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**FATE OF NITROGEN IN SALMON-INFLUENCED RIPARIAN FOREST SOILS AND VEGETATION – A  
<sup>15</sup>N TRACER STUDY**

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**ABSTRACT**

We introduced a <sup>15</sup>N-NH<sub>4</sub><sup>+</sup> tracer to the riparian forest of a salmon-bearing stream (Kennedy Creek, Washington, USA) to quantify the cycling and fate of a late-season pulse of salmon-N, and, ultimately, mechanisms regulating potential links between salmon abundance and tree growth. The <sup>15</sup>N tracer simulated deposition of 7.25 kg of salmon (fresh) to 4, 50-m<sup>2</sup> plots. We added NH<sub>4</sub><sup>+</sup> (the initial product of salmon carcass decay) and other important nutrients provided by carcasses (P, S, K, Mg, Ca) to soils in late October 2003, coincident with local salmon spawning. We followed the <sup>15</sup>N tracer through soil and tree pools for one year. Biological uptake of the <sup>15</sup>N tracer occurred quickly; 64% of the <sup>15</sup>N tracer was bound in soil microbiota within 14 days, and roots of the dominant riparian tree, western redcedar (*Thuja*

*plicata*), began to take up  $^{15}\text{N}$  tracer within 7 days. Root uptake continued through the winter. The  $^{15}\text{N}$  tracer content of soil organic matter reached a maximum of ~52%, 5 weeks after the application, and a relative equilibrium of ~40% within 5 months. Six months after the addition, in spring 2004, at least 37% of the  $^{15}\text{N}$  tracer was found in tree tissues: ~23% in foliage, ~11% in roots and ~3% in stems. Within the stems, xylem and phloem sap contained ~96% of the tracer N, and ~4% was in structural xylem N. After one year, at least 28% of the  $^{15}\text{N}$  tracer was still found in trees, and loss from the plots was only ~20%. The large portion of tracer N taken up in the fall and reallocated to leaves and stems the following spring provides mechanistic evidence for a 1-year lagged tree-growth response to salmon nutrients. Salmon nutrients have been deposited in the Kennedy Creek system each fall for centuries, but the system shows no evidence of nutrient saturation. Rates of N uptake and retention are a function of site history, disturbance, and may also be the result of a legacy effect, in which annual salmon nutrient addition may lead to increased efficiency of nutrient uptake and use.

Key words: ecophysiology;  $^{15}\text{N}$ -tracer; nitrogen; nitrogen uptake, *Oncorhynchus*; riparian; riparian trees; salmon-derived nutrients; soils

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**INTRODUCTION**

Reproduction of Pacific salmon (*Oncorhynchus* spp.) occurs in freshwater, and depends on the continued existence of quality stream habitat and associated riparian areas. Streams, in turn, are influenced by salmon; in-stream nutrient capital and productivity are increased by salmon contributions (e.g. Wipfli et al. 1999, Bilby et al. 1996, 1998; Kline et al. 1990), and the influence of salmon also reaches surrounding riparian areas. High densities of predators and scavengers are drawn to salmon rivers for a few months each year, and can move a seasonal pulse of salmon-derived nutrients and energy upslope (e.g. Stockner 2003, Hilderbrand et al. 1999, Ben-David et al. 1998). Salmon nutrients are deposited in the riparian forest by predators and scavengers in excreta, and as partially eaten salmon carcasses and skeletons. Carcasses can be moved upslope by floods, and salmon nutrients dissolved in stream water can be transported in the forest by subsurface flow. Salmon-derived nutrients are now believed to encourage the growth of some riparian trees, which provide shading, bank stabilization, and, ultimately, large woody debris to streams, thereby improving habitat for freshwater life stages of salmon (Naiman et al. 2002). Positive feedbacks between salmon and the riparian forest may then be important for the long-term productivity of salmon populations. But although these ostensible feedbacks are widely discussed, they are not well quantified or understood. Developing a mechanistic understanding of nutrient-based feedbacks is the essential next step in understanding salmon-riparian interactions. Mechanistic studies are also needed to resolve management questions, particularly about the role of “nutrient enhancement”, or adding nutrients to waters and watersheds to improve conditions for salmon (see Lackey 2003).

The existing evidence of a link between salmon and riparian trees is empirical. At least one study showed that the nitrogen (N) content of vegetation on salmon-influenced stream

reaches was higher than in vegetation upstream of barriers to salmon (Bilby et al. 2003). Salmon abundance has also been related to the radial growth of riparian trees (Drake et al. 2002, Helfield and Naiman 2001). But most of the evidence linking salmon and riparian vegetation is based on natural abundance of N isotopes; 1-4‰ isotopic enrichment of foliage along salmon streams relative to reference sites upstream of waterfalls has been demonstrated in many systems (e.g., Bartz and Naiman 2005, Bilby et al. 2003, Helfield and Naiman 2001, Ben-David et al. 1998). Enrichment of vegetation is attributed to uptake and use of salmon N, naturally enriched in  $^{15}\text{N}$  by 10-15‰ relative to most terrestrial N sources. But these comparisons are based on subtle differences in N isotope composition that are potentially confounded by soil processes unrelated to the presence of salmon. For example, microbial denitrification can result in enrichment of residual soil N by 28-33‰ (Pinay et al. 2003, Robinson 2001), and is more likely to occur in low gradient, occasionally inundated soils, i.e. the lower river reaches more likely to support salmon.

While it seems reasonable that some portion of salmon in a stream ultimately become fertilizer for riparian trees, local conditions will regulate fertilizer effects, e.g. the timing and size of salmon populations, numbers and types of predators, soil processes, and loss of mineral nutrients via leaching. Most salmon spawning occurs in the fall and winter, while many plants are dormant. Mineralization of nutrients bound in salmon tissues and uptake of these nutrients involves lag-times and transformations that will vary with climate and soil characteristics.

We used a pulse of  $^{15}\text{N}$ -labeled  $\text{NH}_4^+$  to simulate the movement and fate of salmon-derived N in a salmon-influenced riparian forest. Isotopically labeled N is widely used to examine the fate of N. For example, silviculturists employ  $^{15}\text{N}$  isotope tracers (reviewed by Nason and Myold 1992), to quantify tree uptake and loss of fertilizer N.  $^{15}\text{N}$  tracers are also used to examine ecosystem-level processes by following  $^{15}\text{N}$  applications through soil/plant systems

(e.g. Perakis and Hedin 2001, Nadelhoffer et al. 1999, Tietema et al. 1998). But N tracers have not yet been used to examine the widely discussed feedbacks between salmon and riparian forests. Our  $^{15}\text{N}$  tracer application was designed to approximate a salmon nutrient contribution to a riparian system; we followed the  $^{15}\text{N}$  tracer through soil and tree pools for one year to answer two sets of questions:

1) Is a late-season pulse of N available to trees, or is it immobilized in recalcitrant soil pools or lost from the system before becoming available to trees? What portion of a late season salmon N pulse do trees take up and use? Is uptake immediate or does it occur gradually through successive seasons as N is cycled through soils?

2) When and how do trees respond to a fall pulse of salmon nutrients, and what are the patterns of N allocation to roots, stems, and foliage over the following year?

## **METHODS**

### *Study Area*

Kennedy Creek, WA is ~ 12 km northwest of Olympia, WA (47°05'N, 123°08'W). The climate is typical of the Pacific Coastal Ecoregion: mean annual precipitation is ~250 cm and the mean monthly air temperature range is ~ 0.5 – 25.0° C. Kennedy Creek is small, with a mean daily flow of ~18.5 m<sup>3</sup>/sec (1990-1999), but it supports a large chum salmon population: escapement averaged 41,000 adults from 1992-2001, 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.

The study site is on a terrace ~1.5 m (vertical) and 5-30 m (horizontal) from the active channel. Kennedy Creek is surrounded by a second growth (~ 80 year-old) Douglas-fir (*Pseudotsuga menzeisii*) forest. Western redcedar (*Thuja plicata*) is used in this study; it is the

late-successional dominant species at the site, replacing Douglas-fir within 20 m of the river. Western redcedar is an important riparian species in the Pacific salmon range and grows under a wide range of conditions; tolerating alkaline to acid soils (Rollinson 1988), wet to dry conditions (Boyd 1965), and low nutrient availability (Radwan and Harrington 1986). Sword fern (*Polystichum munitum*) and several mosses dominate the understory at the study site, but vine maple (*Acer circinatum*) and salmonberry (*Rubus spectabilis*) are also common. Red alder (*Alnus rubra*) and bigleaf maple (*Acer macrophyllum*) occur in patches, but we selected study plots at least 10 m from red alder to minimize any confounding effects that this N-fixing species might have on N dynamics. The soil is a loamy sand texture of the Indianola Series, deep and well-drained, formed in sandy glacial drift (Pringle 1990). Soils are rich in organic matter with a ~4 cm O horizon and a ~14 cm A horizon with ~18% organic matter. Mean soil pH is 5.9 in deionized water. Cation exchange capacity (CEC) is 163 cmol<sub>c</sub>/kg in the O horizon and 90.2 cmol<sub>c</sub>/kg in the A horizon; these values are close to average for salmon-influenced riparian forest soils (Drake 2005). Pre-experimental summer foliage N content of western redcedar was 12.3 (+/- 0.002) g N/kg, higher than average for coastal western redcedar (10.6 g N/kg; Radwan and Harrington 1986), but below the deficiency level for seedlings (15 g N/kg; Walker and Gessel 1990).

#### *Field Methods*

We applied a pulse of 10 atom% <sup>15</sup>N ammonium sulfate [(NH<sub>4</sub>)<sup>2</sup>SO<sub>4</sub>] at a rate of ~3.6 g NH<sub>4</sub>-N/ m<sup>2</sup> to 4, 50-m<sup>2</sup> circular plots (~8.5 m diameter), each centered on 1 mature western redcedar. Two reference plots (receiving no nutrients) were also established. Each plot contained only one tree stem, although roots from other stems extended into the plots. We applied Hoagland solution containing N, P, K, Mg, S and Ca, modified to mimic the nutrient

composition of salmon tissues (Hoagland and Arnon 1950; Table 1). The nutrient application was equivalent to ~7.25 kg spawned-out, fresh chum salmon per plot, was dissolved in approximately 20 L of deionized water, and was applied with a pressurized hand sprayer from 23 – 29 October 2003 (25% of the total addition was applied at 0, 2, 4 and 6 days). Although a 6-day application period is short relative to the release of nutrients during salmon carcass decay (Drake et al. 2005), this was required for accurate calculation of short-term (< 7 days) fluxes.

We collected organic soil, mineral soil, roots in each soil fraction, and foliage from each plots twice prior to tracer additions (-30 days and -7 days) and 11 times after the initial tracer addition (0, 2, 7, 14, 21, 40, 144, 182, 213, 294, and 368 days). Samples were collected from two reference plots on a subset of dates (-30, -7, 7, 14, 40, 213, 294 and 368 days). The collection at 0 days was made approximately 4 hours after the  $^{15}\text{N}$  application, and the collection at 2 days was made prior to the second  $^{15}\text{N}$  application. We collected samples more frequently immediately after the  $^{15}\text{N}$  addition and during the following spring, when rates of N redistribution were expected to be high. On each sampling date, two organic (O horizon) and two mineral (A horizon) samples were collected from each plot, locations were selected randomly but within at least 0.5 meters of the plot boundaries. Organic soil was removed to the A horizon, using a hand shovel and shears (for cutting fiber and roots), from within a guide ring (diameter = 22 cm). Mineral soil samples were collected by driving a 4.8 cm diameter stainless steel cylinder into the A horizon to a depth of 10 cm. All soil samples were weighed separately, fresh and passed through a 4 mm sieve in the field; large surface litter such as sticks and stones were discarded; senesced foliage that would not pass through the sieve was cut up with a scissors and added to the sieved material. All roots were retained for sorting in the laboratory. We collected xylem, phloem and sap (i.e. wood) samples from the  $^{15}\text{N}$  and reference plots with a 5.1

mm diameter increment borer. **Live leaves were collected from sun-exposed branches that** Wood samples were collected on only 4 dates to limit damage to the trees; 1 month prior to tracer addition, and 1, 7, and 12 months after the  $^{15}\text{N}$  addition. Senescent leaves were collected from branches at the end of the experiment (12 months), after they turned brown but prior to abscission.

*Laboratory methods*

Organic and mineral soil samples were processed separately to account for fundamental differences in N retention, depth and bulk density. Subsamples (~10 g) were dried at 100° C for at least 48 hours to determine gravimetric moisture, and then ashed at 550° C for organic matter content. Cedar roots were identified, washed in deionized water, oven dried at 100°C for at least 48 hours, and ground using a Wig-1-bug amalgamator. Foliage was rinsed in deionized water, dried and ground. Total soil N was determined from dry, ground samples. All ground samples and encapsulated in tin for  $\delta^{15}\text{N}$  analysis.

We determined organic and mineral soil N content and  $\delta^{15}\text{N}$  for each soil pool, within each plot, on each date. Soil samples collected on day 0 were processed within 10 hours of the  $^{15}\text{N}$  tracer application, and soil samples collected on all other dates were processed within 24 hours of collection. We used standard methods (Robertson et al. 1999) with 2M potassium chloride (KCl) to perform all soil extractions. Microbial N and  $\delta^{15}\text{N}$  were determined from chloroform-treated soils; chloroform vapor was drawn through soils under a vacuum, and the samples were incubated in a chloroform atmosphere for 7 days. This resulted in cell lysis and release of cellular contents into the soil solution (after Paul et al. 1999). The soil samples were extracted, and organic N in the extracts was oxidized to mineral N via persulfate digestion (modified from Sparling et al. 1996). Extracts (30 – 50 ml) were pipetted into 125 ml glass jars,



potassium persulphate (0.6 g) was added, and the extracts were processed at 121°C and ~1.05 kg/cm<sup>2</sup> for 40 minutes in a pressure-cooker. N was isolated from the processed samples for  $\delta^{15}\text{N}$  analysis using a microdiffusion technique developed for soil extracts (Brooks et al. 1989).

Sap and its solutes were extracted from ground xylem by bleaching in a basified hydrogen peroxide solution, and repeated rinsing in deionized water. All  $\delta^{15}\text{N}$  analyses were conducted at the Stable Isotope Facility, University of California, Davis. Standard error for replicate samples was <0.0002 atom% (<sup>15</sup>N labeled samples).

#### *<sup>15</sup>N mass balances*

The biomass of foliage, xylem and phloem (Appendix A) was determined using allometric equations (e.g. Brown 1978) developed specifically for western redcedar and based on diameter at breast height. Root biomass was assumed to be a plot-specific constant, and was calculated from root dry weights of 26 samples collected from each plot over the experiment. Foliage N content and  $\delta^{15}\text{N}$  were measured on each sampling date, and were corrected for the N content of new and previous year's foliage (i.e. because new foliage was relatively N-rich proportions of new and old foliage were multiplied by respective %N). Root N and  $\delta^{15}\text{N}$  were also corrected for relative biomass and N content of new and old roots. Soil volume and bulk density estimates were based on overall averages (26 samples from each plot) and were also assumed to be constant over the experiment.

Recovery of tracer N from each pool was calculated using the equation:

$$^{15}\text{N}_{\text{rec}} = m_{\text{pool}}(\text{atom \% } ^{15}\text{N}_{\text{pool}} - \text{atom \% } ^{15}\text{N}_{\text{ref}}) / (\text{atom \% } ^{15}\text{N}_{\text{tracer}} - \text{atom \% } ^{15}\text{N}_{\text{ref}}) \quad [1]$$

where  $^{15}\text{N}_{\text{rec}}$  = mass of the <sup>15</sup>N tracer recovered in the labeled N pool,  $m_{\text{pool}}$  = N mass of the pool;  $\text{atom \% } ^{15}\text{N}_{\text{pool}}$  = atom percent <sup>15</sup>N in the labeled N pool,  $\text{atom \% } ^{15}\text{N}_{\text{ref}}$  = atom % <sup>15</sup>N in the reference (pre-labeled) N pool, and  $\text{atom \% } ^{15}\text{N}_{\text{tracer}}$  = atom percent of the applied tracer.

#### *Statistical analyses*

Pre-experimental atom%<sup>15</sup>N values of soil and tree pools did not vary considerably between plots (Appendix A). Additionally, the strength of the <sup>15</sup>N tracer signal made any fluctuations in natural abundance irrelevant. We therefore assumed, for statistical analyses, that the reference (pre-experiment) N content of each pool was a fixed value ( $m_{\text{pool}}$  and atom %<sup>15</sup>N<sub>ref</sub> from equation 1, Appendix A). Atom%<sup>15</sup>N of reference site N pools did not vary considerably from baseline over the experiment, although total N content of pools varied seasonally. These data were used to distinguish seasonal changes in total N from experimental effects.

We calculated total N and %<sup>15</sup>N of each pool within each plot separately. Tracer recovery is summed for the four plots, and is expressed as a proportion of the total <sup>15</sup>N tracer applied through the time of sample collection. We calculated microbial N by subtracting extractable N from chloroform-labile N, and applied a conservative recovery correction of 1.05 to microbial N estimates to account for the fraction of soil microbiota not lysed by chloroform fumigation and N immobilization during incubation (i.e. total microbial N values are much more likely to be underestimated than overestimated). Soil organic matter (SOM) atom% and N content was calculated by subtracting microbial and extractable pools from bulk soil values.

We used repeated measures analysis of variance to test for changes in total N and <sup>15</sup>N over time. N content data were log transformed to improve normality. The significance level for all tests was  $\alpha = 0.05$ , and all analyses were performed with EXCEL 9.0.2720 software.

## **RESULTS**

### *Fate of tracer N in soils and trees*

A marked redistribution of the <sup>15</sup>N tracer between soil pools (extractable, microbial and SOM) occurred during the 6 weeks following the application (Figure 1a); initially, most of the

$^{15}\text{N}$  tracer was recovered from the soil solution (i.e. the extractable pool), but about 40% of the tracer N was not accounted for in the first two samplings (23 and 25 October 2003). However, by the third sampling (30 October) close to 100% tracer N was accounted for in soil pools, suggesting that a portion of the tracer N was initially “stuck” on the surface of forest litter (sticks, recently-fallen maple leaves), and was not fully incorporated into soil samples until rainwater dissolved and moved the  $\text{NH}_4^+$  from the surface into deeper soils: 3.42 cm of rain fell between the second and third sampling dates.

Extractable and microbial  $^{15}\text{N}$  maxima occurred within days of application in the organic horizon, and 3-4 weeks later in the mineral horizon, demonstrating an unsurprising downward movement of the N tracer through the soil profile (Appendix B).

Microbial pool tracer content reached a maximum of ~63%, 14 days after the initial application, decreased rapidly, and stabilized at ~4% within 5 months (Figure 1a). Rapid cycling of tracer N through the microbial pool resulted in immobilization of ~60% of the tracer N in SOM within 6 weeks, and the SOM pool tracer N content reached a relative equilibrium of ~40% within 5 months (Figure 1b).

Western redcedar roots started to take up the  $^{15}\text{N}$  tracer within 7 days, and the tracer content of roots increased throughout the winter samplings (Figure 1b). Only roots within the sample plots were accounted for, and we suspect that tracer N was redistributed throughout the root system (laterally beyond the plot boundaries and into deeper soils), likely resulting in underestimation of total root tracer N content. The tracer N was redistributed from roots into the stems and foliage the following spring (Figure 1b).

Vigorous growth of new roots occurred in both fertilized and reference trees from October 2003 – February 2004. New roots contained more tracer N per unit dry weight than old

roots; 6 weeks after the tracer application new root growth accounted for ~17% of total root mass (dry weight), but 28% of N in the new roots N was tracer N, while only 11% of the N in old roots was tracer N. By May, new roots were no longer visibly distinguishable from old.

Seven months after the  $^{15}\text{N}$  application (the following spring), 2.3% of the tracer N was found in the stems of western redcedar (wood, bark and sap, Figure 1b). Most of the tracer N in stems was in xylem sap (~80%), and only ~4% was incorporated into structural components of xylem. The remaining ~16% of the  $^{15}\text{N}$  tracer in the stem was in phloem (Appendix C).

Total recovery of the tracer N from tree and soil pools was ~75% one year after the addition (Figure 2). When an estimate of N in the leaves of sword ferns (the dominant understory species) is included, total minimum retention of tracer N within the plots over 1 year is ~79%. This conservative estimate does not include N in fern roots or the less common plant species, or N in roots that extended beyond the plot boundaries. At least 37% of the  $^{15}\text{N}$  tracer was taken up and used by western redcedar within one year (maximum N tracer content in the trees occurred 7 months after the addition), and after 12 months, ~44% of the tracer N retained in the plots was bound in SOM. Loss of tracer N from the plots over one year was ~20% or less.

We used N uptake, storage and loss measured here to produce a semi-quantitative model of N cycling (Figure 3). Salmon N input of 35 kg/ha is equivalent to the experimental addition rate. We estimated red alder N fixation by multiplying area coverage by N fixation rates of pure alder stands (Cole 1995). Atmospheric deposition of N in the region is low, ~1.5 kg N/ha (National Atmospheric Deposition Program 2005). Maximum potential denitrification rates in riparian soils of Alaskan salmon streams were 9-60 kg N/ha, and were linked strongly to soil characteristics (Pinay et al. 2003), leading us to suggest a low denitrification rate of 2 kg N/ha in the well-drained soils at the study site. In forest ecosystems, a large portion of biomass is wood,

supporting relatively low rates of herbivory and small consumer biomass (Chapin et al. 2002). We estimated a herbivory rate of 1.25% of western redcedar annual GPP, and a consumer N standing stock of ~ 2 kg/ha (soil microbiota are included only in soil N estimates). The model suggests net annual accumulation of N in both soils and trees.

*Effects of the N addition on tree-pool dynamics*

The tracer addition did not affect the overall N content of most tree tissues over the year. For example, mean foliar N of both tracer and reference trees varied from 12.9 g N/kg in October - March, to ~19 g N / kg in the spring. The exception to this general pattern of similarity was in phloem; the N content of phloem was significantly lower (by about 40%) in the tracer trees at 7 and 12 months after the tracer N application. The difference in the phloem pool is not significant in terms of total tree N, but it may signify a physiological response to the nutrient addition.

Prior to senescence, about half of the foliage N was withdrawn into the trees. A majority of the tracer N was removed from the leaves; average  $^{15}\text{N}$  atom% of green leaves was 0.370 prior to the experiment, 0.670 in green leaves (of all ages) at 12 months, but only 0.385 in senesced leaves at 12 months - almost returning to the baseline value, and suggesting that the recently acquired N was more accessible.

**DISCUSSION**

*Riparian trees took-up a large portion of the fall N pulse*

Western redcedar on Kennedy Creek are clearly able to take advantage of a late-season pulse of  $\text{NH}_4^+$  provided by salmon. Our data demonstrate high rates of N uptake over the fall and winter, provide mechanistic evidence for a 1-year lagged growth response to salmon nutrients, and corroborate empirically established links between salmon and riparian trees.

The rate of N uptake demonstrated here is notable because it is substantially higher than most published values from regional forest fertilization studies. Western redcedar at Kennedy Creek took-up at least 37% of the N tracer within 6 months and still contained at least 28% of the tracer 12 months after the addition (Figure 2). Another study of western redcedar N uptake reported only 4.1% - 7.7% recovery of <sup>15</sup>N tracer 18 months after application (Chang et al. 1996). Average recovery of fertilizer <sup>15</sup>N from other coniferous species is 18.4% (range 1.9% - 44.0% for 20 studies including root fractions; Nason and Myrold 1992). But few forests receive salmon nutrients – in most forests, more than 90% of N and P absorbed by plants are from litter sources (Chapin et al. 2002). Litter releases nutrients slowly - the mean residence time of plant organic matter in temperate coniferous forest floors is ~18 years (Cole and Gessel 1992). Compared to litter, salmon tissues are nutrient rich and are more easily broken down by decomposers, particularly where desiccation or freezing do not impede decay; e.g. salmon carcasses placed in the Kennedy Creek forest were completely skeletonized within 10 weeks of death (Drake et al. 2005). Salmon contribute nutrients to riparian soils and vegetation at high rates but to small areas over short periods. Feces and urine containing salmon nutrients are also arguably available for uptake in large quantities but over small areas (e.g. Overrein 1968). Patchy, short-lived delivery of salmon nutrients might be expected to lead to high rates of loss, but the data show the opposite - uptake and retention of N was high.

The relatively high rate of N uptake demonstrated here has several potential explanations. First, the <sup>15</sup>N tracer was applied in the fall, a time of vigorous root growth in western redcedar. Chang et al. (1996) applied <sup>15</sup>N fertilizer in the spring, the time of low root growth (although not necessarily uptake). Second, the addition of other important nutrients (Ca, K, Mg, P, S) with N may increase the rate of N uptake. Positive (and negative) interactions between nutrient

combinations are known to occur; for example, addition of N plus P stimulated a higher rate of root growth in Sitka spruce (*Picea sitchensis*) than addition of either nutrient alone (Philipson and Coutts 1977). Third, trees that receive annual pulses of nutrients may, over time, develop physiological mechanisms (a legacy effect) that allow more efficient uptake of nutrients. Several studies have shown that the *efficiency* of resource use by trees is positively correlated with *rates* of resource use over time. For example, chronically fertilized oaks (*Quercus spp.*) and red pine (*Pinus resinosa*) take-up N 4 to 5 times more efficiently than unfertilized trees (Nadelhoffer et al. 1999). *Eucalyptus* plantation case studies also show that more productive sites tend to use resources more efficiently than less productive sites (Binkley et al. 2004, Stape et al. 2004).

All three potential explanations for high rates of tracer N uptake shown here apply to salmon-derived nutrients. First, most salmon spawn in the fall and winter, this is consequently when most salmon-derived nutrients are added to riparian forests. Fall/winter root activity may therefore confer a competitive advantage in salmon systems. Mild, wet winters in the Pacific Coastal Ecoregion allow significant winter physiological activity – conifers can fix 30 to 65% of their annual total carbon during winter months (e.g. Waring and Franklin 1979). Second, salmon tissues contain many nutrients important for plant growth, and a companion study demonstrated that decaying carcasses at Kennedy Creek contributed significant amounts of N, S and P to soils within 50 cm (Drake et al. 2005). Third, Kennedy Creek has supported a large, annual spawning salmon population for at least several hundred years, certainly a chronic nutrient addition.

#### *Allocation of tracer N within trees*

One year after the application of the <sup>15</sup>N tracer, roots of western redcedar were still the most enriched tree tissue, with an average tracer N content of 6.59%. Tracer N content of the foliage was lower, 1.87%, but foliage is relatively N-rich and contained about 60% of the <sup>15</sup>N

tracer in trees. Structural N in sapwood showed the smallest response to environmental N isotope availability (was the least enriched tree pool) with a maximum tracer content of only 0.54%. Translocation of the labeled N between the sapwood and heartwood was low relative to western hemlock (*Tsuga heterophylla*; Drake 2005). When sap and its solutes were removed from heartwood (formed 1973 -1983), structural  $^{15}\text{N}$  atom% was equal to pre-experiment values.

Phloem data suggest that the tracer addition resulted in a physiological response; trees that received the tracer had significantly less N in their phloem than the reference trees 7 to 12 months after the addition (40% less,  $P < 0.01$ ). While not significant in terms of total tree N, retention of recently acquired N in leaves over the winter, instead of reallocation to the winter-active roots, suggests a shift in physiological priority. Decreasing nutrient limitation is generally associated with a shift in the allocation of plant biomass from belowground to aboveground pools (Chapin et al. 2002). Trees allocate biomass and resources to optimize resource capture and use (Binkley et al. 2004); the shift in N allocation from roots to foliage suggests reprioritization of light capture. This also suggests a potential mechanism for a lagged growth response of 2 or more years; allocation of salmon nutrients to foliage growth or quality may result in increased growth of the whole tree over several years.

*Western redcedar N requirements and salmon*

Annual N demand by western redcedar is difficult to determine because of potential storage and translocation of N from senescing leaves and between tissues. An average value for annual N uptake by conifers is  $39 \text{ kg ha}^{-1}$  (Cole and Rapp 1981), and the suggested rate of N application for forest fertilization in the Pacific Northwest is  $220 \text{ kg ha}^{-1}$  every 5-10 years (Cole 1995). We applied N at  $\sim 36 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , a rate well within the range of potential salmon input. The addition increased the total N in surface soils by  $\sim 3\%$ , but increased the amount of plant-



available (extractable) N by a factor of 60 initially (calculated 48 hours after the final  $^{15}\text{N}$  addition). This is consistent with rates of N contribution from salmon decay, where cumulative availability of N was ~250x greater within 20 cm of decaying carcasses (Drake et al. 2005). Collectively, these data suggest that a salmon N contribution of  $36 \text{ kg ha}^{-1}$  provides a significant amount of the average annual N demand by western redcedar.

*Riparian ecosystem dynamics and salmon nutrients*

Salmon can potentially provide a large portion of annual tree nutrient requirements. Data presented here corroborate empirical studies suggesting that up to 30% of the N in riparian plants near salmon streams is salmon-derived (e.g. Bartz and Naiman 2005, Bilby et al. 2003, Ben-David et al. 1998). This study also provides mechanistic evidence for empirical links between salmon abundance and tree-ring growth (Drake et al. 2002, Helfield and Naiman 2001).

The high retention rate of  $^{15}\text{N}$  tracer within the plots at Kennedy Creek is, at least partly, a function of forest and site age. Accumulation of N in soils and trees (Figure 3) is not unexpected; this is a relatively young forest (~80 years), likely in an aggrading stage of secondary succession. Frequent fluvial disturbance, removal of vegetation and resetting of the soil surface, is a defining characteristic of riparian forests in the Pacific Coastal Ecoregion (Benda et al. 1998), so under natural conditions, a large portion of a riparian forest is in early successional (aggrading) stages. This study examines salmon N dynamics over one year – but salmon have been contributing nutrients to riparian forests for thousands of years. Over decades, salmon contributions, although patchy, can potentially far exceed the requirements of riparian forests, even those in aggrading stages. Patterns and frequency of disturbance must, therefore, play an important role in the long-term, large-scale ecological effects of salmon nutrients.

*Chronic nutrient loading – a broader perspective*

In contrast to the perception of nutrient deficits caused by salmon extirpation in the Pacific Northwest, anthropogenic increases in reactive N and P are a serious concern in most of the world (Vitousek et al. 1997). Globally, bioactive N increased twice and P increased four times over background levels (Falkowski et al. 2000). Predicted effects of atmospheric NO<sub>x</sub> deposition on forest ecosystems include non-linear responses to initial fertilization, followed by increased NO<sub>3</sub><sup>-</sup> leaching leading to degradation of water bodies, and eventually by declines in forest productivity due to acidification, toxic Al concentration, and loss of base cations (Aber 1998, Nadelhoffer et al. 1999). We find it noteworthy that salmon systems in the Pacific Northwest have experienced N inputs for thousands of years at rates several times greater than those associated with atmospheric deposition in industrial areas and continue to maintain productive riparian forests. Nutrients contributions from salmon, however, differ fundamentally from industrial pollution (Table 2), most notably in that atmospheric deposition tends to include large amounts of SO<sub>4</sub><sup>2-</sup>. Although Kennedy Creek soils have received salmon nutrients for hundreds or thousands of years, they show no evidence of acidification associated with atmospheric nutrient loading; in fact, both CEC and pH are relatively high **compared to**). Salmon, especially their bones, may contribute significant quantities of base cations and P, whose depletion is associated with forest decline in other N-saturated forests. Fenn et al. (1998) suggested that in eastern USA forests, N loading may result in shifts from conifers to deciduous species, which are faster-growing and better adapted to fast N cycling. There is, however, no data to support increased prevalence of local deciduous species (e.g. big-leaf maple) on salmon streams in the conifer-dominated Pacific Northwest.

This study shows that riparian trees can take advantage of the patchy, short-lived, late-season nutrient pulse provided by salmon, but the details of N uptake and cycling will vary depending on site characteristics. Recently, an intense focus on perceived salmon nutrient deficits in Pacific Northwest rivers has drawn attention from other important wild salmon issues, but habitat destruction is still one of the most pressing threats to wild salmon. This study demonstrates the mechanistic basis for links between the riparian forest and salmon, and reinforces the importance of maintaining natural, functioning, riparian forests.

### **ACKNOWLEDGEMENTS**

We thank Dr. Peter Bisson and Phil Peterson for their help in establishing and planning this study, and Simpson Timber Company for allowing this work on their land. We also thank Dr. Marshall Otter and Amy Miller for helpful suggestions on the manuscript. Funding was provided by the Hall-Ammerer Foundation, the Pacific Northwest Research Station of the US Forest Service, the University of Washington Sea Grant Program, and the National Science Foundation (DEB 98-06575).

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### APPENDIX A

A table listing natural  $^{15}\text{N}$  abundance and native N in plant and soil pools of study plots.

### APPENDIX B

A figure showing  $^{15}\text{N}$  tracer maxima in extractable and microbial soil pools.

### APPENDIX C

A table listing recovery of the  $^{15}\text{N}$  tracer from western redcedar stem pools.

Table 1. Nutrient solution composition. Major nutrients in salmon carcasses were applied to the four riparian forests plots (each  $\sim 50\text{ m}^2$ ) over a period of 6 days (23 – 29 October 2003) to simulate the addition of 7.25 kg salmon (spawned-out) tissue per plot. P, K, Mg, and Ca were added at a 72% rate over this period because these elements enter soil pools from decaying bone more slowly than N from soft tissue decay (Drake et al. 2005). S was added at a 78% rate for the same reason and because it was a constituent of both K and Mg salts.

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Nutrient	% by wet weight in whole salmon (spawned-out)	Kg nutrient in 7.25 kg salmon (spawned-out)	actual application of nutrient per plot	Nutrient form
N	2.50	0.1815	0.1815	$(\text{NH}_4)_2 \text{SO}_4$
P	0.43	0.0312	0.0275	$\text{Ca}(\text{H}_2\text{PO}_4)_2 \text{H}_2\text{O}$
K	0.14	0.0102	0.011	$\text{K}_2\text{SO}_4$
Mg	0.03	0.0022	0.0028	$\text{MgSO}_4$
S	0.10	0.0073	0.0128	$\text{K}_2\text{SO}_4$ and $\text{MgSO}_4$
Ca	0.05	0.0036	0.0413	$\text{Ca}(\text{H}_2\text{PO}_4)_2 \text{H}_2\text{O}$

Table 2. Nutrient ion compositions of polluted atmospheric wet deposition in a Northeastern U.S. forest (National Atmospheric Deposition Program, 2005) and salmon inputs (Drake et al. 2005). A value for atmospheric phosphate deposition was not available.

		Atmospheric deposition of ions in a polluted Northeastern U.S. forest <sup>1</sup>	Estimated nutrient contribution from salmon
		kg/ha	kg/ha
Cations	Ca <sup>+</sup>	2.50	8.25
	Mg <sup>+</sup>	0.55	0.55
	NH <sub>4</sub> <sup>+</sup> -N	3.50	12.71
	K <sup>+</sup>	0.60	2.20
Anions	NO <sub>3</sub> <sup>-</sup> -N	4.97	5.45
	SO <sub>4</sub> <sup>2-</sup> -S	9.55	1.65
	PO <sub>4</sub> <sup>-</sup> -P	-	5.50

Figure 1. (A)  $^{15}\text{N}$  tracer recovery from riparian forest soil pools (combined values for mineral and organic horizons) and trees. The shaded area is the period of  $^{15}\text{N}$  tracer addition. (B) Recovery of  $^{15}\text{N}$  tracer from tree pools over one year. A large redistribution of N from belowground to aboveground tissues occurred in the spring. At the end of one year, foliage contained a majority of the tracer N in trees.

Figure 2. Total recovery of  $^{15}\text{N}$  tracer from soils and trees within the experimental plots over one year. The low value for total recovery in March 2004 may be the result of the  $^{15}\text{N}$  tracer being transported to roots beyond bounds of the study plots, appearing the following month as N was redistributed from roots to leaves.

Figure 3. A semi-quantitative model of N dynamics in the salmon-influenced, Kennedy Creek riparian forest. N pools are expressed in  $\text{kg ha}^{-1}$  and fluxes are  $\text{kg ha}^{-1} \text{ yr}^{-1}$ . All soil and tree pools in addition to fluxes marked with black arrows are based on direct measurements made during this study. Atmospheric deposition is regional data from the National Atmospheric Deposition Program (2005). Gray arrows are estimates of N fixation based on regional data (Cole 1995). Denitrification is an estimate based on Pinay et al. (2003). Rates of herbivory and consumer biomass are estimates based on theory of forest ecosystems (Chapin et al. 2002).





