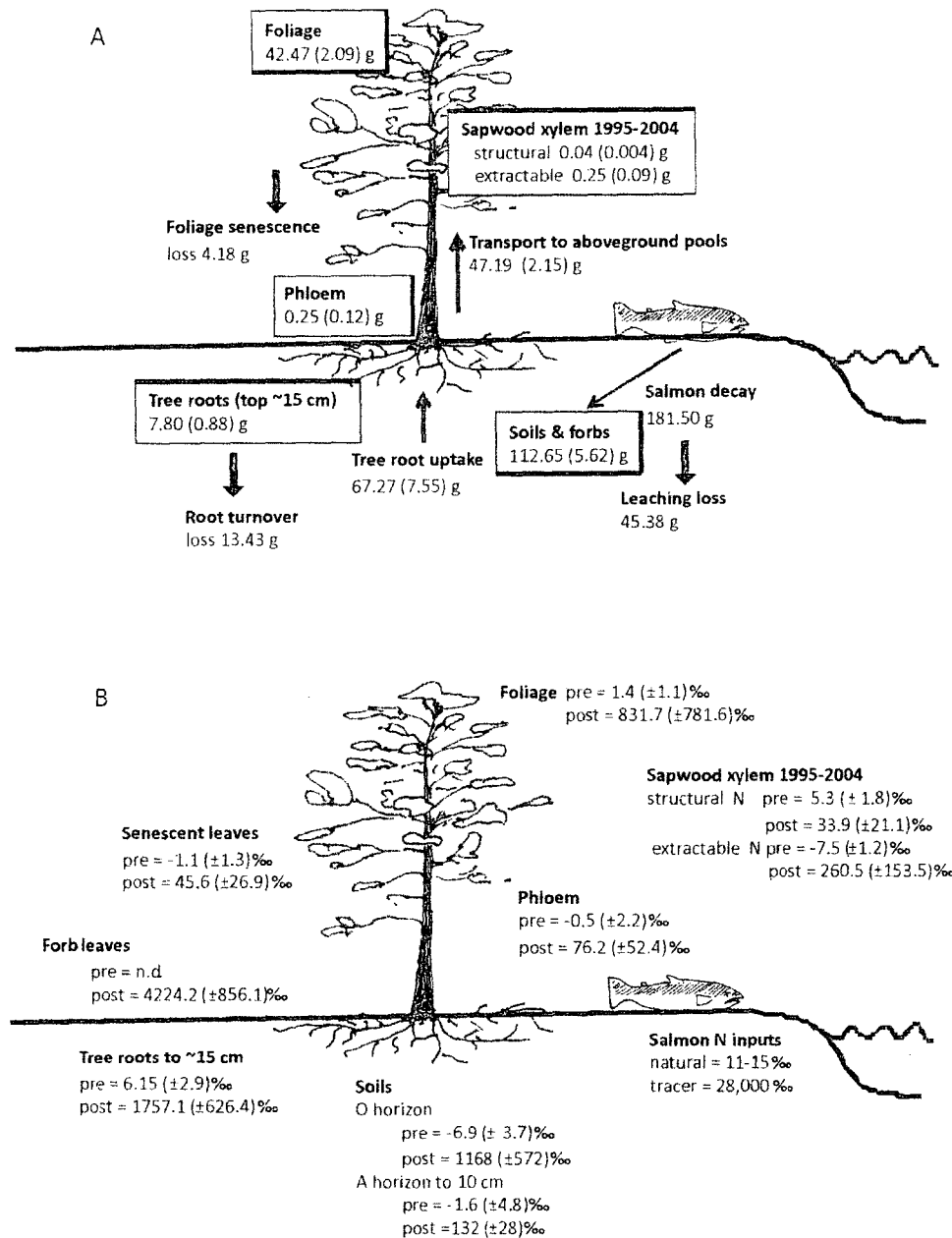
Results

(i) Does sap affect tree-ring ^δ¹⁵N (i.e., does the removal of sap aid in the identification of an isotopic N signal attributable to salmon)?

In all cases examined here, tree-ring ^δ¹⁵N changed when N was extracted from wood, i.e., ^δ¹⁵N_w and ^δ¹⁵N_s were always different. Oxidative extraction removed an average of 45% of the N from tree rings. In sapwood, with a higher proportion of living conductive tissue, as much as 78% of total N was removed, while as little as 20% of total N was removed from heartwood. Extraction reduced the average N content of western redcedar sapwood from 1.50% to 0.52% and that of western hemlock sapwood from 0.73% to 0.35%. All ponderosa pine samples were extracted for analysis, so comparison with unextracted xylem was not possible. Extraction of N increased wood ^δ¹⁵N values in both reference (+6.1‰) and tracer ¹⁵N-labeled (+4.3‰) western hemlock at Mack Creek (Table 2). Nonstructural N was ¹⁵N depleted relative to other tissues in these trees (Table 2) but also showed the highest relative rate of enrichment below the ¹⁵N addition. ^δ¹⁵N_{ns} in trees growing downstream of the addition was, on average, 7.2‰ higher than in reference trees upstream of the addition.

In the Kennedy Creek ¹⁵N tracer study, oxidative extraction decreased the amount of N in xylem by, on average, 65% and decreased the ^δ¹⁵N by 30‰–211‰, demonstrating a high concentration of the ¹⁵N tracer in nonstructural N rel-

Fig. 1. (A) Fate of tracer N ($\pm$ SE) from simulated decay of 7.25 kg of salmon tissue added to 50 m² plots surrounding four individual western redcedar (*Thuja plicata*) trees (mean diameter at breast height 72 cm) in October 2003. Annual N fluxes (arrows) and fate (boxes) of tracer N 1 year after the addition. Units are g N-tree⁻¹ and g-year⁻¹, respectively. Soil and root pools are calculated for 50 m² surrounding each tree. Salmon decay was the exact amount of tracer N added in October of 2003, and leaf senescence and leaching losses were calculated by subtraction. (B) Pre- and post-tracer ¹⁵N addition $\delta^{15}\text{N}$ in tree tissues and other pools prior to the ¹⁵N addition (pre) and 1 year after the ¹⁵N addition (post). "n.d." indicates not determined.



ative to structural N (Fig. 1). Nonstructural N is chemically dynamic and responsive relative to other N pools within trees, and it is not surprising that this extends to isotopic composition. The fact that extraction resulted in N isotope enrichment of the Mack Creek western hemlock and depletion of the Kennedy Creek western redcedar may be a result of the length of time that had elapsed since tracer application. Only 1 year had passed since tracer application at Kennedy Creek, while 5 years had passed at Mack creek, which would allow tracer N more time to be incorporated into structural tissues. Additionally, ¹⁵N tracer was added to the river at

Mack Creek, so it would have left the ecosystem quickly, while at Kennedy Creek, the tracer was soils with a much longer residence time. Physiological differences in the species may also contribute to this pattern.

(ii) Does $\delta^{15}\text{N}_s$ in tree rings reflect a large-scale change in salmon nutrient loading (i.e., extirpation of salmon from the Metolius River)?

We expected Metolius River tree-ring $\delta^{15}\text{N}_s$ to decrease with salmon decline and extirpation and Deschutes River reference site tree-ring $\delta^{15}\text{N}_s$ to remain relatively constant

Table 2. Western hemlock (*Tsuga heterophylla*) $\delta^{15}\text{N}$ above (reference) and below (tracer) the ^{15}N addition on Mack Creek, Oregon.

Tissue	Year of formation	Reference site $\delta^{15}\text{N}$ (‰)	n	Tracer site $\delta^{15}\text{N}$ (‰)	n	P paired t test unequal variance
Unextracted xylem	Prior to 1940	-4.01	3	-3.83	4	0.456
	1993–2003	-5.02	3	-3.75	4	0.004
Extracted xylem	Prior to 1940	2.04	3	-0.40	4	0.019
	1993–2003	1.23	3	1.25	4	0.490
Sap	1993–2003	-17.31	6	-10.08	8	0.058
Foliage	1997–2003	-4.81	5	-3.75	5	0.001
Phloem	Unknown	-4.48	2	-4.78	5	0.546
Roots	Unknown	-4.21	4	-4.00	4	0.808

Note: Xylem (extracted) formed prior to 1940 has significantly lower $\delta^{15}\text{N}$ than that formed between 1993 and 2003 (bold pair in column 5). Bold P values in column 7 denote significant differences between $\delta^{15}\text{N}$ values at reference and tracer addition sites.

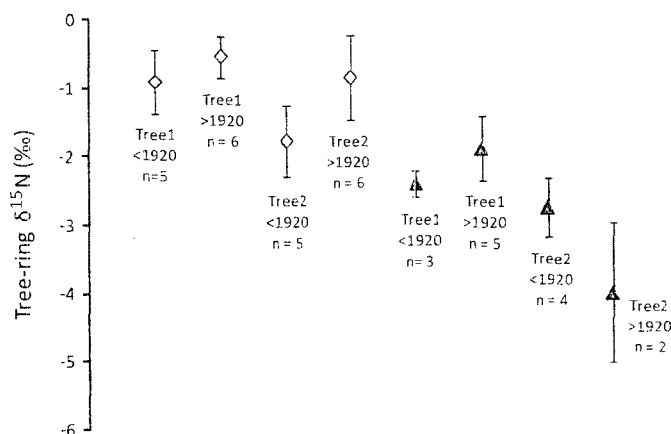
through time. Metolius tree-ring $\delta^{15}\text{N}_s$, however, did not decrease significantly (error bars overlap) with the decline of salmon (reduced to extremely low abundance by 1920; Nehlsen 1995), although a trend toward depletion in one of the Metolius series and a slight enrichment in the reference series are seen after 1920 (Fig. 2). We note that tree-ring $\delta^{15}\text{N}_s$ was unexpectedly $\sim 2\text{‰}$ higher in the reference site trees relative to the salmon site trees over the period of analysis. This could be attributable to differences in soils and (or) microclimates or genetic differences in trees, and this illustrates the type of natural variation that frequently confounds site-to-site comparisons of $\delta^{15}\text{N}$ values for organisms or soils.

(iii) Does tree-ring $\delta^{15}\text{N}_s$ reflect the isotopic composition of N that was taken up during the year of growth? Is there evidence for movement of N between tree rings?

A signal that we assume is attributable to the 1998 ^{15}N tracer addition at Mack Creek was evident in tree rings over the long term (i.e., comparing tree rings formed prior to 1940 with those formed between 1993 and 2003) (Table 2) but was not detectable in individual tree rings formed between 1995 and 2002 (Fig. 3A). This is true for both the extracted (five series) and unextracted (one series) tree rings. Four years after the Mack Creek ^{15}N tracer addition, foliage N, total wood N, nonstructural N, and structural xylem N in trees growing downstream of the tracer addition point were significantly enriched in ^{15}N compared with reference trees (Table 2). Nitrogen in trees that received the ^{15}N tracer was enriched by as much as 7.23‰ relative to reference trees, depending on the tissue, with the greatest enrichment in non-structural N. Phloem $\delta^{15}\text{N}$ and root $\delta^{15}\text{N}$, however, were not statistically different above and below the tracer addition point (Table 2). A significant increase in $\delta^{15}\text{N}_s$ (from -0.40 to 1.25; $P = 0.037$) (Table 2) is also evident between the pre-1940 and 1995–2002 wood and is attributable to the ^{15}N tracer. The unextracted series did not show this pattern. At the reference site, extracted tree-ring $\delta^{15}\text{N}_s$ decreased by 0.82‰ over the same period. The lack of a temporal pattern in unextracted wood suggests that nonstructural N can obscure tree-ring $\delta^{15}\text{N}_s$ patterns over periods as long as 60 years (1940–2000) in western hemlock.

Foliage $\delta^{15}\text{N}$

We initially suspected that the relatively high N content of foliage (1.5%–2.0% N) may make it more likely than tree

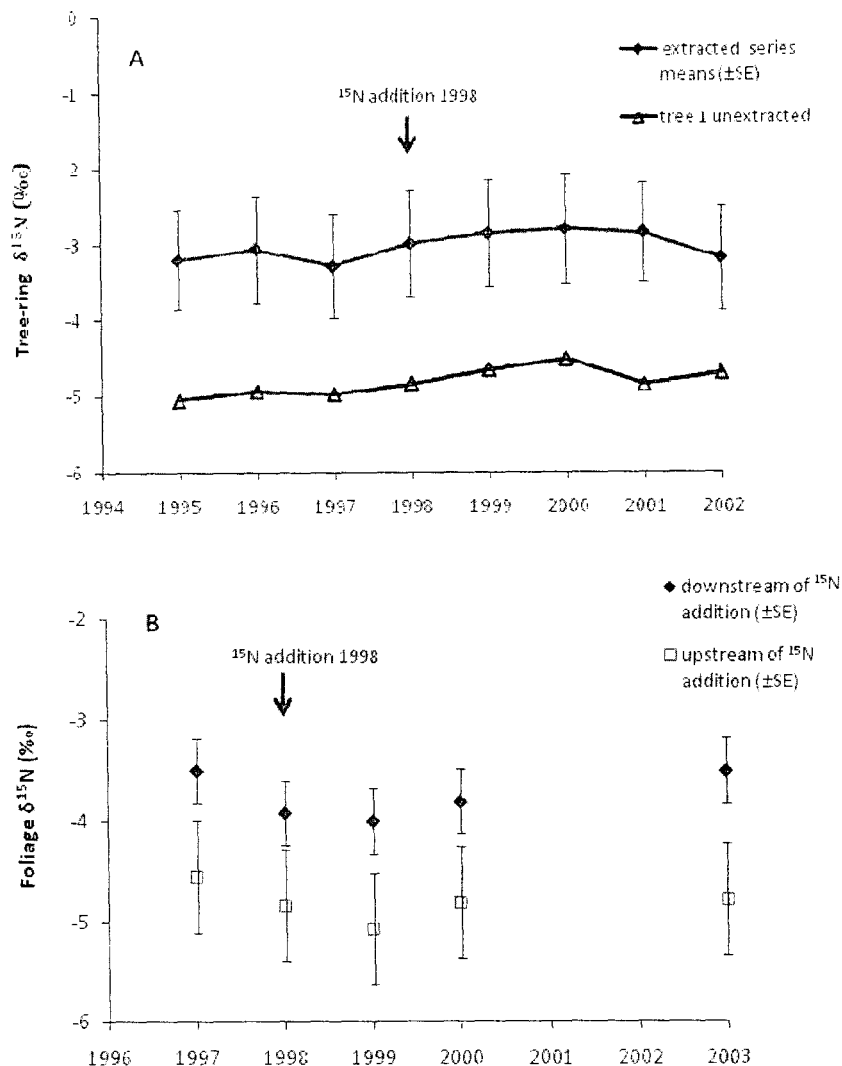
Fig. 2. Extracted xylem $\delta^{15}\text{N} \pm \text{SE}$ before and after 1920. Samples are from trees on the Deschutes River, Oregon, which never supported salmon, and the Metolius River, Oregon, from which salmon were almost entirely eliminated by 1920 (Nehlsen 1995). "n" refers to the number of 10 year segments analyzed from each tree.

rings (0.3%–0.8% N) to reflect the ^{15}N addition. Foliage from trees that received the ^{15}N addition was enriched relative to reference trees (Table 2), but foliage from all year classes analyzed (1997, 1998, 1999, 2000, and 2003) (Fig. 3B) was about equally enriched, suggesting high rates of N movement between foliage year classes. Notably, patterns in foliar $\delta^{15}\text{N}$ of reference and tracer trees are very similar.

(iv) What rate of tree-ring $^{15}\text{N}_s$ enrichment from salmon is possible based on ^{15}N tracer enrichment rates?

Under the assumptions described in the Methods section, the enrichment in the 2004 ring varied from 100‰ to 600‰ in the four western redcedars on Kennedy Creek to which the 28 000‰ tracer was added. $\delta^{15}\text{N}_s$ in tree rings formed from 1995 to 2004 (composited) varied from 11.4‰ to 61.3‰ among the four ^{15}N -labeled trees at Kennedy Creek, while the average $\delta^{15}\text{N}_s$ of 1993–2003 rings collected prior to the ^{15}N addition was 5.3‰ (Fig. 1B). Using eq. 2, if a 28 000‰ application of the tracer N only resulted in structural N in tree rings being enriched to 100‰–600‰, then 20‰ salmon N would only result in an maximum enrichment of tree-ring structural N of 0.08‰–0.43‰.

Fig. 3. (A) Western hemlock (*Tsuga heterophylla*) tree-ring $\delta^{15}\text{N}$ ($\pm\text{SE}$) in trees that received the Mack Creek, Oregon, ^{15}N tracer addition in 1998. Diamonds are mean values for five trees (extracted) and triangles are values from one tree (unextracted). (B) Western hemlock foliage $\delta^{15}\text{N}$ ($\pm\text{SE}$) in years surrounding the 1998 ^{15}N addition at Mack Creek. Values are averaged by year from five trees downstream of the ^{15}N addition (diamonds) four reference trees upstream of the ^{15}N addition (squares).



Discussion

Critical analysis of the tree-ring $\delta^{15}\text{N}$ approach

Using tree-ring $\delta^{15}\text{N}$ to quantify (reconstruct) salmon N additions to riparian forests would have far-reaching implications for salmon ecology and management. The approach has the potential to provide a river-specific understanding of natural cycles of salmon abundance and estimates of salmon abundance in Pacific Northwest systems prior to large-scale salmon decline beginning in the 1800s. The baselines against which we are able to judge the current health of salmon populations are limited to anecdotal records (e.g., Nehlsen 1995) and a few paleoecological studies (e.g., Finney et al. 2002; Drake and Naiman 2007). It is not surprising that the tree-ring $\delta^{15}\text{N}$ approach has attracted much attention and effort — we are aware of at least 15 research groups that have attempted this, including ourselves. Nonetheless, no studies demonstrating correlation between salmon abundance and tree-ring $\delta^{15}\text{N}$ at an annual scale have been published. This fact and the results of the three studies described here suggest

that the approach holds little promise using currently available approaches and methods.

Extraction of nonstructural N clearly changed tree-ring $\delta^{15}\text{N}$ in both of the cases described here, and thus, the presence of nonstructural, mobile N (e.g., sap) in wood fundamentally compromises an annual signal. Nonstructural N accounted for an average of 48% of wood N in western hemlock xylem, with highest concentrations in the most recently formed wood. Our results show that nonstructural N can obscure a signal over periods of 60+ years in western hemlock and that it must be extracted to understand temporal patterns in $\delta^{15}\text{N}$ regardless of the time period, i.e., $\delta^{15}\text{N}_s$ must be examined. Because nitrogenous residue remains even in heartwood, extraction is also recommended for studies examining heartwood. When nonstructural N was extracted from western hemlock, tracer N added in 1998 was found in approximately equal abundance in all individual tree rings formed from 1995 to 2002, demonstrating mobility of N between rings and suggesting that even the N bound in the structural components of wood is somewhat mobile or exchangeable.

Rings formed between 1995 and 2002 $\delta^{15}\text{N}_s$, however, had higher $\delta^{15}\text{N}_s$ than rings formed prior to 1940. We conclude that western hemlock, an extremely common and important riparian species within the range of Pacific salmon, is not a good candidate for high-resolution (subdecadal) tree-ring $\delta^{15}\text{N}$ -based reconstruction, even when N is extracted. A relatively subtle salmon signal (usually 12‰–15‰) is likely lost over all but the longest time scales.

The oxidative extraction method used here was a part of ongoing work designed to improve N removal rates and efficiency. Others have used a solvent-based extraction technique to extract sap and its solutes (Sheppard and Thompson 2000). Solvent (toluene and ethanol) extraction has been reported to remove varying amounts of N from wood and to result in either enriched or depleted $\delta^{15}\text{N}_s$. For example, 6%–13% of wood N was removed from ponderosa pine using solvent extraction, and this resulted in lower $\delta^{15}\text{N}_s$ values (Hart and Classen 2003). Solvent-based extraction of European beech (*Fagus sylvatica* L.) resulted in 36% removal of total wood N and higher $\delta^{15}\text{N}_s$ values (Elhani et al. 2003). In another solvent extraction study, <1% of wood N was removed from American beech (*Fagus grandifolia* Ehrh.) and red spruce (*Picea rubens* Sarg.) (Doucet et al. 2011), which, unsurprisingly, resulted in no change in $\delta^{15}\text{N}$. Relationships between structural and nonstructural N isotope composition clearly vary between species and are likely dependent on extraction efficiency. If ^{15}N tracers are used, results may depend on the isotopic composition of the tracer and the time since application. The salient point here, however, is that the combined data demonstrate that N extraction changes tree-ring $\delta^{15}\text{N}$ and the resulting interpretation of patterns in N isotopes.

Historically, thousands of sockeye salmon spawned in the upper Metolius River, while after 1920, only a few tens or hundreds and eventually none reached Metolius spawning areas. Average tree-ring $\delta^{15}\text{N}$ decreased by less than 1‰ over this period, and this was not statistically significant. Thus, an unambiguous, large, and well-documented change in salmon abundance at the Metolius River was not clearly reflected in tree-ring $\delta^{15}\text{N}$, even at the coarsest resolution. Even if the 1‰ decrease were statistically significant, this would be a very small increment to represent the loss of N contributed by thousands of salmon annually. If little or nothing can be said about this large and well-documented change based on our tree-ring $\delta^{15}\text{N}$ results, any inference about more subtle changes in salmon abundance is unlikely.

Variability in tree-ring $\delta^{15}\text{N}$ occurs independently from salmon inputs. For example, two ~55 year old eastern hemlock (*Tsuga canadensis* (L.) Carrière) trees growing 5 m apart had average xylem $\delta^{15}\text{N}$ of +3.8‰ and +1.0‰ (Poulson et al. 1995). Here, the $\delta^{15}\text{N}$ of Mack Creek western hemlock xylem formed before 1940 varied by 2.4‰ in trees within 100 m of each other. Nitrogen cycle processes play a particularly important role in determining soil N isotope composition (reviewed by Robinson 2001) and vary over small scales. Nitrification, for example, can result in fractionation of soil N by 35‰–60‰. Humification, which occurs patchily in the wet riparian forests of the Pacific Northwest, can result in enrichment of residual N over time (Kramer et al. 2003). Denitrification rates can be elevated near Alaskan salmon streams (Pinay et al. 2003), and enriched residual N (as high

as 28‰–33‰, Robinson 2001) is associated with denitrification in wet areas, potentially confounding a salmon signal in riparian trees. This small-scale variability largely precludes the quantification of a universal relationship between tree-ring $\delta^{15}\text{N}$ and salmon abundance; time-series approaches and site-specific calibration are essential to account for the many factors that affect the isotopic composition of N available to trees.

The $^{15}\text{NH}_4^+$ addition at Kennedy Creek introduced labeled N to soils at a loading rate equivalent to the decay of a 7.25 kg salmon at the base of each study tree, but the N had a very strong isotopic signal — it was greater than 1000 times more detectable than a natural abundance signal from salmon. Nitrogen delivered in the fall of 2003 (during salmon spawning) was taken up and stored in roots over winter and only moved into aboveground tissues the following spring (Drake et al. 2006). Thus, 2004 was the year that the ^{15}N tracer signal was delivered to the xylem. Narrow rings in the study trees required us to analyze composited, 10 year xylem segments, which limited our understanding of N transfer between tree rings in this experiment. However, the maximum potential tree-ring $\delta^{15}\text{N}$ signal that could arise from salmon N contributions calculated using these data was only 0.08‰–0.43‰. The calculations used to derive this estimate assume no movement of N between tree rings after formation and a very high salmon $\delta^{15}\text{N}$ relative to baseline, neither of which can be expected under most circumstances. This range (0.08‰–0.43‰) is similar to the natural range of year-to-year variability. For example, the average standard deviation of tree-ring $\delta^{15}\text{N}$ in four sets of 10 consecutive years from essentially undisturbed eastern hemlock rings was 0.24‰ (data from Poulson et al. 1995). As a whole, the data presented here did not lend support to the idea of reconstructing salmon abundance using only tree-ring $\delta^{15}\text{N}_s$ or $\delta^{15}\text{N}_w$ in western hemlock, ponderosa pine, or western redcedar. This conclusion is strengthened by the fact that despite many efforts by other researchers, no evidence of a positive relationship between salmon abundance and tree-ring $\delta^{15}\text{N}$ over time has been published. Paleolimnological indicators from sediments (e.g., Finney et al. 2002) have provided lower-resolution (decadal), longer-term reconstructions of lake-spawning salmon populations. This approach, however, is usually not possible for reconstruction of stream-spawning salmon populations. High-resolution (approximately annual) reconstruction of stream-spawning salmon abundance was accomplished in five Pacific Northwest rivers using nonclimatic patterns in tree-ring growth (Drake and Naiman 2007). But this and other dendroecological approaches, including tree-ring $\delta^{15}\text{N}$, are frequently limited by the absence of old trees (riparian trees in most of the Pacific salmon range have been harvested in the last 100 years) and a lack of truly comparable reference sites where soil conditions and N cycling are very similar. An N isotope signal from salmon will be diluted by existing N pools in trees to a degree that depends on exchange and mixing, e.g., N delivered and taken up by trees in the fall (during spawning) was stored in roots over the winter and only moved into aboveground tissues the following spring (Drake et al. 2006), mixed with other N stored overwinter in roots. For the measurement and interpretation of N in tree rings to be truly useful in ecological research, new methods for removing mobile N while retaining struc-

tural N will be needed, methods more sophisticated and effective than the solvent extraction or oxidation techniques tried to date.

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