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The Role of Brown Bears (Ursus arctos) in Nutrient Transport Into Forests Near a Salmon Stream in Coastal British Columbia, Canada

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THE ROLE OF BROWN BEARS (*URSUS ARCTOS*) IN NUTRIENT TRANSPORT INTO FORESTS NEAR A SALMON STREAM IN COASTAL BRITISH COLUMBIA, CANADA

by

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ABSTRACT

The Role of Brown Bears (*Ursus arctos*) in Nutrient Transport into Forests Near a Salmon Stream in Coastal British Columbia, Canada

by

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Nutrients from spawned salmon contribute to the fertility of rivers and riparian areas. Adjacent forests, even far from rivers, could receive substantial amounts of nitrogen and other nutrients from salmon. Since brown bears feed heavily on spawning salmon, bears probably influence the movement of nutrients from salmon into surrounding forests. Because salmon-derived nitrogen is high in ^{15}N, increased isotopic enrichment is expected in forest soils and vegetation if this transport is occurring. Based on relative ^{15}N enrichment of spawning areas, a quantitative estimate of marine-derived nitrogen (MDN) can be obtained using a linear two-source mixing model. To evaluate the reliability of MDN estimates based on such a two-source mixing model, we evaluated some assumptions used in mixing model calculations. We determined isotopic changes as nitrogen moved from salmon tissue into brown bear feces and soil where the bears were feeding on salmon near Knight Inlet, British Columbia. We also used a simulation model to evaluate fractionation's effect on MDN estimates. To evaluate

dissemination of MDN by grizzly bears, we determined ^{15}N of vegetation and soil from transects across bear trails and beds along the Koeye River, British Columbia. We expected to find the highest isotopic enrichment closest to bear trails and beds.

We found little difference (about 2‰) between $\delta^{15}N$ of salmon tissue and $\delta^{15}N$ of salmon-derived N in soil. However, $\delta^{15}N$ in other areas was high, even exceeding $\delta^{15}N$ of salmon tissue. Using a simulation model we found that fractionation of N losses from the soil caused gross (more than 70% in some cases) overestimates of MDN. It appeared that ^{15}N fractionation could be large enough under natural conditions to prevent accurate quantification of MDN with a two-source mixing model.

Delta^{15}N at bear trails and beds exceeded $\delta^{15}N$ from several meters away on both sides (by an average of 1.5‰), and $\delta^{15}N$ of a reference transect, supporting the assertion that bears move substantial amounts of MDN upslope. We calculated 5% to 56% MDN in soil within 10 m of bear trails and beds using $\delta^{15}N$ data, compared to 14% MDN based on the ^{15}N difference between reference and spawning sites.

(129 pages)

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CHAPTER 1

INTRODUCTION

Nitrogen often limits the rate of primary production on land and sea (Vitousek and Howarth 1991). Most forests receive N inputs only as atmospheric deposition (in precipitation and particulates) and through nitrogen fixation (conversion of N_2 to NH_4^+ by plants, microorganisms and humans) but some forests, like the northwestern temperate rainforest, may receive large amounts of nitrogen from a marine source.

Spawning pacific salmon (*Oncorhyncus* spp.) move millions of kilograms of nitrogen from the Pacific Ocean into rivers of the northwestern temperate rainforests (Willson and Halupka 1995, Willson et al. 1998, Cederholm et al. 1999, 2000, Naiman et al. 2000). In addition to nitrogen, adult salmon bodies are rich in phosphorous, calcium, and other nutrients (Piorkowski 1995, Kline et al. 1997). As salmon swim upstream, spawn, and die, the nutrients from their bodies are released. Nitrogen is released as organic molecules and as inorganic, mineral N (Piorkowski 1995). In addition to contributing nutrients to freshwater systems, salmon also play a focal role in some northwestern faunal interactions that have probably helped to shape the northwestern Pacific rainforests. For instance, brown bears (*Ursus arctos*) congregate to feed on spawning salmon. The overarching effects of salmon connect traditionally separated ecosystems, and function in what may be an autocatalytic (positive feedback) relationship between salmon, freshwater rivers, brown bears (*Ursus arctos*), and the northwestern American coastal forests.

The rich fish fertilizer of spawned salmon, their eggs, and fry have been shown to increase the productivity of freshwater rivers and lakes where salmon spawn (Juday et

al. 1932, Donaldson 1967, Brickell and Goering 1970, Richey et al. 1975, Mathisen et al. 1988, Piorkowski 1995, Bilby et al. 1996, Kline et al. 1990, 1993, 1997, Gross et al. 1998, Willson et al. 1998, Cederholm et al. 1999, 2000). Nutrients from salmon provide support for primary producers (autotrophs), direct salmon consumers (e.g., caddisfly larvae; Trichoptera: Limnephilidae *Ecclisomyia*), and indirect secondary consumers (e.g., salmon fry that consume caddisfly larvae) (Mathisen et al. 1988, Kline et al. 1990, 1993, 1997, Piorkowski 1995, Bilby et al. 1996). Systems with salmon appear to possess unique aquatic macroinvertebrate community structure and support higher production of aquatic invertebrates and fish, including salmon fry (Piorkowski 1995, Kline et al. 1997). In fact, salmon nutrients have been shown to be so important that as salmon have declined in northwestern American rivers some people have attempted to mimic natural salmon fertilizer by wiring salmon carcasses into rivers, or mechanically spreading inorganic nitrogen fertilizers in freshwater lakes and rivers (Larkin and Slaney 1997, Cederholm et al. 2000). In many cases the effects of such inorganic nutrient dispersal by humans are unsustainable since they depend on continued human intervention. In addition, inorganic fertilizer does not play the same role as salmon at an ecological level.

Salmon-derived nutrients circulate into riparian areas and other terrestrial forests, in addition to dispersing through freshwater rivers and lakes. Recent studies document movement of MDN into northwestern Pacific forests (Piorkowski 1995, Willson and Halupka 1995, Bilby et al. 1996, Ben-David et al. 1998a, 1998b, Willson et al. 1998, Cederholm et al. 1999, 2000, Hilderbrand et al. 1999a, 1999b, Naiman et al. 2000, Helfield and Naiman 2001). Marine nitrogen from salmon can move into terrestrial

systems with the bulk flow of water (flooding and hyporheic flow), through the atmosphere (volatilization followed by N-fixation, or as particulates), through plant uptake, and as waste products or carcasses of salmon predators (Cederholm et al. 1989, Ben-David et al. 1998a, Naiman et al. 2000, Edwards and O'Keefe 2001). Thirty-five terrestrial wildlife species (mammals, birds, amphibians, and a reptile) are known to be directly supported by spawning salmon, carcasses, and/or eggs (Willson et al. 1998, Cederholm et al. 2000). As terrestrial predators consume and transport salmon and eggs they may transport tons of marine nutrients from oceans into forests over an unknown area (Willson and Halupka 1995, Willson et al. 1998, Cederholm et al. 1999).

Brown bears, the largest terrestrial salmon predators, are so large and mobile that they can distribute substantial amounts of marine-derived nitrogen over long distances. As brown bears congregate where salmon spawn they consume fish then move through the forest to day beds or to other feeding areas where their excrement and discarded salmon parts contribute marine-derived nutrients to the land. Hilderbrand et al. (1999a) estimated that an average female brown bear on the Kenai Peninsula in Alaska consumed about 37 kg of salmon-derived nitrogen during the spawning season and excreted more than 35 kg of that nitrogen on land. Since bears congregate at spawning rivers, the total N contribution to forests may be high, both as a function of many bears and as a result of localized areas of high activity (Olson et al. 1997, Hilderbrand et al. 1999a). In addition to excreted nitrogen, bears often leave salmon on shore (Quinn and Kinnison 1999, Ruggerone et al. 2000). Evidence suggests that when salmon are plentiful bears preferentially feed on the most energy-rich parts of the salmon (brain, eggs, and skin) leaving the rest of the carcasses for scavengers or to rot (Gende et al.

2001). Since bears catch so many salmon, their scraps may be an important source of nutrients for terrestrial ecosystems. Olson et al. (1997) observed brown bears capturing sockeye salmon at an average rate of 18 fish per hour per bear in the Brook's River, Alaska. At that rate, a bear fishing one hour each day for 30 d would capture over 3000 kg of salmon (about 110 kg nitrogen). Salmon are available in the Brook's River for more than 30 d each season, and bears fish more than one hour each day, so the total number of salmon killed by bears may be even greater. A substantial number of those fish may be transported into the forest. One study reported that black bears (*Ursus americana*) transported more than 60% of all the fish in an entire salmon run into the forest on Gwaii Haanas Island in British Columbia (Reimchen 1994).

The rich fish fertilizer of salmon in the forest may contribute to the productivity and diversity of terrestrial communities (Cederholm et al. 2000, Naiman et al. 2000). Helfield and Naiman (2001), who have done the only study to date on the effects of salmon on vegetation in the forest, found that trees along rivers where salmon spawned grew faster than trees along rivers where salmon did not spawn. Inputs of marine-derived nitrogen, phosphorous, and other nutrients to the soil could also change forest structure or function in other ways, for example by increasing productivity of other plants, changing nutrient-acquisition relationships, creating localized areas of high nutrient concentration, increasing plant litter decomposition rates, and subsidizing microbial communities which affect N turnover rates (Kirchner 1977, Piorkowski 1995, Ben-David et al. 1998a). In addition, animals attracted by spawning salmon can modify the forest in more ways than just dispersal of marine-derived nutrients. Brown bears can change the forest through their interactions with other salmon-predators and herbivores,

which directly influences riparian herbivory, as well as through digging and trail formation, which has been shown to change nutrient cycling (Butler 1995, Tardiff and Stanford 1998, Berger et al. 2001).

The effects of brown bears, salmon, and terrestrial forests may be mutually beneficial. Forests contribute to salmon spawning success by affecting shade, streambank stabilization, sediment control, litter input, large woody debris, nutrient input, and microclimate (Cederholm et al. 2000). It appears that a reduction in forest productivity would lead to a decrease in salmon spawning success (Naiman et al. 2000, Helfield and Naiman 2001). Forests also directly provide brown bears with both refuge and food. Successful salmon spawning runs contribute to the productivity of streams, lakes, and probably forests, which facilitate further spawning runs as well as the continuance of brown bears and other salmon-predators. Bears excrete or discard most (99%) of the MDN they consume (Hilderbrand et al. 1999a). Discarded nitrogen and other nutrients from salmon, if they increase production and diversity of terrestrial forests, could help to replenish salmon. It may be that brown bears, which help to link salmon and forest, influence the success of both aquatic and terrestrial ecosystems.

Understanding the role of brown bears in the movement of salmon-derived nitrogen into terrestrial forests is of importance where management decisions intend to preserve or increase the productivity of northern coastal forests, salmon spawning runs, or brown bears themselves. In northwestern temperate rainforests, ecological relationships exist that are not constrained to just saltwater, freshwater, or land (Pringle 2001). A salmon-ecosystem occurs in northwestern temperate rainforests, in which ocean, freshwater rivers, lakes, and forest systems are components. Matter and energy,

important for the functioning of each system, are transferred across the boundaries of traditionally separate ecosystems (Willson and Halupka 1995, Willson et al. 1998, Pringle 2001). Evaluating the consequences of manipulating this large-scale salmon ecosystem requires a perspective of nutrient transfer and the interactions of salmon vectors at different levels. For instance, if a manager does not consider the role of terrestrial processes in salmon reproduction, and/or the role of salmon carcasses on terrestrial processes, then salmon harvest decisions might not preserve sustainable numbers of salmon (Cederholm et al. 2000, Naiman et al. 2000). In a similar vein, artificially mimicking a nutrient flux into northern coastal forests by spreading inorganic fertilizer may not encourage the ecologically ascendent relationships (i.e., interactive processes at different scales; Ulanowicz 1997) between salmon predators and salmon. In the case of Pacific salmon, the package (salmon bodies) may be as valuable as the product (N and other nutrients), and salmon consumers themselves may contribute to the sustenance of forests and rivers.

One step toward understanding the relationships between salmon, brown bears, and the forest is to determine the amount of marine-derived nitrogen transferred to different components of the ecosystem. Nitrogen is relatively easy to analyze and may serve as an index for other marine nutrients. Currently the most common way to directly measure the presence and amount of marine-derived nitrogen in ecosystems is to use naturally occurring, stable nitrogen (N) isotopes as tracers or integrators of N transfers (Helfield and Naiman 2001, Robinson 2001).

Stable isotopes provide information about the marine source of nitrogen and other nutrients since the ratio of heavy to light isotopes is greater in salmon than

terrestrial vegetation. Pacific salmon has $\delta^{15}N$ in the range +11‰ to +14‰, and $\delta^{13}C$ of about -18‰ (Kline et al. 1990, 1993, Bilby et al. 1996, Ben-David et al. 1998a, 1998b, 1997, unpublished data, Hilderbrand et al. 1999a, Kline 2001). Northwestern American freshwater primary producers, terrestrial vegetation, and soil are usually substantially lower in $\delta^{15}N$ and $\delta^{13}C$ than salmon (Mathisen et al. 1988, Kline et al. 1990, 1993, Ben-David et al. 1998a, 1998b). Although it is impossible to specify generally accurate typical values for vegetation or soil (Handley and Scrimgeour 1997, Hogberg 1997, Ben-David et al. 1998a, 1998b) studies in Alaska and Washington have found that vegetation and soil $\delta^{15}N$ values are often negative, and $\delta^{13}C$ is often as low as -26‰ (Bilby et al. 1996, Ben-David et al. 1997, 1998a, Hilderbrand et al. 1999a, Helfield and Naiman 2001). The difference between salmon natural isotopic abundance ($\delta^{15}N \approx$+13‰, $\delta^{13}C \approx$-18‰) and terrestrial plant and soil isotope levels ($\delta^{15}N$ often close to zero or negative, $\delta^{13}C$ often less than -25‰) suggests that stable isotopes may be used to quantify marine contributions to terrestrial environments. If a baseline terrestrial $\delta^{15}N$ and $\delta^{13}C$ signature can be determined, then terrestrial $\delta^{15}N$ and $\delta^{13}C$ above the baseline could indicate the presence of marine-derived nitrogen and carbon. Delta^{13}C has not been used to trace marine-derived carbon into vegetation since plants obtain most of their C from the atmosphere, but $\delta^{15}N$ has been used to trace marine-derived nitrogen into terrestrial vegetation.

Based on studies prior to 1998 (Willson and Halupka 1995, Bilby et al. 1996, Hilderbrand et al. 1996, Ben-David et al. 1997) we hypothesized that brown bears transported substantial amounts of marine-derived nitrogen and carbon into forests near

salmon spawning rivers. Bear behavior suggests that isotopic enrichment from salmon-derived nitrogen and carbon would follow a pattern of greater concentrations on bear trails and beds. Brown bears feeding on salmon do not preferentially defecate or urinate in certain "latrine" areas. Most bear feces are found along bear trails and near day beds where single bears or family groups (sow with cubs) rest during the day. Therefore we expected that soil and vegetation near bear trails and beds would be enriched in $\delta^{15}N$ and $\delta^{13}C$ relative to soil and vegetation away from trails or in areas where bears do not consistently travel. We also predicted locally high levels of ^{15}N and ^{13}C near bear trails further away from the river where bears moved between feeding areas or away from the river.

Since 1998, three studies (Ben-David et al. 1998b, Hilderbrand et al. 1999a, Helfield and Naiman 2001) have raised questions about $\delta^{15}N$ distribution relative to areas of high bear concentrations and provide an interesting context for our study. Ben-David et al. (1998b), Hilderbrand et al. (1999a), and Helfield and Naiman (2001) concluded that marine-derived nutrients had entered terrestrial ecosystems near salmon-spawning streams and mentioned the possibility that salmon-predators (specifically brown bears for Hilderbrand et al. 1999a) had acted as MDN vectors. Ben-David et al. (1998b) and Hilderbrand et al. (1999a) reported higher levels of ^{15}N where piscivore activity (noted by feces or telemetry) appeared to be highest. These researchers explained correlation between high levels of ^{15}N and piscivore activity by suggesting that salmon predators had moved salmon into the forests, thus enriching ^{15}N in the areas where they spent the most time and urinated and defecated most frequently.

None of these studies (Ben-David et al. 1998b, Hilderbrand et al. 1999a, Helfield and Naiman 2001) provided data on a scale less than 10 m. If bears comprise a major vector for salmon-derived nutrients into forests then we would expect to find evidence of salmon-derived nutrients on a scale corresponding to areas of locally high bear activity, which appears to be along bear trails and beds in the forest. Hilderbrand et al. (1999a) mentioned the possibility, but did not provide measurements, of highly localized nitrogen distribution patterns where bears focus their activity, for instance, where fishing is most profitable. We add that high activity also occurs on trails and in beds when bears move between or away from profitable fishing areas.

Ben-David et al. (1998b), Hilderbrand et al. (1999a), and Helfield and Naiman (2001) focused on large-scale patterns of $\delta^{15}N$ in vegetation so they did not include soil analysis. Measuring isotopic enrichment of soil benefits isotope tracing because: (1) In soil two isotopes (^{15}N and ^{13}C) can be used to estimate marine inputs, while in vegetation only ^{15}N provides useful information about marine inputs; (2) Soil samples can be collected at regular intervals while plants' occurrence, abundance, and rooting patterns are less uniform and less predictable; and (3) Isotopic fractionation (discrimination between heavy and light isotopes) during plant uptake or internal N translocations might help to obscure or counterfeit a marine salmon ^{15}N signature. Reliable quantification of marine nitrogen in plants requires an estimate of the amount of isotopic fractionation between salmon-nitrogen sources, soil, and vegetation.

To quantify MDN in terrestrial systems other researchers used a two-source isotopic mixing model (Bilby et al. 1996, Hilderbrand et al. 1999a, Helfield and Naiman 2001) similar to Kline et al. (1990, 1993). Riparian vegetation with $\delta^{15}N$ somewhere

between $\delta^{15}N$ of marine and terrestrial N was assumed to contain a mixture of N from both a marine and a terrestrial source. The relative similarity of vegetation $\delta^{15}N$ to the $\delta^{15}N$ of either source was used to calculate the relative quantity of N derived from the two sources based on the following equation:

$$\%MDN = (N_{veg} - N_{terr})/(N_{MDN}-N_{terr}) \cdot 100$$

where %MDN is the percent of N in vegetation that is derived from marine sources, N_{veg} is the $\delta^{15}N$ of the vegetation, N_{MDN} is the $\delta^{15}N$ that vegetation would have if marine-derived N was the only source of N, and N_{terr} is the $\delta^{15}N$ vegetation would have if terrestrially derived N was the only N source. The primary difficulty in using this approach is obtaining accurate estimates of the $\delta^{15}N$ of vegetation grown exclusively on one source or the other. All reported studies have assumed that the $\delta^{15}N$ of vegetation grown solely on MDN is the same as $\delta^{15}N$ of salmon bodies (a questionable assumption). The $\delta^{15}N$ of vegetation grown solely on terrestrial N has been assumed to be constant between similar landscapes (also a questionable assumption). Terrestrial $\delta^{15}N$ was estimated by measuring the $\delta^{15}N$ of vegetation growing in "reference sites" which are either riparian stretches where salmon do not spawn (Bilby et al. 1996, Helfield and Naiman 2001), or sites far away from spawning rivers (Hilderbrand et al. 1999a). Estimates of %MDN made with mixing models have ranged from 15.5% (Hilderbrand et al. 1999a) to 24% (Helfield and Naiman 2001) (Table 1), but the accuracy of these estimates is as questionable as the coarse assumptions used in the mixing model.

TABLE 1. Reported marine-derived nitrogen calculations.

	Sample $\delta^{15}N$[1]		Marine Source $\delta^{15}N$[2]		Terrestrial Source $\delta^{15}N$[3]		Calculated %MDN[4]
Bilby et al. (1996)	Riparian vegetation Washington, USA	+0.7‰	Salmon	+14.1‰	Riparian veg from river without salmon (above impassable falls)	-2.2‰	**17.5 %**
Hilderbrand et al. (1999a)	Spruce Needles from within 500 meters of spawning river along two transects at each river Kenai Peninsula Alaska, USA	Killey River -3.5‰	Salmon	+13.2‰	Spruce needles from the same (2) transects far from each river (>1000 m)	Killey River -6.5‰	**15.5%**
		Mystery Creek -2.2‰	Salmon	+13.2‰		Mystery Creek -5.5‰	**17.8%**
Helfield and Naiman (2001)	Riparian vegetation along two rivers Chichagof Island Alaska, USA	Sitka spruce +0.63‰	Salmon	+13.4‰	Riparian vegetation along the same two rivers where salmon did not spawn (one above impassable falls, the other in small tributaries above spawning reaches)	Sitka spruce -3.34‰	**24% (16%-32%)**
		Devil's club +2.24‰	Salmon	+13.4‰		Devil's club -0.91‰	**22% (12%-32%)**
		Fern +0.62‰	Salmon	+13.4‰		Fern -3.05‰	**22% (13%-32%)**
		Red alder -0.91‰	Salmon	+13.4‰		Red alder -1.04‰	**1% (-2%-4%)**

[1]Sample material thought to contain both marine-derived and terrestrial nitrogen.
[2]The marine source $\delta^{15}N$ estimates $\delta^{15}N$ of marine-derived N found in sample material.
[3]The terrestrial source $\delta^{15}N$ estimates $\delta^{15}N$ of terrestrial N found in sample material.
[4] %MDN = $100 \cdot (O-A)/(B-A)$; O = target, or observed $\delta^{15}N$ of sample in question, A = lower endmember (terrestrial source $\delta^{15}N$), B = upper endmember (marine source $\delta^{15}N$) of a linear two source mixing model.

Complications of quantifying MDN include variable and unpredictable isotopic changes as well as non-uniform microsite characteristics and nutrient processing (Handley and Scrimgeour 1997, Hogberg 1997). Delta^{15}N of salmon-derived nitrogen may not be the same in soil, vegetation, and salmon because fractionation occurs during N cycling and transfer (Handley and Scrimgeour 1997, Hogberg 1997, Kendall 1998). In addition, δ^{15}N may not be the same at spawning sites and reference sites even without salmon-N, because fractionation varies with temperature, moisture, acidity, N concentration, and many other factors (Peterson and Fry 1987, Lajtha and Marshall 1994, Handley and Scrimgeour 1997, Hogberg 1997, Kendall 1998, Neilson et al. 1998). It appears that δ^{15}N signatures in soil or vegetation are relatively unpredictable and site specific (Handley et al. 1999, Robinson 2001).

The δ^{15}N difference between salmon and terrestrial N may still provide unique information about nutrient sources if variation in δ^{15}N of one N source does not obscure δ^{15}N of the other source. In addition, nitrogen isotopes can provide information about N sources when isotopic fractionation rates are known, so that changes in isotope levels can be attributed to fractionating processes or different N sources (Handley and Scrimgeour 1997, Hogberg 1997, Ben-David et al. 1998a, 1998b, Robinson 2001). Since we were interested in quantifying MDN inputs to the forest, we evaluated the potential for error in δ^{15}N mixing model calculations. To test the assumption that isotope levels remain constant between salmon and soil or plants we measured δ^{15}N relationships in MDN between salmon, brown bear feces, soil, and vegetation. No other study has reported these changes under field conditions, nor have they reported δ^{15}N of

both soil and vegetation growing under a suspected regime of salmon-derived nitrogen inputs. As part of this study we also used a simulation model to consider whether fractionating losses could mimic or obscure $\delta^{15}N$ signatures from MDN. We modeled N losses with various fractionation rates to see whether MDN estimates changed significantly while the amount and ^{15}N enrichment of N inputs remained constant. We also considered how changing salmon presence over time would effect MDN calculations by modeling soil $\delta^{15}N$ after a sudden, persistent change in salmon inputs.

The second part of our study was to investigate the pattern and magnitude of MDN distribution by brown bears along a river in the northwestern temperate rainforest. We compared N and C concentrations, and their isotopic enrichments, in soil and vegetation on bear trails and beds to adjacent areas where bear activity was not as concentrated. Our objective was to determine whether there were measurable patterns of ^{15}N and ^{13}C enrichment relative to highly localized areas of brown bear activity, and if so, to determine how much nitrogen and carbon was distributed by bears.

Willson and Halupka (1995) wrote that studies are needed to work out the details, especially to quantify, the linkages between aquatic and terrestrial systems. Only when connections between biota and abiotic components of the environment are elucidated can we hope to manage natural systems in a positive way, or even assure that our effects contribute to stated objectives at all (Pringle 2001). Ascendent characteristics of ecosystems make tweaking individual plant or animal populations a risky business. In the case of northwestern Pacific rainforests, salmon and brown bears are both potentially important links between marine and terrestrial ecosystems, and both are strongly declining. The forest, which both shapes and reflects communities of animals like

salmon and bears, may depend, through those communities, on the ocean for perpetuating itself. Changes in the populations of brown bears and/or salmon may lead to changes in the forest, and vice versa. Our objective with this study is to provide more information on a piece of the link between ocean, animals, and the forest.

References

Ben-David, M., R. T. Bowyer, L. K. Duffy, D. D. Roby, and D. M. Schell. 1998a. Social behavior and ecosystem processes: river otter latrines and nutrient dynamics of terrestrial vegetation. Ecology **79**:2567-2571.

Ben-David, M., T. A. Hanley, D. R. Klein, and D. M. Schell. 1997. Seasonal changes in diets of coastal and riverine mink: the role of spawning Pacific salmon. Canadian Journal of Zoology **75**:803-811.

Ben-David, M., T. A. Hanley, and D. M. Schell. 1998b. Fertilization of terrestrial vegetation by spawning pacific salmon: the role of flooding and predator activity. Oikos **83**:47-55.

Berger, J., P. B. Stacey, L. Bellis, and M. P. Johnson. 2001. A mammalian predator-prey imbalance: grizzly bear and wolf extinction affect avian neotropical migrants. Ecological Applications **11**:947-960.

Bilby, R. E., B. R. Fransen, and P. A. Bisson. 1996. Incorporation of nitrogen and carbon from spawning coho salmon into the trophic system of small streams: evidence from stable isotopes. Canadian Journal of Fisheries and Aquatic Science **53**:164-173.

Brickell, D. C., and J. J. Goering. 1970. Chemical effects of salmon decomposition on aquatic ecosystems. Pages 125-138 *in* R. S. Murphy, editor. First International Symposium on Water Pollution Control in Cold Climates. US Govt. Printing Office, Washington, District of Columbia, USA.

Butler, D. R. 1995. Zoogeomorphology: animals as geomorphic agents. Cambridge University Press, Cambridge, Massachusetts, USA.

Cederholm, C. J., D. B. Houston, D. L. Cole, and W. J. Scarlett. 1989. Fate of coho salmon (*Oncorhynchus kisutch*) carcasses in spawning streams. Canadian Journal of Fisheries and Aquatic Science **46**:1347-1355.

Cederholm, C. J., D. H. Johnson, R. E. Bilby, L. G. Dominguez, A. M. Garrett, W. H. Graeber, E. L. Greda, M. D. Kunze, B. G. Marcot, J. F. Palmisano, R. W. Plotnikoff, W. G. Pearcy, C. A. Simenstad, and P. C. Trotter. 2000. Pacific salmon and wildlife-ecological contexts, relationships, and implications for management. Special edition technical report, prepared for D. H. Johnson and T. A. O'Neil (management directors), wildlife-habitat relationships in Oregon and Washington. Washington Department of Fish and Wildlife, Olympia, Washington, USA.

Cederholm, C. J., M. D. Kunze, T. Murota, and A. Sibatani. 1999. Pacific salmon carcasses: essential contributions of nutrient and energy for aquatic and terrestrial ecosystems. Fishery Bulletin **24**:6-15.

Donaldson, J. R. 1967. The phosphorus budget of Iliamna Lake, Alaska, as related to cyclic abundance of sockeye salmon. PhD Thesis. University of Washington. Seattle, Washington, USA.

Edwards, R. T., and T. C. O'Keefe. 2001. Evidence for hyporheic marine-derived nutrient storage in sockeye streams in southwest Alaska. Abstract *in* American Fisheries Society 2001 International Conference April 24-26: Restoring Nutrients to Salmonid Ecosystems. Eugene, Oregon, USA. http://www.gpafs.org/confnutr/

Gende, S. M., T. P. Quinn, and M. F. Willson. 2001. Consumption choice by bears feeding on salmon. Oecologia **127**:372-382.

Gross, H. P., W. A. Wurtsbaugh, and C. Luecke. 1998. The role of anadromous sockeye salmon in the nutrient loading and productivity of Redfish Lake, Idaho. Transactions of the American Fisheries Society **127**:1-18.

Handley, L. L., A. T. Austin, D. Robinson, C. M. Scrimgeour, J. A. Raven, T. H. E. Heaton, S. Schmidt, and G. R. Stewart. 1999. The ^{15}N natural abundance ($\delta^{15}N$) of ecosystem samples reflects water availability. Australian Journal of Plant Physiology **26**:135-210.

Handley, L. L., and C. M. Scrimgeour. 1997. Terrestrial plant ecology and ^{15}N natural abundance: the present limits to interpretation for uncultivated systems with original data from a Scottish old field. Advances in Ecological Research **27**:133-212.

Helfield, J. M., and R. J. Naiman. 2001. Effects of salmon-derived nitrogen on riparian forest growth and implications for stream productivity. Ecology **82**:2403-2409.

Hildebrand, G. V., S. D. Farley, C. T. Robbins, T. A. Hanley, K. Titus, and C. Servheen. 1996. Use of stable isotopes to determine diets of living and extinct bears. Canadian Journal of Zoology **74**:2080-2088.

Hilderbrand, G. V, T. A. Hanley, C. T. Robbins, and C. C. Schwartz. 1999a. Role of Brown Bears (*Ursus arctos*) in the flow of marine nitrogen into a terrestrial ecosystem. Oecologia **121**:546-550.

Hilderbrand, G. V., S. G. Jenkins, T. A. Hanley, C. T. Robbins, and C. C. Schwartz. 1999b. Effect of seasonal differences in dietary meat intake on changes in body mass and composition in wild and captive brown bears. Canadian Journal of Zoology **77**:1623-1630.

Hogberg, P. 1997. Tansley Review No. 95 ^{15}N natural abundance in soil-plant systems. New Phytologist **137**:179-203.

Juday, C., W. H. Rich, G. I. Kemmerer, and A. Mann. 1932. Limnological studies of Karluk Lake, Alaska, 1926-1930. Bulletin of the Bureau of Fisheries **47**:407-436.

Kendall, C. 1998. Tracing nitrogen sources and cycling in catchments. Pages 519-576 *in* C. Kendall and J. J. McDonnell, editors. Isotope tracers in catchment hydrology. Elsevier Science B. V., Amsterdam, Netherlands.

Kirchner, T. B. 1977. The effects of resource enrichment on the diversity of plants and arthropods in a shortgrass prairie. Ecology **58**:1334-1344.

Kline, T. C., Jr. 2001. Assessing the role of salmon carcasses in the nitrogen budget of sockeye salmon (*Onchorhyncus nerka*) nursery lakes using stable isotopes. Abstract *in* American Fisheries Society 2001 International Conference April 24-26: Restoring nutrients to salmonid ecosystems. Eugene, Oregon, USA. http://www.gpafs.org/confnutr/

Kline, T. C., Jr., J. J. Goering, O. A. Mathisen, P. H. Poe, and P. L. Parker. 1990. Recycling of elements transported upstream by runs of pacific salmon: I. $\delta^{15}N$

and $\delta^{13}C$ evidence in Sashin Creek, southeastern Alaska. Canadian Journal of Fisheries and Aquatic Science **47**:136-144.

Kline, T. C., Jr., J. J. Goering, O. A. Mathisen, P. H. Poe, P. L. Parker, and R. S. Scalan. 1993. Recycling of elements transported upstream by runs of pacific salmon: II. $\delta^{15}N$ and $\delta^{13}C$ evidence in the Kvichak river watershed, Bristol Bay, southwestern Alaska. Canadian Journal of Fisheries and Aquatic Science **50**:2350-2365.

Kline, T. C., Jr, J. J. Goering, and R. J. Piorkowski. 1997. The effects of salmon carcasses on Alaskan freshwaters. Pages 179-203 *in* A. M. Milner and M. W. Oswood, editors. Freshwaters of Alaska: ecological synthesis. Springer-Verlag, New York, New York, USA.

Lajtha, K., and J. D. Marshall. 1994. Sources of variation in the stable isotope composition of plants. Pages 1-21 *in* K. Lajtha and R. Michener, editors. Stable Isotopes in ecology and environmental science. Blackwell Science, Oxford, UK.

Larkin, G. A., and P. A. Slaney. 1997. Implications of trends in marine-derived nutrient influx to south coastal British Columbia salmonid production. Fisheries **22**:16-24.

Mathisen, O. A., P. L. Parker, J. J. Goering, T. C. Kline Jr., P. H. Poe, and R. S. Scalan. 1988. Recycling of marine elements transported into freshwater by anadromous salmon. Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie **23**:2249-2258.

Naiman, R. J., R. E. Bilby, and P. A. Bisson. 2000. Riparian ecology and management in the Pacific coastal rainforest. BioScience **50**:996-1011.

Neilson, R., D. Hamilton, J. Wishart, C. A. Marriott, B. Boag, L. L. Handley, C. M. Scrimgeour, J. W. McNicol, and D. Robinson. 1998. Stable isotope natural abundances of soil, plants and soil invertebrates in an upland pasture. Soil Biology and Biochemistry **30**:1773-1782.

Olson, T. L., B. K. Gilbert, and R. C. Squibb. 1997. The effects of increasing human activity on brown bear use of an Alaskan river. Biological Conservation **82**:95-99.

Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics **18**:293-320.

Piorkowski, R. J. 1995. Ecological effects of spawning salmon on several south-central Alaskan streams. PhD Thesis. University of Alaska, Fairbanks, Alaska, USA.

Pringle, C. M. 2001. Hydrologic connectivity and the management of biological reserves: a global perspective. Ecological Applications **11**:981-998.

Quinn, T. P., and M. T. Kinnison. 1999. Size-selective and sex-selective predation by brown bears on sockeye salmon. Oecologia **121**:273-282.

Reimchen, T. E. 1994. Further studies of predator and scavenger use of chum salmon in stream and estuarine habitats at Bag Harbour, Gwaii Hanaas. Page 57 *in* Report prepared for Canadian Parks Service, Islands Ecological Research. Queen Charlotte City, British Columbia, Canada.

Richey, J. E., M. A. Perkins, and C. R. Goldman. 1975. Effects of kokanee salmon (*Oncorhynchus nerka*) decomposition on the ecology of a subalpine stream. Journal of the Fisheries Research Board Canada **32**:817-820.

Robinson, D. 2001. $\delta^{15}N$ as an integrator of the nitrogen cycle. Trends in Ecology & Evolution **16**:153-162.

Ruggerone, G. T., R. Hanson, and D. E. Rogers. 2000. Selective predation by brown bears (*Ursus arctos*) foraging on spawning sockeye salmon (*Oncorhynchus nerka*). Canadian Journal of Zoology **78**:974-981.

Tardiff, S. E., and J. A. Stanford. 1998. Grizzly bear digging effects on subalpine meadow plants in relation to mineral nitrogen availability. Ecology **79**:2219-2228.

Ulanowicz, R. E. 1997. Ecology, the ascendent perspective. Columbia University Press, New York, New York, USA

Vitousek, P.M., and R. W. Howarth. 1991. Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry **13**:87-115.

Willson, M. F., S. M. Gende, and B. H. Marston. 1998. Fishes and the forest. Bioscience **48**:455-462.

Willson, M. F., and K. C. Halupka. 1995. Anadromous fish as keystone species in vertebrate communities. Conservation Biology **9**:489-497.

CHAPTER 2

AN EVALUATION OF ERROR IN LINEAR TWO-SOURCE MIXING MODELS USED TO QUANTIFY MARINE-DERIVED NITROGEN IN TERRESTRIAL ECOSYSTEMS[1]

Introduction

Pacific salmon (*Oncorhynchus* spp.) spawn and die in northwestern rivers where their decomposing bodies contribute important nutrients to spawning streams (Mathisen et al. 1988, Kline et al. 1990, 1993, 1997, Bilby et al. 1996, Willson et al. 1998, Cederholm et al. 1999, 2000). Salmon-derived nutrients have been shown to increase primary and secondary productivity of freshwater rivers so much that people have even attempted to mimic natural salmon occurrence by placing salmon carcasses in rivers where salmon runs have declined (Larkin and Slaney 1997, Cederholm et al. 2000). However, not only aquatic systems benefit from spawned salmon. In fact, declining salmon runs may also deprive the land of marine-derived nutrients. Salmon nutrients can be transferred onto land through the action of such abiotic vectors as flooding, hyporheic water flow, and wind, and through biotic vectors such as terrestrial salmon predators (Willson et al. 1998, Cederholm et al. 1999). For thousands of years terrestrial predators such as brown bears (*Ursus arctos*), the largest terrestrial salmon predators, have moved marine nitrogen far into terrestrial ecosystems (Hilderbrand et al. 1999a). As bears and other vectors have dispersed marine nutrients from salmon on land, they

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have probably contributed to the productivity of terrestrial communities, which in turn have contributed to the success of salmon runs (Cederholm et al. 2000, Naiman et al. 2000, Helfield and Naiman 2001).

Understanding the mutually reinforcing relationship between salmon, salmon predators, and northwestern forests requires an idea of the amount of salmon-derived nutrients in terrestrial forests. Currently the most common way to directly measure the presence and amount of marine-derived nitrogen in ecosystems is to use naturally occurring, stable isotopes as tracers or integrators of nitrogen (N) transfers (Helfield and Naiman 2001, Robinson 2001). Stable isotopes may provide information about the marine source of nitrogen and other nutrients since the ratio of heavy to light isotopes is greater in marine sources, including salmon, than terrestrial vegetation.

Pacific salmon $\delta^{15}N$ is in the range +11‰ to +14‰, and $\delta^{13}C$ is about -18‰ (Kline et al. 1990, 1993, Bilby et al. 1996, Ben-David et al. 1997, 1998b, unpublished data, Hilderbrand et al. 1999a, Kline 2001). Northwestern American freshwater primary producers, terrestrial vegetation, and soil are usually substantially lower in $\delta^{15}N$ and $\delta^{13}C$ than salmon (Mathisen et al. 1988, Kline et al. 1990, 1993, Ben-David et al. 1998a). Although it is impossible to specify generally accurate typical values for vegetation or soil (Handley and Scrimgeour 1997, Hogberg 1997, Ben-David et al. 1998b) studies in Alaska and Washington have found that vegetation and soil $\delta^{15}N$ values are often negative, and $\delta^{13}C$ is often as low as -26‰ (Bilby et al. 1996, Ben-David et al. 1998b, Hilderbrand et al. 1999a, Helfield and Naiman 2001). The difference between salmon natural isotopic abundance ($\delta^{15}N \approx +13‰$, $\delta^{13}C \approx -18‰$) and terrestrial

plant and soil isotope levels ($\delta^{15}N$ often close to zero or negative, $\delta^{13}C$ often less than -25‰) suggests that stable isotopes may be used to evaluate marine contributions to terrestrial environments. If a baseline terrestrial $\delta^{15}N$ and $\delta^{13}C$ signature can be determined, then terrestrial $\delta^{15}N$ and $\delta^{13}C$ above the baseline could indicate the presence of marine-derived nitrogen and carbon. Delta^{13}C has not been used to trace marine-derived carbon into vegetation since plants obtain most of their C from the atmosphere, but $\delta^{15}N$ has been used to trace marine-derived nitrogen into terrestrial vegetation.

Recent studies have found isotopic evidence for the movement of marine (salmon) derived N into northwestern Pacific forests (Piorkowski 1995, Bilby et al. 1996, Ben-David et al. 1998a, 1998b, Hilderbrand et al. 1999a, Helfield and Naiman 2001). These studies have found elevated $\delta^{15}N$ in vegetation near salmon spawning streams compared to locations where salmon did not spawn (Bilby et al. 1996, Ben-David et al. 1998b, Helfield and Naiman 2001), or far from spawning streams (Hilderbrand et al. 1999a). Vegetation samples collected near spawning sites had 2.9‰ to 4.0‰ higher $\delta^{15}N$ on average than reference sites. Higher $\delta^{15}N$ in vegetation near salmon spawning streams was interpreted as an indication of marine-derived nitrogen (MDN) in that vegetation (Bilby et al. 1996, Ben-David et al. 1998b, Hilderbrand et al. 1999a, Helfield and Naiman 2001).

While elevated $\delta^{15}N$ near salmon spawning areas supports the assertion that salmon-derived N has been transferred to terrestrial ecosystems, accurately quantifying that MDN is not straightforward. Although quantitative estimates of MDN have appeared in both popular and scientific literature (Rasmussen 1996, Salmon 1997,

Cederholm et al. 2000, Naiman et al. 2000, Bothwick 2001, Chadwick 2001, Reimchen 2001), only three studies have reported the methods used for their estimates (Bilby et al. 1996, Hilderbrand et al. 1999a, Helfield and Naiman 2001). A critical assessment of the methods and assumptions used in the MDN estimates of these studies will illustrate the complicated nature of quantifying MDN.

All studies for which methods have been reported used a linear two-source mixing model similar to that outlined by Kline et al. (1990, 1993) to quantify salmon-derived nitrogen in terrestrial vegetation. In a two-source mixing model, endmembers represent two N sources, in this case terrestrial-N and marine-N. The model relates $\delta^{15}N$ of a sample to $\delta^{15}N$ of the endmembers using the following equation:

$$\%MDN = (SAM-TEM)/(MEM-TEM) \cdot 100$$

where %MDN is the percentage of marine-derived N in the sample, SAM is $\delta^{15}N$ of the sample, TEM is $\delta^{15}N$ of the terrestrial endmember (or $\delta^{15}N$ of the terrestrial N source as it would appear in a sample with 0% marine-derived N), and MEM is $\delta^{15}N$ of the marine endmember (or $\delta^{15}N$ of 100% marine-derived N as it would appear in the sample). The primary difficulty in using a mixing model approach to estimate the amount of MDN in vegetation has been obtaining accurate estimates of the $\delta^{15}N$ of vegetation grown exclusively on one source or the other. All reported studies have represented $\delta^{15}N$ of vegetation grown solely on MDN by using $\delta^{15}N$ of salmon bodies (a questionable assumption). The $\delta^{15}N$ of vegetation grown solely on terrestrially-derived N (TEM) has been estimated by measuring the $\delta^{15}N$ of vegetation growing in "reference sites" which are either riparian stretches where salmon do not spawn (Bilby et al. 1996, Helfield and

Naiman 2001), or sites far away from spawning rivers (Hilderbrand et al. 1999a). Assuming that $\delta^{15}N$ of terrestrial N at a reference site is the same at a spawning site is also a very questionable assumption. Estimates of %MDN made with this approach have ranged from 15.5% (Hilderbrand et al. 1999a) to 24% (Helfield and Naiman 2001) (Table 1), but the accuracy of these estimates is as questionable as the coarse assumptions used in the mixing model.

Two-source mixing models provide an easy method for numerical estimations because of their simple mathematical structure. However, both sampling error and fractionation can lead to problems with mixing model MDN estimates. Sampling error may result from high variability within samples, or from unknowingly sampling different N pools, each with its own, different ^{15}N level. In addition, $\delta^{15}N$ of a source may change regardless of inputs from any other sources, as N isotopes fractionate during transfer into the sample.

Previous researchers have estimated MDN by assuming that sampling error and fractionation error were negligible. However, doubt has been raised in other cases about the appropriateness of similar assumptions used in ^{15}N tracing at natural abundance levels (Handley and Scrimgeour 1997, Hogberg 1997, Robinson 2001). To aid in determining whether or not two-source mixing model estimates of MDN are accurate we can consider the assumptions specific to MDN calculations in light of current knowledge about $\delta^{15}N$ variability, predictability, and fractionation. Key assumptions used in mixing models affect the difference between source signatures (i.e., the difference between endpoints on the isotopic gradient) and a sample's position relative to the sources. These assumptions are important because mixing model calculations are mathematically

sensitive to both the isotopic differences between source signatures and the isotopic value of a presumed mixture relative to those sources.

It is helpful to consider the effects of three key assumptions used in mixing models of MDN in terrestrial systems: (1) Delta^{15}N of a marine-N source can be established, and it remains the same after transfer to vegetation; (2) Delta ^{15}N of a terrestrial-N source can be established, and it remains constant or changes predictably across the landscape; and (3) Delta ^{15}N of vegetation samples near a spawning stream represents a mixture of only two distinct N sources that retain their δ^{15}N signatures during mixing.

1. Delta^{15}N of a marine-N source can be established, and it remains the same after transfer to vegetation. Delta^{15}N of the marine source has been represented in all reported studies by δ^{15}N of salmon tissue. Under this assumption δ^{15}N of salmon tissue represents δ^{15}N of the majority of salmon-N transported into the forest. In addition, for δ^{15}N to remain the same in vegetation it was assumed that no net fractionation occurred between δ^{15}N of salmon tissue and sampled vegetation. These assumptions are often incorrect, although N fractionation between salmon tissues and vegetation appears to hold more potential for affecting mixing model calculations than does ^{15}N variability between salmon species or parts.

Pacific salmon appear to vary in δ^{15}N by about 3‰ (+11‰ to +14‰) between sites and species, although δ^{15}N of samples from adults of the same species at single sites do not seem to vary by more than 1‰ (Kline et al. 1990, 1993, Bilby et al. 1996, Ben-David et al. 1998a, Hilderbrand et al. 1999a). In addition, parts of the same salmon

may have different $\delta^{15}N$ signatures. For instance, average egg $\delta^{15}N$ was 0.67 (SE=0.29‰) higher than muscle $\delta^{15}N$ in pink salmon from Chichagof Island, Alaska (difference $P < 0.01$, $n = 9$; Ben-David unpublished data). Isotopic differences have been reported between skin, hair, bone, blood, and muscle of other vertebrates, reflecting enzymatic processes that fractionate N (Kelly 2000), so it is also reasonable to expect differences in $\delta^{15}N$ between salmon parts. If salmon parts differ in $\delta^{15}N$, the $\delta^{15}N$ signature of salmon-N distributed by bears could change as a reflection the consumption of different parts, which may be influenced by salmon abundance or stream conditions. Error in establishing a marine $\delta^{15}N$ signature for N transported by bears could result from using $\delta^{15}N$ of salmon flesh when bears preferentially fed on eggs or skin. An incorrect value for $\delta^{15}N$ of the marine source may also result from using $\delta^{15}N$ of salmon tissue from other locations or from other salmon species. At this time the magnitude of $\delta^{15}N$ differences between individual salmon at different locations and between salmon parts remains unknown, although reported values cited above suggest that differences between parts of individual salmon, and between whole salmon of the same species are likely to be on the order of about 1‰.

A more serious problem for determining $\delta^{15}N$ of the marine endmember (MEM) probably arises from fractionation of N isotopes between salmon and vegetation. In general, chemical and physical processes transfer heavy isotopes at slower rates than light isotopes when they are available in equal parts. While there appears to be little or no fractionation during plant uptake (Hogberg 1997, Robinson 2001) fractionation occurs between salmon and vegetation as a result of fractionation during N transfer

between salmon and soil or within the plant itself. Each time an N transfer occurs in salmon tissue, in soil pools, or between plant N pools the $\delta^{15}N$ signature can change. Nitrogen volatilization, for example, via denitrification, has been shown to leave source pools more than 30‰ enriched relative to sink $\delta^{15}N$ (The difference between salmon tissue $\delta^{15}N$ and reported northwestern coastal vegetation $\delta^{15}N$ is at most around 20‰.) (Handley and Scrimgeour 1997, Hogberg 1997, Kendall 1998). In soil, fractionation also occurs between nitrogen pools during nitrogen fixing, decomposition of dead organic matter, uptake and assimilation of nitrogen by microorganisms, ammonia volatilization, nitrification, denitrification, and leaching (Handley et al. 1999). In vascular land plants, fractionations of nitrogen isotopes may occur with internal allocations and remobilization, and losses from the plant (Handley and Scrimgeour 1997). Unique localized fractionation is common, resulting from variations in temperature, moisture, acidity, N concentration, and many other factors (Handley and Scrimgeour 1997, Hogberg 1997, Neilson et al. 1998, Kendall 1998, Handley et al. 1999, Handley and Chang 2000, Robinson 2001) and it (the fractionation) may change with time (Helfield and Naiman 2001). Therefore, $\delta^{15}N$ of nitrogen from salmon in soil, plants, or other organisms may not match $\delta^{15}N$ of the original salmon.

Generally N sinks (vegetation in this case) are ^{15}N depleted relative to sources (Mariotti et al. 1981, Nadelhoffer and Fry 1988, Kendall 1998, Neilson et al. 1998). However, fractionation during N loss from soil could potentially leave soil $\delta^{15}N$ very enriched relative to salmon $\delta^{15}N$ (Hogberg 1997, Kendall 1998, Bronson et al. 1999) so that salmon-derived N in vegetation growing on that soil would have higher $\delta^{15}N$ than

$\delta^{15}N$ of N in salmon tissues. The magnitude of net fractionations between salmon tissue and salmon-N in plants or soil has not yet been reported for any site.

2. Delta^{15}N of a terrestrial-N source can be established, and it remains constant or changes predictably across the landscape. To estimate $\delta^{15}N$ of a terrestrial source it has been assumed that $\delta^{15}N$ measured in vegetation at a reference site far from spawning salmon represents $\delta^{15}N$ of all terrestrial-N, which is often incorrect. Under that assumption, terrestrial $\delta^{15}N$ is constant at similar sites, so it is the same at spawning and reference sites. Although much data is not available on isotopic variation between apparently similar riparian sites, the general unpredictability of N isotope fractionation indicates that assuming a constant terrestrial $\delta^{15}N$ level is incorrect.

Natural abundance of terrestrial ^{15}N is actually not constant within plants even of the same species, between species, or at different sites, and terrestrial $\delta^{15}N$ may change with time. Plant parts often differ in $\delta^{15}N$ as a result of internally fractionating processes so that $\delta^{15}N$ of leaves, for example, does not match $\delta^{15}N$ of roots (Handley and Scrimgeour 1997, Robinson 2001). New leaf $\delta^{15}N$ of non-nodulated soybeans exceeded (by about 2‰) $\delta^{15}N$ of applied fertilizer N, while roots of the same plants generally had lower (by about 2‰) $\delta^{15}N$ than N of the fertilizer (Bergersen et al. 1988). Lower $\delta^{15}N$ of roots than shoots was also found in non-N fixing Komatsuna plants (Yoneyama and Kaneko 1989), and root and shoot differences have been observed to vary unpredictably by as much as 5.2‰ in other plants (Handley, unpublished data cited in Neilson et al. 1998).

Delta^{15}N is not constant within plants of different species, even when they appear to be growing under the same conditions. Ben-David et al. (1998a) documented different δ^{15}N signatures in different plants growing in the same areas of Alaska, suggesting that some plants utilized MDN while others did not. We add that fractionation between or within soil or plant N pools could have changed δ^{15}N of MDN enough for it to be unrecognizable in some vegetation.

In fact, δ^{15}N varies even in the same parts of plants grown under the same conditions. Differences in δ^{15}N of 1.3‰ have been documented between individuals of the same species grown hydroponically on a source of known, constant δ^{15}N (1‰) (Robinson 2001). In terrestrial ecosystems δ^{15}N in plants and soil also typically represent N of several different molecular species and/or from several different sources, all of which have experienced potentially different ^{15}N fractionations. Delta^{15}N of the resulting mixture is unpredictable (Robinson 2001). If individuals of the same (or different) species obtain N from pools at different depths, from pools of different chemical composition (e.g., amino acids versus nitrate), or from pools separated in other ways, then δ^{15}N of a purely terrestrial source will not be the same in different individuals (Nadelhoffer et al. 1996, Handley and Scrimgeour 1997). Since δ^{15}N has been shown to vary with soil depth and other environmental characteristics, regardless of MDN (Nadelhoffer and Fry 1988, Nadelhoffer et al. 1996, Handley and Scrimgeour 1997), non-uniform rooting depths, or utilization of different N pools may lead to changes in δ^{15}N between individual plants that falsely appears to be a difference in MDN (Nadelhoffer et al. 1996, Handley and Scrimgeour 1997, Robinson 2001). For example,

Juniper (*Juniperus communis*) foliage from a single site in Scotland varied up to 11‰ between individuals (Hill et al. 1996). In another study, $\delta^{15}N$ of individual grain plants (*Triticum aestivum*) grown in a field in Saskatchewan Canada, differed by as much as 2‰ when separated by only 2 m, indicating that $\delta^{15}N$ variability can be quite high even between the same species at the same sites (Sutherland et al. 1991).

Another serious problem with determination of a terrestrial $\delta^{15}N$ signature is that $\delta^{15}N$ may not be the same at spawning sites and reference sites even when salmon are not present. So many factors influence apparent isotope enrichment that a constant $\delta^{15}N$ signature of N from any source throughout an area is not assured. Currently it is impossible to predict the magnitude or extent of fractionations that change $\delta^{15}N$ (Handley and Scrimgeour 1997, Kendall 1998, Robinson 2001). Therefore it is difficult to establish a set of reference samples that reliably represent $\delta^{15}N$ of the terrestrial source across sites. In fact, spatial differences in terrestrial $\delta^{15}N$ can be large enough to mask or mimic $\delta^{15}N$ differences due to MDN inputs. For instance, Garten (1993) reported a decrease in $\delta^{15}N$ of about 3‰ in foliage of red maple (*Acer rubrum*) and dogwood (*Cornus florida*) with increasing elevation and dryness in Tennessee, where no salmon spawn. Nadelhoffer et al. (1996), found about 2‰ difference in individuals of a single sedge species collected at 10 tundra sites along a 600-km transect in Alaska. In addition to spatial differences in $\delta^{15}N$, temporal differences in fractionation, and/or historical MDN inputs that are currently not recognized, may create $\delta^{15}N$ of a reference site that is not representative of all terrestrial $\delta^{15}N$ in other areas.

The evidence for terrestrial $\delta^{15}N$ differences in vegetation from the same and different sites appears to create uncertainty in TEM estimates of at least +/- 2‰. However, differences in total soil $\delta^{15}N$ between sites are often lower than differences in $\delta^{15}N$ of terrestrial foliage (Garten 1993, Hogberg 1997). To date, $\delta^{15}N$ of terrestrial soil near spawning streams has not been reported, nor have isotopic relationships between these soils and vegetation been adequately described.

3. Delta^{15}N of vegetation samples near a spawning stream represents a mixture of two distinct N sources that retain their $\delta^{15}N$ signatures during mixing. To calculate the percentage of MDN in a sample thought to contain salmon-N, researchers have assumed that $\delta^{15}N$ of a sample represents only a mixture of N from two sources. Under this assumption, $\delta^{15}N$ of vegetation containing some salmon-derived N must be discernibly different from $\delta^{15}N$ of vegetation with no salmon-derived nitrogen or containing only salmon-derived nitrogen.

Actually, since $\delta^{15}N$ of vegetation samples can reflect $\delta^{15}N$ of N from several pools, samples collected near spawning sites do not necessarily reflect only a single terrestrial-N source combined with MDN. For example, as mentioned above, $\delta^{15}N$ has been shown to vary with soil depth and other environmental characteristics, regardless of MDN (Nadelhoffer and Fry 1988, Nadelhoffer et al.1996, Handley and Scrimgeour 1997). When different quantities of N are obtained from these different pools and/or the N undergoes fractionation, $\delta^{15}N$ differences between marine and terrestrial N sources may not be discernible in samples. The unpredictable masking effect of N mixing and fractionation has been called the "Achilles heel of natural ^{15}N tracer approaches"

(Robinson 2001) because it leads to uncertainty about what $\delta^{15}N$ measurements mean. In addition, high MDN inputs may even lead to an increase in $\delta^{15}N$ variability in vegetation. Ben-David et al. (1998b) explained higher than normal $\delta^{15}N$ differences within and between vegetation species from river otter's latrine sites as a reflection of patchy deposition of MDN and possibly the different distribution of roots. They speculated that plants obtained N from different pools or in different amounts at otter latrine sites compared to non-latrine sites.

Fractionation can even result in $\delta^{15}N$ signatures higher in reference (non-salmon) areas than in areas expected to contain MDN, and/or in $\delta^{15}N$ signatures higher in vegetation or soil than in salmon (Lajtha and Marshall 1994). For example, in another study at a spawning site near the Koeye River we measured $\delta^{15}N$ in soil and vegetation 1‰ to 5‰ higher than $\delta^{15}N$ of pink salmon that spawned in the Koeye River (salmon tissue $\delta^{15}N = 12.40‰$) (see next chapter). If fractionation(s) or other factors have led to reference $\delta^{15}N$ exceeding $\delta^{15}N$ of target samples (samples of presumed MDN and terrestrial N mixture) then we calculate negative MDN. If target sample $\delta^{15}N$ exceeds $\delta^{15}N$ of the marine endmember (typically assumed to be $\delta^{15}N$ of salmon tissue) then mixing model calculations result in estimation of greater than 100% MDN. Conditions leading to the calculation of less than zero or more than 100% MDN illustrate the wide range of effects of ^{15}N natural abundance on MDN calculations. When $\delta^{15}N$ of samples results in calculation of negative or more than 100% MDN it is obvious that fractionation or N mixing has created conditions that make at least one assumption of the mixing model inaccurate. Similar fractionating or mixing could occur at lower levels in

other samples although the elevated $\delta^{15}N$ would only be attributed to MDN if it didn't cause MDN estimates that were obviously too large or too small. The magnitude of these effects cannot be predicted at this time, but even relatively small differences in source and sample $\delta^{15}N$ values may result in quite different MDN estimates.

Marine-derived N calculations are most sensitive to the actual $\delta^{15}N$ (or other isotope ratio) separation of sources so that for a given amount of variability in measurements, the uncertainty increases as $\delta^{15}N$ of sources becomes more similar (Phillips and Gregg 2001). In simulations, doubling the difference between sources reduced uncertainty by half (Phillips and Gregg 2001). The sensitivity of mixing model calculations to the $\delta^{15}N$ difference between sources shows that changes in the isotopic value of marine or terrestrial endmembers seriously affect MDN estimates. The largest reported difference in $\delta^{15}N$ between endmember $\delta^{15}N$ values was 19.7‰ (Hilderbrand et al. 1999a). A change of 1‰ in their terrestrial endmember would change the MDN estimate by about 4%, and a change of 1‰ in their marine endmember would lead to a change of about 1% MDN. Other researchers reported a smaller $\delta^{15}N$ difference between sources, which would cause greater changes in their MDN estimates if they changed their endmember values, but their estimates would still show less than an 8% change in MDN per 1‰ change in $\delta^{15}N$ of either endmember.

Marine-derived N calculations are also sensitive to the relative mathematical $\delta^{15}N$ distance from the source to the mixture. When the sample's $\delta^{15}N$ is close to the terrestrial endmember's $\delta^{15}N$, changes in $\delta^{15}N$ of the terrestrial endmember affect MDN estimates more than changes in $\delta^{15}N$ of the marine endmember, and vice versa. All

MDN calculations we located were most sensitive to changes in $\delta^{15}N$ of the terrestrial source because $\delta^{15}N$ of vegetation near spawning streams was much closer to the terrestrial source than to the marine source (about 3‰ difference between sample $\delta^{15}N$ (SAM) and terrestrial source $\delta^{15}N$ (TEM); Bilby et al. 1996, Hilderbrand et al. 1999a, Helfield and Naiman 2001). Therefore, a 3‰ error in determining $\delta^{15}N$ of the target sample (SAM) or terrestrial source (TEM) could produce absolute error in MDN estimates of more than 12%.

Other researchers have recognized the potential for error in quantifying MDN. Helfield and Naiman (2001) reported MDN ranges (mean +/- about 10%), and also wrote that $\delta^{15}N$ signatures may reflect long term rather than current MDN inputs. Ben-David et al. (1998b) measured decreasing $\delta^{15}N$ away from salmon spawning rivers and interpreted that as evidence of a diminution of MDN with distance from the rivers, but did not quantify MDN because of the potential for $\delta^{15}N$ variation with changing elevation and wetness. Ben-David et al. (1998b) suggested that MDN calculations are better used as an index of relative MDN contributions rather than as an absolute measure of salmon N. These researchers wrote that quantitative MDN estimates depend on additional information about site-specific fractionation rates.

To aid in evaluating N and C isotopes as quantitative MDN tracers in northern forests we undertook a field study in British Columbia, Canada, to measure changes in $\delta^{15}N$ between salmon, bear feces, soil, and vegetation. We also used a reiterative spreadsheet design to model soil $\delta^{15}N$ changes resulting from fractionation of N due to ^{15}N discriminating N losses. The objective of our study was to determine levels of

fractionation in some N pools affected by salmon under field conditions in the northwestern Pacific rainforest, and also to determine the extent to which fractionation could affect MDN estimates.

Methods

Measurement of Change in Marine-Derived Nitrogen $\delta^{15}N$

Study Sites

We evaluated changes in $\delta^{15}N$ of MDN using samples from two different rivers in British Columbia, Canada: the Koeye River, and Glendale River, both on the mainland. Both rivers supported runs of 20,000 or more pink salmon (*Oncorhynchus gorbuscha*), which were preyed on by 3 to 25 brown bears in the areas from which we collected samples (about 2 km along each river).

The Koeye River Watershed (51° 46' N 127° 53' W) is one of the least disturbed areas of coastal temperate rainforest in British Columbia, Canada. Recently protected by federal agreement, the Koeye was described by the British Columbia Land Use Coordination Office (LUCO 1999) as having an unusually productive forest resulting in high biological diversity, grizzly bear habitat, and salmonid values. Mean annual rainfall at the Koeye River exceeds 300 cm. Western hemlock (*Tsuga heterophylla*), coastal Douglas fir (*Pseudotsuga menziesii*), western redcedar (*Thuja plicata*), Sitka spruce (*Picea sitchensis*), and yellow-cedar (*Chamaecyparis nootkatensis*) predominate in the forest, with an understory of salal (*Gaultheria shallon*), salmonberry (*Rubus spectabilis)*, and fern (*Blechnum splicant*). Alder (*Aldus rubra*) was not common on the

lower Koeye River where we established transects, although it can be found in the estuary. Moss (probably *Rhytidiadephus loreus, Hylocomium splendens,* and/or *Kindbergia oregana)* was common on the forest floor and on many structures throughout the study area.

The Glendale River's forest is similar to the Koeye River forest except that there has been a greater human presence around Knight Inlet where the Glendale River is found, including logging, tourism, and commercial fishing. Near the mouth of Glendale River an artificial spawning channel has been constructed for use by pink salmon. The embankments of this channel were constructed from light-colored, sandy, gravelly soil. Grass grows on all the embankments. Alders have grown in many places, especially on embankments where vehicles do not travel. When we did our study brown bears had congregated to feed on pink salmon below a weir that controls the number of salmon allowed into the channel. Most of our samples were collected along the sides of a vehicle track along the top of one embankment.

Sample Collection: Salmon, Feces, and Soil

Since $\delta^{15}N$ of 100% MDN has not been directly measured in soil and vegetation under field conditions we attempted to measure fractionation relationships that would allow determination of $\delta^{15}N$ of 100% salmon-N in terrestrial soil or plants. We attempted to quantify how much isotope fractionation actually occurs in the field setting as nitrogen and carbon move from salmon to feces, from feces to soil, from soil to vegetation, and then within vegetation. Based on these relationships $\delta^{15}N$ of a marine-N source can be calculated in the sample material of choice for use in MDN estimates.

To determine fractionation between salmon and bear feces we compared $\delta^{15}N$ of pink salmon tissue to brown bear feces. We collected tissue samples of three spawned male pink salmon from the Koeye River in the fall of 1997. We also obtained samples of one female and one male pink salmon from Glendale River in the fall of 2001. Total N, total C, and their isotopic enrichments in salmon from the Koeye River were compared to fresh (less than 24 hr old) feces we sampled at the Glendale River.

To determine fractionation between bear feces and soil we compared total N, total C, and their isotopic enrichments underlying soil while feces decomposed (Fig. 1). Changes in N, C, $\delta^{15}N$, and $\delta^{13}C$ were noted during decomposition by analyzing samples collected on the first day and then again after 5 d. Reference samples were obtained from soil one meter away from each fecal pile. We used a stainless steel trowel to collect fecal and soil samples. After a portion of feces was placed in a plastic zip-shut bag, we removed a portion of soil from directly under where we had gathered the fecal sample. Another soil sample was collected of about the same volume and depth from a reference area one meter away from the feces. We chose reference locations so that they represented characteristics of soil under the corresponding feces (same groundcover, slope, and soil appearance). We chose reference sites so that no other feces were visible near the non-feces sample locations. Repeat samples were collected from the same fecal piles, but not touching other sampling holes.

To provide information about fractionation between soil and vegetation we collected soil samples together with vegetation samples at the Koeye River. Samples were collected in the spring and fall along transects that we established across bear trails and beds as part of another study (see next chapter). We sampled soil by driving a 5-cm

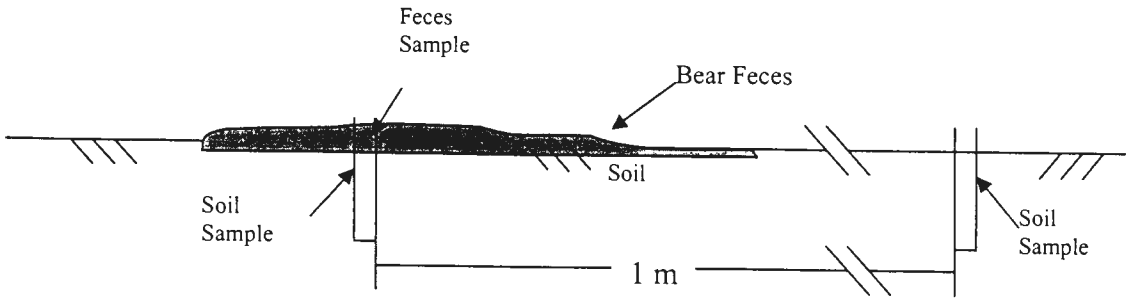

FIG. 1. Fecal and Soil Sampling.

diameter stainless steel corer into the soil to a depth of 15 cm. Moss and litter were removed prior to sampling. Leaves or leaf pieces were collected from one or more species of plants growing within 0.5 m of soil samples. At each sampling location we collected parts of leaves or whole leaves from false lily of the valley (*Maianthemum dilatatum*), bunchberry (*Cornus canadensis*), salal (*Gaultheria shallon*), and salmonberry (*Rubus spectabilis*) as they were available.

To consider $\delta^{15}N$ differences within plants we compared samples of leaf tips to leaf bases. Leaf tip samples were from the distal half of the leaf. Leaf base samples came from the proximal half of the leaf (closest to the stem). We also compared $\delta^{15}N$ and total N in leaves, shoots and roots from four plants of two species (salmonberry and false lily of the valley) at one location at the Koeye River.

Sample Handling and Analysis

We measured total N, $\delta^{15}N$, total C, and $\delta^{13}C$ in salmon, feces, soil, and most vegetation samples (some vegetation samples were only analyzed for total N and $\delta^{15}N$) by continuous-flow direct combustion and mass spectrometry using a Europa Scientific SL2020 system (PDZ Europa, Cheshire, UK).

Salmon pieces were frozen after collection, and remained frozen until they were freeze-dried in the Utah State University Laboratory. After freeze-drying, skin, bones, and flesh were separated. Bones and flesh were crushed with mortar and pestle. Intermuscular fascia did not crush easily and was separated from other muscle tissue for separate analysis in four samples from fish collected in the fall of 2001. Skin samples were not crushed, but cut into fine pieces with a stainless steel scalpel. Subsamples of 2 to 6 μg of each material were weighed into 8 mm by 5 mm tin (Sn) capsules for mass spectrometric analysis.

Soil and feces from the Glendale River were frozen in zip-shut plastic bags within 3 hr of collection and kept frozen until processed in the laboratory, where they were thawed at 5° C for approximately 24 hr. Soil samples were then homogenized by hand in the same bags while wearing clean latex gloves. Fecal samples were kneaded while still in their closed bags. A portion of each sample was placed in a glass jar and oven dried at 70° C for at least 48 hr. The dried samples were then crushed by grinding with steel roller bars for at least 24 hr. After crushing, soil was weighed (1-10 ug) into tin 8 mm by 5 mm tin capsules for N and C analysis.

Soil samples from the Koeye River were also frozen in zip-shut plastic bags prior to analysis, although some remained cool but unfrozen during transport from the field to the laboratory. Just before analysis soil samples were thawed at 5° C for approximately 24 hr, then homogenized, and prepared for analysis as described for soil from the Glendale River.

Vegetation samples were folded into #40 Whatman filter paper at each sampling location, and then slipped into the pouches of a plastic slide sheet or a plastic zip-shut

bag. Samples were kept cool or frozen until drying at the Utah State University Laboratory. Unfrozen plant samples were oven dried at 70°C for at least 48 hr. Frozen samples were freeze-dried for at least 24 hr. Most dried vegetation samples were crushed and placed directly into 8-mm by 5-mm tin capsules or 24-mm diameter tin disks. Some stems and roots were first crushed with mortar and pestle, and then weighed into tin capsules.

Data Analysis: Calculation of Fractionation

To determine fractionation between salmon and feces we compared $\delta^{15}N$ of salmon to $\delta^{15}N$ of feces. To determine fractionation between feces and soil we compared $\delta^{15}N$ in feces with $\delta^{15}N$ of N entering the soil from fecal decomposition. The amount of N (and ^{15}N) entering the soil from fecal decomposition was calculated from the change in total soil N (and $\delta^{15}N$) beneath the fecal material during the 5-d period, after correcting for background changes in total N (and $\delta^{15}N$) that were unrelated to fecal decomposition. Background change in N was measured as the change of N in soil one meter away from the feces from the first day to the last day. We assumed that whatever happened in the soil one meter away from feces also happened in soil under the feces. We used the following equation to calculate ^{15}N enrichment of salmon-derived nitrogen entering the soil from fecal decomposition:

$$A_{new} = (N_{B0}A_{B0} - N_{A0}A_{A0} - (N_{B1}A_{B1} - N_{A1}A_{A1}))/(N_{B0} - N_{A0} - (N_{B1} - N_{A1}))$$

where A is the atom % ^{15}N, and N is total nitrogen (grams total N per gram of dry soil). We used a two-letter code in the subscript to differentiate soil samples. The first letter in the subscript indicates the sampling time: A is the beginning (1^{st} day), B is the end (5^{th}

day). The second letter is the spatial location: 0 is directly under feces, 1 is one meter away. Atom % ^{15}N (A), multiplied by the grams total N per gram of dry soil (N), gives the grams of ^{15}N per gram of oven dry soil. The term $N_{A0}A_{A0}$ indicates ^{15}N in the original soil pool. $N_{B0}A_{B0}$ indicates ^{15}N in the final pool after some is lost and/or gained. The difference between the final and starting pools in soil away from feces, ($N_{B1}A_{B1}$-$N_{A1}A_{A1}$), is our estimate for change of total nitrogen independent of the feces. We used the same process, substituting total C for total N and $\delta^{13}C$ for $\delta^{15}N$ to calculate ^{13}C fractionation between feces and soil.

Although we did not measure urine-N, fractionation between urine-N and soil-N is important if urine contains the majority of excreted salmon-derived N. Hilderbrand et al. (1999a, 1999b) estimated that an average female brown bear excreted 96% of its MDN intake as urine, 3% as feces, and 1% was assimilated. We calculated $\delta^{15}N$ of the urine-N by assigning our measured values for fecal $\delta^{15}N$ to the 3% excreted as feces, and assuming that $\delta^{15}N$ of the 1% assimilated N increased 3‰ relative to salmon (Handley and Scrimgeour 1997, Kelly 2000). That means that $\delta^{15}N$ of urine N had to be low enough to compensate for elevated $\delta^{15}N$ in bears' feces and muscles, so $\delta^{15}N$ of the total N in urine, feces, and muscle would equal $\delta^{15}N$ of salmon tissue.

To determine fractionation between soil and vegetation we compared $\delta^{15}N$ in vegetation and $\delta^{15}N$ in soil within 0.5 m of the soil samples. To evaluate fractionation between locations on plants we compared $\delta^{15}N$ in leaf tips to $\delta^{15}N$ in leaf bases, as well as $\delta^{15}N$ in leaves compared to $\delta^{15}N$ in stems and roots.

Marine-Derived Nitrogen Calculations

We calculated MDN using a two-source, linear mixing model after Kline et al. (1990, 1993). Marine and terrestrial source signatures were calculated using the regression relationship between soil and false lily of the valley. We chose to use false lily of the valley for these calculations because it had the widest range of $\delta^{15}N$, including samples from soil that had $\delta^{15}N$ less than 0‰ up to soil with $\delta^{15}N$ greater than salmon $\delta^{15}N$. Delta^{15}N of leaves representing 100% terrestrial nitrogen (0% MDN) was measured at a reference site at the Koeye River where no bear activity was likely and none was evident. Bears probably did not use the reference area very much because salmon were not easily accessible in the adjacent river and bears did not need to pass through the reference area to go from one good fishing spot to another. We compared MDN estimates made with two different values for the marine source $\delta^{15}N$ signature (MEM): (1) Leaves containing 100% MDN were assumed to have $\delta^{15}N$ equal to $\delta^{15}N$ of salmon tissue (MEM_{salm}); and (2) Leaves containing 100% MDN were assumed to have $\delta^{15}N$ of salmon-N adjusted for fractionation occurring as the N moved through bears and into the soil (MEM_{soil}). As part of the spreadsheet model analysis, reported below, we also computed MDN estimates for soil, assuming that the ^{15}N difference between reference and spawning sites represented ^{15}N only from salmon (MEM_{diff}).

Statistical Analysis

Relationships between salmon, bear feces, soil, and plant parts were evaluated using paired t-tests or ANOVA. The relationship between soil $\delta^{15}N$ and leaf $\delta^{15}N$, and differences in this relationship among species, were assessed using a general linear

model of leaf $\delta^{15}N$, including as explanatory variables soil $\delta^{15}N$ on a continuous scale, species on a categorical scale, and the interaction of these two factors. Essentially, the statistical model fit a separate regression line for each species, and permitted statistical comparison of regression coefficient estimates among species. Pairwise comparisons of slope estimates were made using contrasts within the full model. Computations were done using PROC MIXED in SAS/STAT.

Spreadsheet Model

To evaluate the effects of fractionation and input variations on MDN calculations we simulated a simple system using an iterative spreadsheet model. Soil $\delta^{15}N$ without MDN (i.e., "reference site") was compared to soil $\delta^{15}N$ with MDN inputs (i.e., "spawning site"). We modeled total soil ^{14}N and ^{15}N concentrations assuming that there were two possible N inputs and one output (Fig. 2). One input was salmon-derived nitrogen which was set to zero to simulate reference sites, or set at 0.72 kg N/ha/yr to simulate spawning sites, matching numerical estimates from Hilderbrand et al. (1999a) for MDN dispersed by brown bears. We assumed the second source of N to be a combination of atmospheric deposition and N-fixation, set at 2.14 kg/ha/yr for both reference and spawning sites, based on values from Hilderbrand et al. (1999a). Total N output was first order, with the first order rate constant initially set at 0.00025 yr^{-1}. Total N output was intended to represent the sum of processes of N loss, such as denitrification and ammonia volatilization. We modeled ^{15}N and ^{14}N using separate submodels. Assigning ^{15}N a different rate constant represented fractionation. The ratio

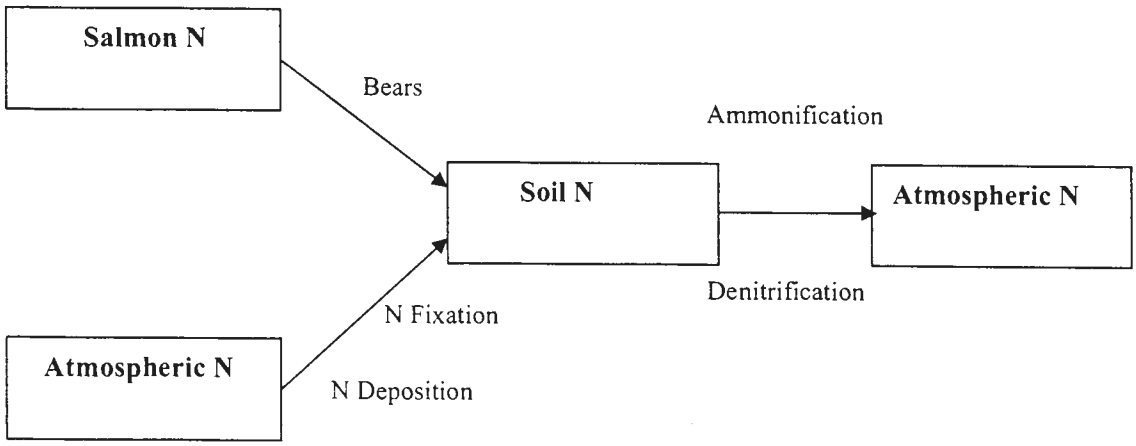

FIG. 2. Schematic of simplified nitrogen flow for spreadsheet model. Nitrogen input to soil was constant, N output from soil was first order. We modeled ^{14}N and ^{15}N separately. Fractionation was represented by assigning ^{14}N and ^{15}N different output rates.

of ^{14}N to ^{15}N output rate constants we denoted as β, the fractionation factor (i.e., $\beta=K_{14N}/K_{15N}$ where K is the rate constant). Using a value of β greater than one left the soil pool ^{15}N enriched. Different fractionation factors were applied to the output so we could evaluate the effects of fractionation on steady state $\delta^{15}N$ and determine the error introduced in MDN mixing model calculations. We chose N-loss fractionation rates that were theoretically realistic based on existing literature (Heaton 1986, Garten 1992, Lajtha and Marshall 1994, Handley et al. 1999, Nadelhoffer et al. 1999). For example, we set fractionation rates in the range reported for denitrification ($\beta<1.04$ for our model; Hogberg 1997, Kendall 1998).

Using yearly time steps we ran the model with a certain fractionation rate until it reached steady state, at which point N outputs equaled total N inputs. Then we calculated %MDN using the two-source linear mixing model equation described earlier. The terrestrial source $\delta^{15}N$ was represented by the total $\delta^{15}N$ predicted by the model for

the "reference" soil, with zero marine-N inputs. We evaluated the accuracy of MDN estimates by using two different $\delta^{15}N$ estimates for the marine-N source. First we used $\delta^{15}N$ of salmon tissue as the marine source signature (MEM_{salm}), and secondly, we used $\delta^{15}N$ of salmon-N as it actually appeared in the soil N pool (MEM_{soil}). Delta^{15}N of salmon was determined from pink salmon collected at the Koeye River ($\delta^{15}N$ = 12.40‰; mean N for bones, skin, flesh, and brains). Nitrogen-15 enrichment of MEM_{diff} was calculated by subtracting soil ^{15}N and ^{14}N predicted by the model for the "reference site" from the soil ^{15}N and ^{14}N predicted by the model for the "spawning site."

We also evaluated the temporal stability of MDN estimates. After the soil $\delta^{15}N$ reached steady state we reduced salmon inputs by half (0.36 kg N/ha/yr rather than 0.72 kg N/ha/yr) and ran the model to evaluate the effects of residual salmon-N on soil $\delta^{15}N$.

To determine whether the variability of MEM_{diff} values calculated from real samples would be small enough for reliable MDN estimates, we evaluated the sensitivity of MDN estimates to MEM_{diff} variability. We allowed $\delta^{15}N$ and total N (units = g N/g dry soil) of the "spawning site" to vary while holding $\delta^{15}N$ and total N of the reference site constant, and vice versa. We also calculated MDN while allowing $\delta^{15}N$ and total N to vary at both spawning and reference sites. To vary $\delta^{15}N$ and total N for a parameter within realistic bounds, we generated 5,000 random values for $\delta^{15}N$ and total N from normal distributions with means and SD's equal to the means and SD's of soil samples collected as part of another project near the Koeye River (see next chapter). Delta^{15}N, total N, and their SD's for samples from the spawning site (SAM) were obtained by averaging soil samples collected from bear trails and beds along the Koeye River where

brown bears were feeding on spawning salmon. Delta^{15}N, total N, and their SD's for the terrestrial endmember (TEM) were obtained from a reference site at the Koeye River where bear activity was minimal due to the lack of good fishing sites, and which was not between feeding areas where bears regularly traveled (Table 2).

After generating 5,000 random values from the spawning site, we calculated ^{15}N and ^{14}N pools in each sample by breaking down total N into its constituent isotopic pools. Namely we calculated atom% ^{15}N from δ^{15}N and multiplied that by total N to find the total ^{15}N pool. Then we subtracted ^{15}N from total N to find the amount of ^{14}N present in the sample. By subtracting ^{15}N and ^{14}N at the reference area from ^{15}N and ^{14}N found in samples from the spawning site we found the amount of ^{15}N and ^{14}N in each sample that was not present at the reference area. Assuming that ^{15}N and ^{14}N not found in the reference area comprised salmon-N in the soil after fractionation, we used the ratio between ^{15}N and ^{14}N ($R = {}^{15}N/{}^{14}N$) to calculate δ^{15}N, which is MEM_{diff}. We followed a similar procedure to calculate MDN while SAM varied and TEM was fixed. Finally we calculated MDN while allowing both SAM and TEM to vary.

TABLE 2. Nitrogen data used for calculation of MEM_{diff}.

	Total N (g N/ g soil)	SD	δ^{15}N (‰)	SD
Reference Site (TEM)	0.0109	0.0013	-0.07	0.92
Spawning Site (SAM)	0.0134	0.0029	5.21	4.35

Results

Measurement of Change in Marine-Derived Nitrogen $\delta^{15}N$

Flesh, skin, and brain (the salmon parts consumed by bears) of three pink salmon collected in 1998 from the Koeye River averaged +12.40‰ (SE = 0.49). This is the value we used for all subsequent MDN and fractionation calculations. Delta^{15}N of pink salmon from the Koeye River was not substantially different from $\delta^{15}N$ of the same tissue from two pink salmon collected in 2001 from Glendale River (+12.45‰, SE = 0.26, n = 2). There was also no difference ($P < 0.05$) in $\delta^{15}N$ between brain, flesh, or skin within salmon. Delta^{15}N of intermuscular fascia was significantly lower than $\delta^{15}N$ of other muscle tissue (by about 1‰, $P < 0.001$, n = 4), but when averaged the total muscle $\delta^{15}N$ (fascia plus other tissue) was not significantly different from brain or skin. Average $\delta^{13}C$ for flesh, skin, and brain differed by about 2‰ between salmon collected from the Koeye River (–20.02‰, SE = 0.66, n = 3) and those collected at Glendale River (-22.54‰; SE = 0.54‰, n = 2).

Average fecal ^{15}N enrichment for 13 feces at Knight Inlet was +14.14‰ (SE = 0.18‰). Feces were significantly ($P < 0.001$) ^{15}N enriched by about 2‰ relative to pink salmon bodies (α = 1.14; α as used here denotes $\delta^{15}N$product/$\delta^{15}N$source; 14.14/12.40). Based on the assumption that brown bear bodies were 3‰ greater than salmon tissue (Handley and Scrimgeour 1997, Kelly 2000), and using Hilderbrand et al. (1999a) estimates of relative N excretion and assimilation rates (3% excreted as feces, 1% assimilated as muscle, 96% excreted as urine), we estimated that brown bear urine was

less than 0.20‰ $\delta^{15}N$ depleted relative to salmon $\delta^{15}N$ (urine $\delta^{15}N$ was +12.29‰, salmon $\delta^{15}N$ was +12.40‰).

All of the feces we sampled changed appearance and decreased in size during the 5-d sampling period. Two of the fecal piles disintegrated completely, leaving only dark marks. It rained twice during the five days. We calculated ^{15}N enrichment from feces entering the soil using the equation for A_{new} described above. Two of the 8 feces we tracked through decomposition yielded very unusual effective $\delta^{15}N$ values (+49‰ and -83‰) so they were excluded from fractionation calculations. Converting A_{new} to $\delta^{15}N$ gave a value of +13.96‰ (SE = 1.13‰) entering the soil from feces, compared to +14.33‰ (SE = 0.34‰) measured in fecal material. A *t*-test indicated this soil to feces difference (0.37‰) was not significant ($P > 0.40$, $n = 6$). Therefore no fractionation was detectable between feces and soil.

Stable isotopes of carbon fractionated during digestion and during fecal to soil transfer. Feces were 3‰ depleted relative to salmon tissue ($\alpha = 1.16$). Fecal $\delta^{13}C$ was -23.22‰ (SE = 0.25‰, $n = 13$) as opposed to –20.02‰ (SE = 0.66‰, $n = 3$) in salmon. Further fractionation occurred as feces decomposed into the soil. Delta^{13}C of feces as it appeared in the soil was 4.15‰ depleted relative to fresh fecal $\delta^{13}C$ ($\alpha = 1.18$). Salmon $\delta^{13}C$ as it appeared in the soil was –27.37‰ (SE = 0.40‰, $n = 6$) as opposed to -23.22‰ (SE = 0.38‰) for the 6 feces tracked during decomposition. Delta^{13}C differences between salmon and feces were statistically significant, as were $\delta^{13}C$ differences between feces and soil (*t*-test, $P < 0.01$, Fig. 3).

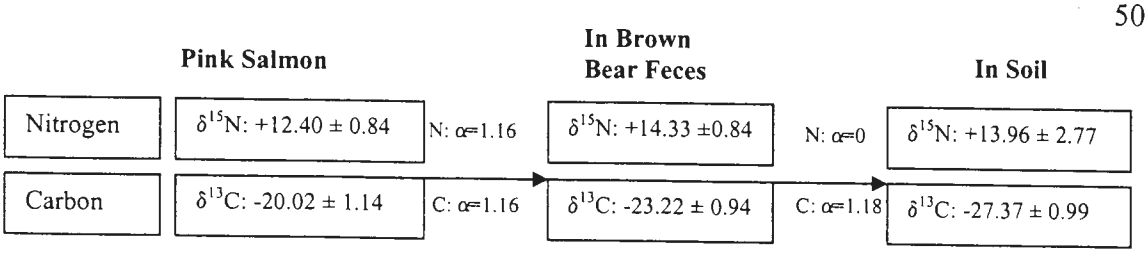

FIG. 3. ^{15}N and ^{13}C enrichment: salmon to feces to soil.
Delta^{15}N Mean +/- 1 standard deviation, $\alpha = (\delta$ product$)/(\delta$ source).

Generally, leaf $\delta^{15}N$ was less than total soil $\delta^{15}N$ in the 0-10 cm soil layer from within 0.5 m of the plant. The magnitude of the difference depended on the species and the soil $\delta^{15}N$ (Fig. 4). Nitrogen-15 enrichment of vegetation increased as $\delta^{15}N$ of total N in the nearby soil increased ($P < 0.001$). The slope of the linear relationship between leaf $\delta^{15}N$ and soil $\delta^{15}N$ differed among species (test of interaction between soil $\delta^{15}N$ and species, $P = 0.015$). The regression relationship between false lily of the valley and its underlying soil was: leaf $\delta^{15}N = -6.79 + 1.201 \cdot$soil $\delta^{15}N$ (SE = 0.17, $P < 0.001$). Pairwise comparisons of slopes among species indicated no apparent difference between salmonberry and false lily of the valley, or among salal, bunchberry, and salmonberry. The slope of false lily of the valley exceeded the slopes of salal and bunchberry.

Analysis of data from 18 plants (bunchberry $n = 12$, salal $n = 3$, false lily of the valley $n = 2$, salmonberry $n = 1$) using a two-way ANOVA showed no $\delta^{15}N$ difference ($P = 0.317$) between leaf tips and bases (Fig. 5). Data from three false lily of the valley plants and one salmonberry plant at a single location near the Koeye River indicated that leaves had $\delta^{15}N$ values 1.25‰ higher than roots ($P < 0.02$, $n = 4$).

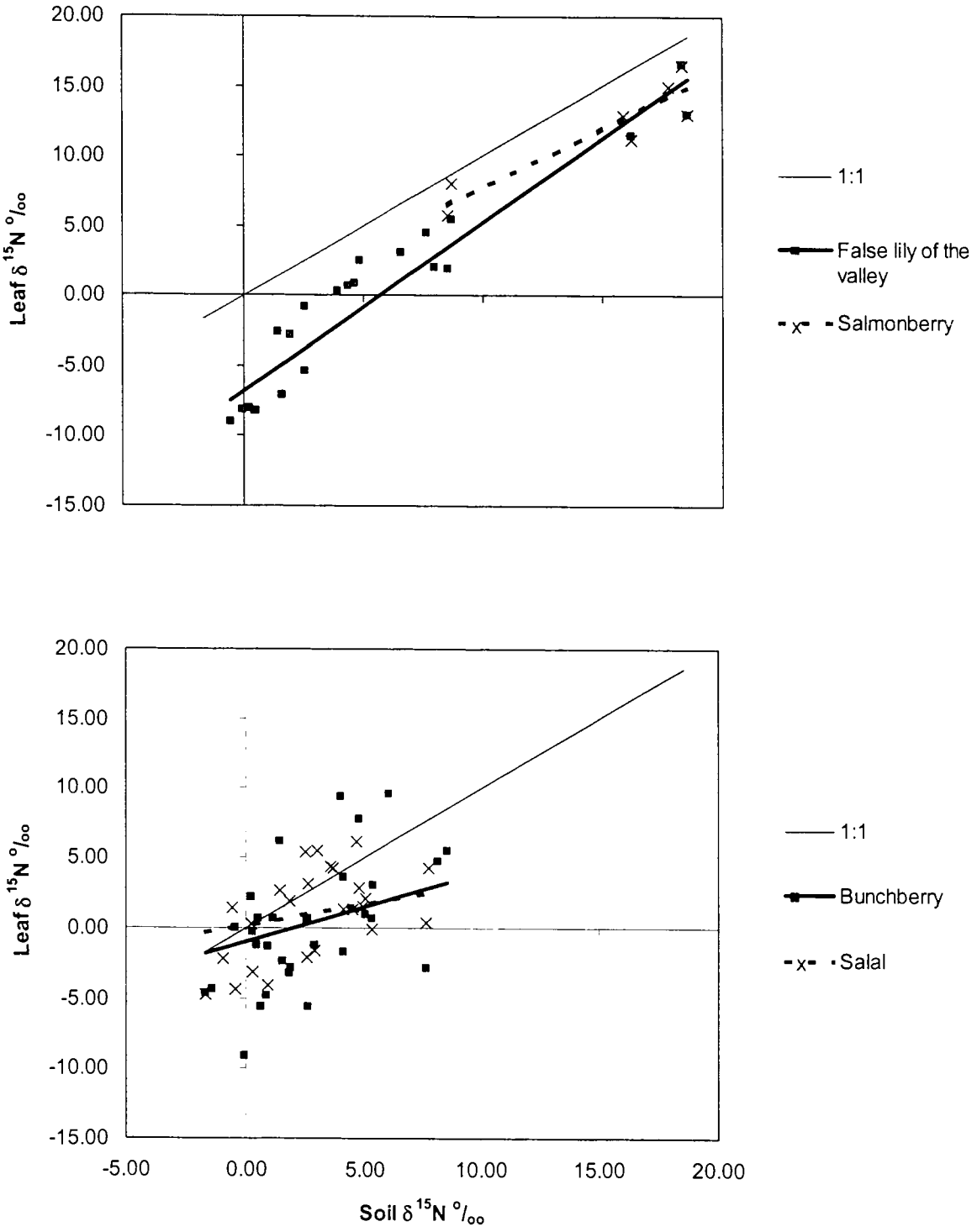

FIG. 4. Relationship between soil $\delta^{15}N$ and vegetation $\delta^{15}N$ at Koeye River.

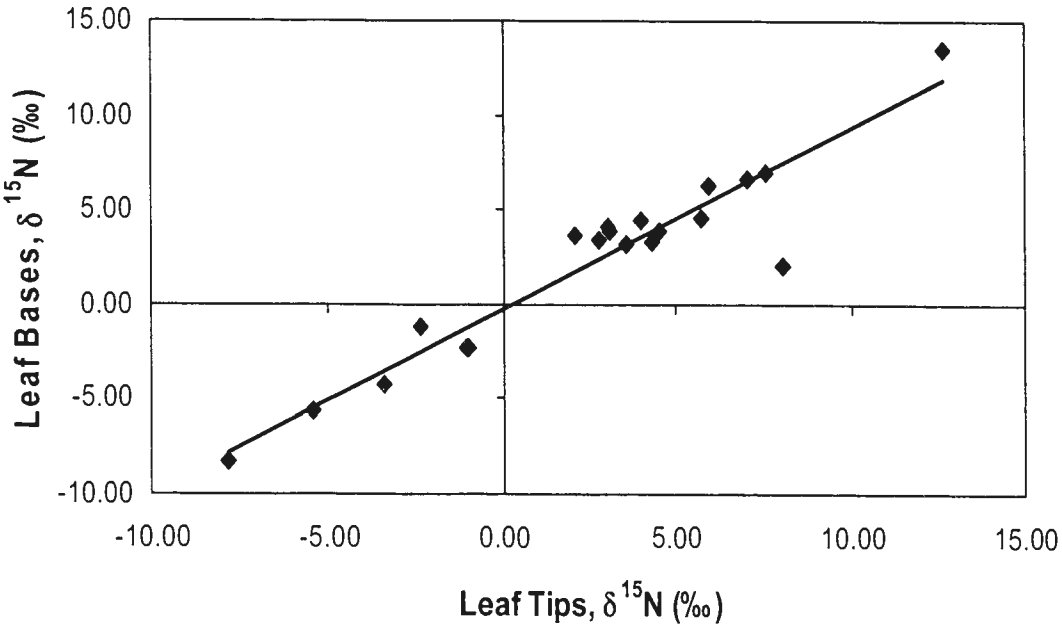

FIG. 5. ^{15}N Comparison Between Leaf Tips and Bases.
Base $\delta^{15}N = 0.9611 \cdot (\text{Tip } \delta^{15}N) - 0.2653$, $R^2 = 0.8992$

The terrestrial source $\delta^{15}N$ signature as it appeared in false lily of the valley at a reference site at the Koeye River was –8.10‰ (SE = 0.14‰, n = 4). The marine source $\delta^{15}N$ signature as it appeared in vegetation was estimated using two methods: (1) Delta^{15}N of pink salmon tissue ($\delta^{15}N$ = +12.40‰) represented the signature of the marine source; and (2) Delta^{15}N of salmon-N in the soil after fractionating through bear feces represented the signature of the marine source ($\delta^{15}N$ = +13.96‰).

The MDN estimates made with the assumption that MEM was equal to the $\delta^{15}N$ of salmon tissue were 7% higher (relative difference) than MDN estimates made after correcting for fractionation. The 7% relative difference between MDN estimates translates into an absolute difference in %MDN of 0% to about 7% depending on $\delta^{15}N$ of the target sample (SAM). For instance, when SAM was 0‰ our MDN

estimates using δ15N of salmon tissue and δ15N of salmon-N in soil under bear feces, were 40% and 37%, respectively. However, when SAM was +12‰ our MDN estimates were 98% and 91% (Fig. 6).

Spreadsheet Model

Mixing model calculations based on theoretical steady state $\delta^{15}N$ for "reference" and "spawning" sites accurately predicted true marine-derived N inputs when there was no fractionation. When N outputs were fractionated, the soil total N pool became so enriched that mixing model calculations overestimated MDN by as much as 77% when

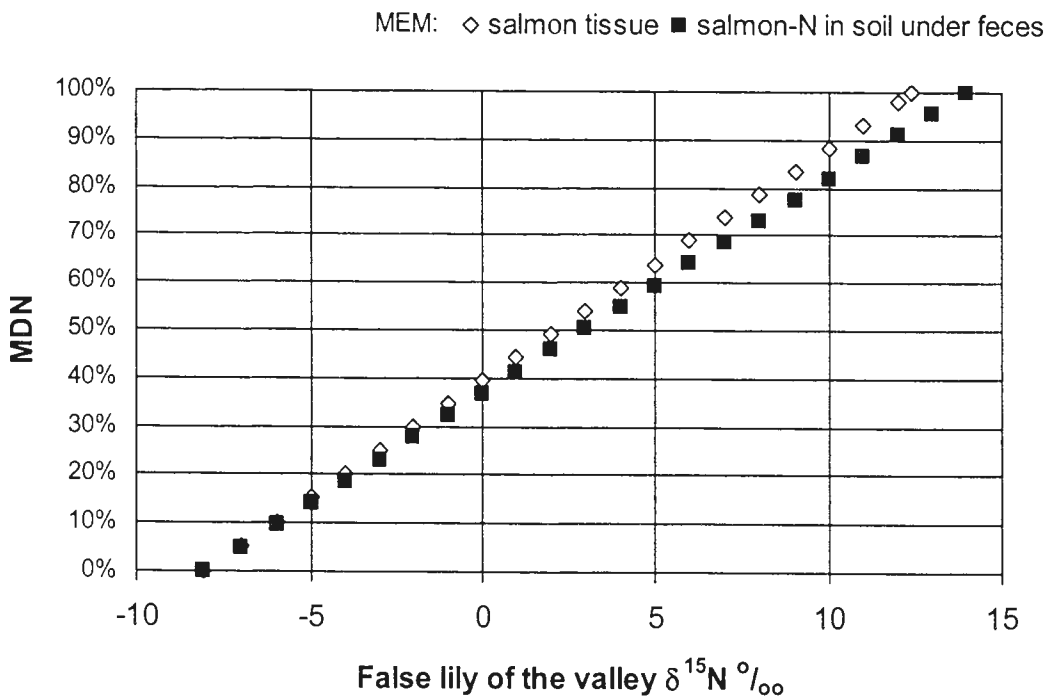

FIG. 6. Magnitude of differences between marine-derived nitrogen calculations for samples of various $\delta^{15}N$, using different marine source $\delta^{15}N$ signatures (MEM).

salmon $\delta^{15}N$ was used as the marine source (Table 3). Output fractionation at β of 1.02 resulted in $\delta^{15}N$ at spawning and reference sites exceeding $\delta^{15}N$ of salmon tissue so that MDN estimates were negative. When we used our estimate of MEM_{diff}, (i.e., $\delta^{15}N$ calculated from the ^{15}N and ^{14}N difference between "spawning" and "reference" sites) as $\delta^{15}N$ of the marine source, the mixing model calculations predicted the actual %MDN regardless of the fractionation constant.

TABLE 3. Results of spreadsheet model.

Output Fractionation β[1]	Calculated MDN[2]
1	25.1%[3]
1.005	38.1%
1.01	77.9%
1.02	-73.1%[4]

[1] The fractionation factor, β, is K_{14N}/K_{15N}, where K is the first order rate constant. β=1 indicates no fractionation.
[2] MDN calculation was based on a linear two-source mixing model with salmon $\delta^{15}N$ as the upper endmember.
[3] This is the actual MDN, i.e. the true relative proportion of marine-derived N to terrestrial N in the model.
[4] Negative MDN resulted from target and reference soil $\delta^{15}N$ exceeding salmon $\delta^{15}N$.

When we ran the model to steady state and then decreased salmon-inputs by half, persistent MDN led to 10%-25% overestimation of MDN, ($1 < \beta \leq 1.02$), even after 100 years (100 iterations with salmon inputs at 0.36 kg N/ha/yr). In other words, even though salmon input remained at a constant low level, residual ^{15}N in the soil kept $\delta^{15}N$ high enough that it appeared as if 10% to 20% more salmon-derived N was present.

Based on $\delta^{15}N$ and total N of soil samples from the Koeye River, MEM_{diff} was calculated and used to estimate MDN. The mean MDN estimate using MEM_{diff} as the marine endmember was 14% (both SAM and TEM varied) in contrast to an estimate of 42% (SD = 0.35%) MDN using $\delta^{15}N$ of salmon tissue (MEM_{salm}) as the marine endmember with the same values for TEM and SAM. Standard deviation of MDN estimates were 0.22% to 0.24% when either SAM varied ($\delta^{15}N$ SD = 4.35‰, total N SD = 0.0029 g N/ g soil) or both SAM and TEM varied (TEM: $\delta^{15}N$ SD = 0.92‰, total N SD = 0.0013 g N/ g soil; SAM same as above), or 0.10% when only TEM varied.

Discussion

Measurement of Change in Marine-Derived Nitrogen $\delta^{15}N$

The isotopic similarity we measured between pink salmon from two locations, collected on two different years suggests that, at least over the distance of several hundred kilometers and the period of several years, a distinct marine $\delta^{15}N$ signature exists for tissue from adult pink salmon. We found no difference in $\delta^{15}N$ between salmon collected in 1997 at the Koeye River, compared to salmon collected in 2001 at Glendale River. Delta^{13}C did appear to differ between salmon we collected from the Koeye and Glendale Rivers (by about 2‰), but the difference is small relative to fractionation of $\delta^{13}C$ between salmon tissue and total soil C.

Although reports of preferential feeding by bears on different salmon parts (Quinn and Kinnison 1999, Ruggerone et al. 2000, Gende et al. 2001) illustrate the potential for isotopic signatures to vary along a spawning river where different bears

feed and where salmon abundance varies, we found no significant $\delta^{15}N$ or $\delta^{13}C$ difference between brain, skin, or flesh. Therefore, preferential feeding on brain, skin, or flesh would not have changed $\delta^{15}N$ of salmon-N transported by bears along the two rivers where we collected samples.

Delta^{15}N of bears' salmon diets could have reflected $\delta^{15}N$ of salmon roe as much as it reflected $\delta^{15}N$ of salmon flesh at the Glendale River. We observed bears at Glendale River eating what seemed to be a high proportion of eggs relative to meat. We estimate that well over half of the fish attacked were female, and that most bears feeding in our view consumed more than 50% eggs. The effect on $\delta^{15}N$ of bears' N excretion due to an egg rich diet was probably not very great. As mentioned above, $\delta^{15}N$ of pink salmon roe from Alaska was only slightly ^{15}N enriched relative to flesh (average egg $\delta^{15}N$ was 0.67‰ greater than muscle $\delta^{15}N$, $n = 9$, SE = 0.29, $P < 0.01$; Ben-David unpublished data), so $\delta^{15}N$ in bears' diets from eggs would not have been appreciably different from $\delta^{15}N$ in bears' diet from salmon flesh (a 100% egg diet would have been 1.28‰ less than $\delta^{15}N$ of bear feces, as opposed to $\delta^{15}N$ of salmon tissue 1.95‰ less than feces' $\delta^{15}N$).

Differences we observed in $\delta^{15}N$ between salmon flesh and brown bear feces agrees with earlier findings for other species (i.e., about 2‰ enrichment of cow feces relative to diet and evidence for similar fractionation in human and pigs) (Steele and Daniel 1978). If feces were not 100% salmon, then $\delta^{15}N$ enrichment we measured in feces was not all due to salmon-N (i.e., it could have been diluted with $\delta^{15}N$ of berries for instance). However, the appearance of fecal material that we sampled supported our

assumption that it contained only salmon-derived nitrogen. Fecal splats that we sampled were uniformly black, translucent, liquid, and smelled of rotten fish. The amount of other foodstuff was probably negligible compared to salmon in the feces we sampled, even though bears are known to eat other things besides salmon along salmon streams (Hilderbrand et al. 1999b, Quinn and Kinnison 1999).

Given bears' fecal ^{15}N enrichment and $\delta^{15}N$ of salmon tissues that we measured, bears' urine would have only been slightly ^{15}N depleted (urine $\delta^{15}N$ was calculated to be 0.11‰ less than salmon $\delta^{15}N$). Even if bears excreted as little as 85% of their N intake as urine (Hilderbrand et al. 1999a), $\delta^{15}N$ of urine-N would still exceed 12‰, which is substantially higher than expected levels of $\delta^{15}N$ in terrestrial soil and vegetation. If most N input to coastal soils where bears are active is in the form of bear urine, $\delta^{15}N$ of the soil's N source is very similar to salmon $\delta^{15}N$. However, fractionating losses due to $NH_3(g)$ volatilization or denitrification (following nitrification of NH_4^+) could leave the urine N fraction in soil enriched relative to fresh urine (Kendall 1998, Bronson et al. 1999). While NH_3 volatilization may be minimal in low pH soils, fractionation during decomposition of urine-N into soil has not been measured under field conditions in northwestern Pacific rainforests.

Since $\delta^{15}N$ differed between salmon tissue and bear feces, MDN estimates that used $\delta^{15}N$ of salmon tissue as the marine endmember (MEM) were higher than MDN estimates that used $\delta^{15}N$ of salmon-N in soil under bear feces as MEM. However, the absolute difference in MDN estimates was less than 5% between our two methods when $\delta^{15}N$ of the target sample (SAM) was within 8‰ of the terrestrial endmember (TEM)

$\delta^{15}N$. It can be seen that a 2‰ change in $\delta^{15}N$ of the marine endmember does not affect MDN estimates very much for samples with $\delta^{15}N$ close to the TEM.

We used TEM we measured in false lily of the valley leaves from our reference site for our sample MDN calculations (Fig. 6) to consider the impacts of changing MEM on MDN estimates. However, our estimate of TEM is susceptible to all of the errors discussed above, particularly the high potential for unpredictable differences in $\delta^{15}N$ between sites regardless of the presence of MDN. If TEM increased 2% then our MDN estimates would have decreased as much as 10% (absolute), when we used $\delta^{15}N$ of salmon tissue as the MEM.

In contrast to very low levels of $\delta^{15}N$ fractionation, $\delta^{13}C$ fractionated more than 7‰ between salmon flesh and soil. Even considering differences in $\delta^{13}C$ between pink salmon from the Koeye River and from Glendale River, $\delta^{13}C$ of salmon-derived C in soil was at least 6‰ less enriched than $\delta^{13}C$ of salmon flesh. Since stable isotopes of carbon fractionated differently than nitrogen, the same level of $\delta^{15}N$ and $\delta^{13}C$ enrichment would not be expected in samples containing the same amount of marine-derived nitrogen and marine-derived carbon. High ^{13}C depletion between salmon flesh and soil means that $\delta^{13}C$ of salmon flesh is not an acceptable estimator for $\delta^{13}C$ of soil's marine-C source. Mixing model calculations using $\delta^{13}C$ of salmon tissue as the marine-C signature would underestimate the amount of marine-C in a soil sample.

Delta^{15}N did not differ between sections of the same leaves, but we did find a significant difference in $\delta^{15}N$ between roots and leaves. The lack of $\delta^{15}N$ difference between leaf tips and bases supports the assumption that leaf samples can be compared

regardless of location on the leaf. However, since roots and leaves showed a significant $\delta^{15}N$ difference, comparisons for MDN detection should be made only with similar plant parts. In a similar way, the difference between $\delta^{15}N$ in vegetation and soil where the plant was growing indicates that $\delta^{15}N$ signatures should be measured in the same type of material for which MDN calculations are done.

Changes in N isotopes that we measured are probably site specific and should not be used as numerically accurate in other salmon/bear systems. We have used empirical fractionation rates here to illustrate the effects of fractionation on MDN estimates. Although fractionation at Glendale River or the Koeye River is probably not the same at other areas or at other times, it serves as an approximation of the relative magnitude of fractionation under the wet, cool conditions of the northwestern Pacific forest.

Spreadsheet Model

Our model supported the use of linear two-source mixing models when there was no net fractionation between N sources and measured sinks. However, our model indicated that even small amounts of fractionation during soil N cycling could lead to large overestimations of MDN when salmon $\delta^{15}N$ was used to estimate the marine $\delta^{15}N$ signature. Error resulting from the use of salmon $\delta^{15}N$ as the marine signature indicates that it is best to determine $\delta^{15}N$ of salmon-derived nitrogen in the sampled material. Using $\delta^{15}N$ of the model's salmon N component in the soil as the marine source signature, which is analogous to using $\delta^{15}N$ of salmon-derived N measured in soil or calculated in vegetation, allowed calculation of the actual marine inputs.

Long stabilization periods after input perturbations could lead to errors in MDN calculations. We observed persistent $\delta^{15}N$ elevation due to salmon inputs for more than 100 years after inputs were decreased in our model. If current non-spawning reaches were used as reference areas to estimate terrestrial source $\delta^{15}N$ values, residual MDN from historical salmon runs at the reference sites could lead to underestimating MDN of the target. Even if a salmon-free reference site were located, persistent MDN from larger historical runs at spawning sites could lead to overestimation of current inputs. On the other hand, persistent MDN signatures would allow for evaluation of historical marine inputs.

Calculating $\delta^{15}N$ of salmon-N by subtracting reference isotopic N pools from the same pools at spawning sites is one way to account for ^{15}N fractionation. The difference between N pools at reference and spawning sites indicated the amount of N above the reference baseline (i.e., the amount of N from salmon-N). Assuming that a reasonable reference area can be located, our analysis indicates that subtracting ^{15}N and ^{14}N at reference sites from ^{15}N and ^{14}N at spawning sites to calculate MEM_{diff} provides acceptable levels of standard error in MDN estimates as long as reference and spawning-site samples' total N pools are separated at least as much as our soil samples from the Koeye River (spawning site total N mean = 0.0134 g N/ g soil, SD = 0.0029; reference total N mean = 0.0109 g N/ g soil, SD = 0.0013). Given the standard deviation of soil samples from the Koeye River, MDN estimates appeared to be more sensitive to variability in $\delta^{15}N$ and total N at the spawning sites than to $\delta^{15}N$ or total N variability at terrestrial reference sites. Even when variability was included for both spawning sites and reference sites, the standard deviation of MDN estimates was less than 0.25%.

In essence, calculating MDN from MEM_{diff} was the same as calculating MDN from total ^{15}N differences, because the mixing model equation for MDN (i.e., (TEM-SAM)/(TEM-MEM)) is mathematically equivalent to the proportional difference of ^{15}N (i.e., ^{15}Nspawning-^{15}Nreference)/(^{15}Nspawning). Therefore, to calculate MDN, given total soil N measurements, it is only necessary to divide the total ^{15}N difference between spawning and reference sites by total ^{15}N at the spawning site. The procedure we undertook for calculating MDN using MEM_{diff} included the extra steps of breaking total N into its constituent isotopic pools (^{15}N and ^{14}N) and converting those pools to $\delta^{15}N$.

Intuitively it makes sense to calculate MDN from total N since the difference in total N between spawning and reference sites should only be marine-derived N. If the difference in total N between spawning and reference sites includes other N besides marine-derived N, then the reference area may not be a valid reference. Soil total N data, whether it is first converted to MEM_{diff} or not, provides a check on the validity of the reference site. If total ^{15}N at reference sites exceeds total ^{15}N at spawning sites then the reference sites may violate the assumption that N conditions at the spawning and reference sites are the same except for the presence of marine-derived N. Theoretically a spawning site could receive less terrestrial N but have the same terrestrial $\delta^{15}N$ as the reference site and could therefore still be used in the traditional mixing model described above. (The assumption for mixing model calculations is only that $\delta^{15}N$ of terrestrial N remains constant between reference and spawning sites.) In actuality, processes that change N levels are strongly associated with fractionation, making it unlikely that soil would have the same $\delta^{15}N$ signature at two sites with different levels of total N, unless the sites received N from different sources. Therefore, if both $\delta^{15}N$ and total N indicate

extra N of elevated $\delta^{15}N$ at a spawning site compared to a reference site, they support the conclusion that MDN is present at the spawning site.

Conclusion

The first objective of our study was to determine levels of fractionation in the field. We found no difference in $\delta^{15}N$ of salmon parts, so consumption of different salmon parts would not be likely to alter $\delta^{15}N$ signatures of salmon-N transported into the forest by brown bears. The difference in $\delta^{15}N$ between salmon and soil was small (about 2‰). If isotopic change between urine N and soil N is similar to fecal N change, then $\delta^{15}N$ of salmon tissue was a reasonable estimator for $\delta^{15}N$ of salmon-N in soil, since most MDN contributions by bears would occur as feces and urine. We also found that salmon-C which had decomposed from bear feces into the soil was at least 6‰ $\delta^{13}C$ depleted relative to salmon tissue. Therefore $\delta^{13}C$ of salmon tissue was not an acceptable estimator of $\delta^{13}C$ of salmon-C in soil, and the same level of $\delta^{15}N$ and $\delta^{13}C$ enrichment would not be expected in samples containing the same amount of marine-derived nitrogen and marine-derived carbon.

The second objective of our study was to determine the extent to which fractionation can affect MDN estimates. Using a reiterative spreadsheet model, we found that fractionation of N losses from the soil caused gross overestimates of salmon-derived nitrogen. Correcting $\delta^{15}N$ of the salmon-N for fractionation revealed that $\delta^{15}N$ of the MDN fraction in soil could be more than two times higher than $\delta^{15}N$ of salmon tissue, so that estimates of MDN which used $\delta^{15}N$ of salmon tissue as the marine

endmember substantially overestimated the amount of MDN present in the system. In our analysis of empirical data from the Koeye River, estimates of MDN in the soil using $\delta^{15}N$ of salmon-N corrected for fractionation (MEM_{diff}), were about 20% (absolute) less than MDN estimates that used $\delta^{15}N$ of salmon tissue as the marine endmember. We also found that residual MDN in the soil caused overestimation of MDN inputs for more than 100 years.

It appears that ^{15}N fractionation and mixing, as well as spatial and temporal $\delta^{15}N$ variability can be large enough under natural conditions to prevent the use of a two-source mixing model as an exact predictor of MDN. Therefore, the numerical results of mixing model calculations are best asserted as ranges or upper limits rather than precise values.

References

Ben-David, M., R. T. Bowyer, L. K. Duffy, D. D. Roby, and D. M. Schell. 1998a. Social behavior and ecosystem processes: river otter latrines and nutrient dynamics of terrestrial vegetation. Ecology **79**:2567-2571.

Ben-David, M., T. A. Hanley, D. R. Klein, and D. M. Schell. 1997. Seasonal changes in diets of coastal and riverine mink: the role of spawning pacific salmon. Canadian Journal of Zoology **75**:803-811.

Ben-David, M.,T. A. Hanley, and D. M. Schell. 1998b. Fertilization of terrestrial vegetation by spawning pacific salmon: the role of flooding and predator activity. Oikos **83**:47-55.

Bergersen, F. J., M. B. Peoples, and G. L. Turner. 1988. Isotopic discriminations during the accumulation of nitrogen by soybeans. Australian Journal of Plant Physiology **15**:407-420.

Bilby, R. E., B. R. Fransen, and P. A. Bisson. 1996. Incorporation of nitrogen and carbon from spawning coho salmon into the trophic system of small streams: evidence from stable isotopes. Canadian Journal of Fisheries and Aquatic Science **53**:164-173.

Bothwick, L. 2001. Forests & salmon & bears, oh my! The Varsity, University of Toronto, Canada. February 1. http://www.varsity.utoronto.ca/archives/121 /feb01/scitech/forest.html

Bronson, K. F., G. P. Sparling, and I. R. P. Fillery. 1999. Short-term dynamics following application of ^{15}N-labeled urine to a sandy soil in summer. Soil Biology and Biochemistry **31**:1049-1057.

Cederholm, C. J., D. H. Johnson, R. E. Bilby, L. G. Dominguez, A. M. Garrett, W. H. Graeber, E. L. Greda, M. D. Kunze, B. G. Marcot, J. F. Palmisano, R. W. Plotnikoff, W. G. Pearcy, C. A. Simenstad, and P. C. Trotter. 2000. Pacific salmon and wildlife-ecological contexts, relationships, and implications for management. Special edition technical report prepared for D. H. Johnson and T. A. O'Neil (managment directors), wildlife-habitat relationships in Oregon and Washington. Washington Department of Fish and Wildlife, Olympia, Washington, USA.

Cederholm, C. J., M. D. Kunze, T. Murota, and A. Sibatani. 1999. Pacific salmon carcasses: essential contributions of nutrient and energy for aquatic and terrestrial ecosystems. Fishery Bulletin **24**:6-15.

Chadwick, D. H. 2001. Grizzly survival: their fate is in our hands. National Geographic Magazine **July**:2-25.

Garten, C. T., Jr. 1992. Nitrogen isotope composition of ammonium and nitrate in bulk precipitation and forest throughfall. International Journal of Environmental and Analytical Chemistry **47**:33-45.

Garten, C. T., Jr. 1993. Variation in foliar ^{15}N abundance and the availability of soil nitrogen on Walker Branch watershed. Ecology **74**:2098-2113.

Gende, S. M., T. P. Quinn, and M. F. Willson. 2001. Consumption choice by bears feeding on salmon. Oecologia **127**:372-382.

Handley, L. L., A. T. Austin, D. Robinson, C. M. Scrimgeour, J. A. Raven, T. H. E. Heaton, S. Schmidt, and G. R. Stewart. 1999. The ^{15}N natural abundance ($\delta^{15}N$) of ecosystem samples reflects water availability. Australian Journal of Plant Physiology **26**:135-210.

Handley, L. L., and S. X. Chang. 2000. Site history affects soil and plant ^{15}N natural abundance ($\delta^{15}N$) in forests of northern Vancouver Island, British Columbia. Functional Ecology **14**:273-280.

Handley, L. L., and C. M. Scrimgeour. 1997. Terrestrial plant ecology and ^{15}N natural abundance: the present limits to interpretation for uncultivated systems with original data from a Scottish old field. Advances in Ecological Research **27**:133-212.

Heaton, T. H. E. 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. Chemical Geology (Isotope Geoscience Section) **59**:87-102.

Helfield, J. M., and R. J. Naiman. 2001. Effects of salmon-derived nitrogen on riparian forest growth and implications for stream productivity. Ecology **82**:2403-2409

Hilderbrand, G. V, T. A. Hanley, C. T. Robbins, and C. C. Schwartz. 1999a. Role of Brown Bears (*Ursus arctos*) in the flow of marine nitrogen into a terrestrial ecosystem. Oecologia **121**:546-550.

Hilderbrand, G. V., S. G. Jenkins, T. A. Hanley, C. T. Robbins, and C. C. Schwartz. 1999b. Effect of seasonal differences in dietary meat intake on changes in body mass and composition in wild and captive brown bears. Canadian Journal of Zoology **77**:1623-1630.

Hill, P.W., L. L. Handley, and J. A. Raven. 1996. *Juniperus communis* L. spp. *communis* at Balnaguard, Scotland: foliar carbon discrimination ($\delta^{13}C$) and ^{15}N natural abundance ($\delta^{15}N$) suggests gender-linked differences in water and N use. Botanical Journal of Scotland **48**:209-224.

Hogberg, P. 1997. Tansley Review No. 95 ^{15}N natural abundance in soil-plant systems. New Phytologist **137**:179-203.

Kelly, J. F. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Canadian Journal of Zoology **78**:1-27.

Kendall, C. 1998. Tracing nitrogen sources and cycling in catchments. Pages 519-576 *in* C. Kendall and J. J. McDonnell, editors. Isotope tracers in catchment hydrology. Elsevier Science B. V., Amsterdam, Netherlands.

Kline, T. C., Jr. 2001. Assessing the role of salmon carcasses in the nitrogen budget of sockeye salmon (*Onchorhyncus nerka*) nursery lakes using stable isotopes. Abstract *in* American Fisheries Society 2001 International Conference April 24-26: Restoring nutrients to salmonid ecosystems. Eugene, Oregon, USA. http://www.gpafs.org/confnutr/

Kline, T. C., Jr., J. J. Goering, O. A. Mathisen, P. H. Poe, and P. L. Parker. 1990. Recycling of elements transported upstream by runs of pacific salmon: I. $\delta^{15}N$ and $\delta^{13}C$ evidence in Sashin Creek, southeastern Alaska. Canadian Journal of Fisheries and Aquatic Science **47**:136-144.

Kline, T. C., Jr., J. J. Goering, O. A. Mathisen, P. H. Poe, P. L. Parker, and R. S. Scalan. 1993. Recycling of elements transported upstream by runs of pacific salmon: II. $\delta^{15}N$ and $\delta^{13}C$ evidence in the Kvichak river watershed, Bristol Bay, southwestern Alaska. Canadian Journal of Fisheries and Aquatic Science **50**:2350-2365.

Kline, T. C., Jr, J. J. Goering, and R. J. Piorkowski. 1997. The effects of salmon carcasses on Alaskan freshwaters. Pages 179-203 *in* A. M. Milner and M. W. Oswood, editors. Freshwaters of Alaska: ecological synthesis. Springer-Verlag, New York, New York, USA.

Lajtha, K., and J. D. Marshall. 1994. Sources of variation in the stable isotope composition of plants. Pages 1-21 *in* K. Lajtha and R. Michener, editors. Stable Isotopes in ecology and environmental science. Blackwell Science, Oxford, UK.

Larkin, G. A., and P. A. Slaney. 1997. Implications of trends in marine-derived nutrient influx to south coastal British Columbia salmonid production. Fisheries **22**:16-24.

LUCO (British Columbia Land Use Coordination Office). 1999. Appendix V: revised study areas descriptions. Koeye. *In* Central Coast Land and Resource Management Plan: The Central Coast Protected Area Strategy (PAS) Report. http://www.luco.gov.bc.ca/lrmp/cencoast/reports/ccpasrpt/ccpasap5.htm#KOEYE

Mariotti, A., J. C. Germon, P. Hubert, P. Kaiser, R. Letolle, A. Tardieux, and P. Tardieux. 1981. Experimental determination of nitrogen kinetic isotope fractionation: some principles; illustration for the denitrification and nitrification processes. Plant and Soil **62**:413-430.

Mathisen, O. A., P. L. Parker, J. J. Goering, T. C. Kline Jr., P. H. Poe, and R. S. Scalan. 1988. Recycling of marine elements transported into freshwater by anadromous salmon. Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie **23**:2249-2258.

Nadelhoffer, K. J., B. A. Emmett, P. Gundersen, O. J. Kjonaas, C. J. Koopmans, P. Schleppi, A. Teitema, and R. F. Wright. 1999. Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests. Nature **398**:145-148.

Nadelhoffer, K. J., and B. Fry. 1988. Controls on natural nitrogen-15 and carbon-13 abundances in forest soil organic matter. Soil Science Society of America Journal **52**:1633-1640.

Nadelhoffer, K., G. Shaver, B. Fry, A. Giblin, L. Johnson, and R. McKane. 1996. ^{15}N natural abundances and N use by tundra plants. Oecologia **107**:386-394.

Naiman, R. J., R. E. Bilby, and P. A. Bisson. 2000. Riparian ecology and management in the Pacific coastal rainforest. BioScience **50**:996-1011.

Neilson, R., D. Hamilton, J. Wishart, C. A. Marriott, B. Boag, L. L. Handley, C. M. Scrimgeour, J. W. McNicol, and D. Robinson. 1998. Stable isotope natural abundances of soil, plants and soil invertebrates in an upland pasture. Soil Biology and Biochemistry **30**:1773-1782.

Phillips, D. L., and J. W. Gregg. 2001. Uncertainty in source partitioning using stable isotopes. Oecologia **127**:171-179.

Piorkowski, R. J. 1995. Ecological effects of spawning salmon on several south-central Alaskan streams. PhD Thesis. University of Alaska, Fairbanks, Alaska, USA.

Quinn, T. P., and M. T. Kinnison. 1999. Size-selective and sex-selective predation by brown bears on sockeye salmon. Oecologia **121**:273-282.

Rasmussen, M. 1996. Dead salmon do more than stink. High Country News. August 19, Vol. **28**(15). http://www.hcn.org/servlets/hcn.Article?article_id=2708

Reimchen, T. E. 2001. The salmon forest. February 7 *in* D. Suzuki, host. The Nature of Things. Canadian Broadcasting Company Television, Canada.

Robinson, D. 2001. $\delta^{15}N$ as an integrator of the nitrogen cycle. Trends in Ecology & Evolution **16**:153-162.

Ruggerone, G. T., R. Hanson, and D. E. Rogers. 2000. Selective predation by brown bears (*Ursus arctos*) foraging on spawning sockeye salmon (*Oncorhynchus nerka*). Canadian Journal of Zoology **78**:974-981.

Salmon, D. K. 1997. Oceanography of the eastern north Pacific. Pages 7-23 *in* D. K. Schoonmaker, B. Von Hagen, and E. C. Wolf, editors. The rainforests of home. Island Press, Washington, District of Columbia, USA.

Steele, K. W., and R. M. Daniel. 1978. Fractionation of nitrogen isotopes by animals: a further complication to the use of variations in the natural abundance of ^{15}N for tracer studies. Journal of Agricultural Science. Cambridge **90**:7-9.

Sutherland, R. A., C. van Kessel, D. J. Pennock. 1991. Spatial variability of nitrogen-15 natural abundance. Soil Science Society of America Journal **55**:1339-1347.

Willson, M. F, S. M. Gende, and B. H. Marston. 1998. Fishes and the forest. Bioscience **48**:455-462.

Willson, M. F., and K. C. Halupka. 1995. Anadromous fish as keystone species in vertebrate communities. Conservation Biology **9**:489-497.

Yoneyama, T., and A. Kaneko. 1989. Variations in natural abundance of ^{15}N in nitrogeneous fractions of Komatsuna plants supplied with Nitrate. Plant Cell Physiology **30**:957-962.

CHAPTER 3

EVIDENCE OF SALMON-DERIVED NITROGEN TRANSPORT BY BROWN BEARS (*URSUS ARCTOS*) IN BRITISH COLUMBIA BASED ON NITROGEN AND CARBON ISOTOPES[2]

Introduction

Brown bears (*Ursus arctos*) are prominent in the fauna of northern forests. They are the largest terrestrial predators in the world, but their numbers have been declining over much of their range. The effects of this decline on high latitude ecosystems are not well known. Brown bears' size, mobility, and habits help change soil and forest ecosystem dynamics where they live (Butler 1995, Tardiff and Stanford 1998). In Alaska, brown bears have been implicated in the flow of salmon-derived nutrients into coastal forests (Hilderbrand et al. 1999a). The potential role of brown bears in maintaining or restoring biological productivity and diversity is of special interest where large forest disturbances have occurred or are planned.

Salmon contribute substantial amounts of organic matter to the aquatic ecosystems where they spawn (Mathisen et al. 1988, Kline et al. 1990, 1993, 1997, Piorkowski 1995, Bilby et al. 1996, Allendorf et al. 1997, Cederholm et al. 2000). Other vectors, including brown bears, move salmon nutrients up into riparian areas and forests (Bilby et al. 1996, Ben-David et al. 1998b, Cederholm et al. 1989, 1999, 2000, Hilderbrand et al. 1999a).

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Brown bears consume large amounts of salmon where runs are plentiful (Hilderbrand et al. 1996, 1999a, 1999b, 1999c, Olson et al. 1997). When brown bears congregate on salmon spawning streams most of their diet consists of salmon (Gilbert and Lanner 1995, Willson and Halupka 1995, Hilderbrand et al. 1996). Bears may consume all or part of a salmon, often leaving part of the carcass on shore (Quinn and Kinnison 1999, Ruggerone et al. 2000). Evidence suggests that when salmon are plentiful bears preferentially feed on the most energy-rich parts of the salmon (brain, eggs, and skin), leaving the rest of the carcass for scavengers or to rot (Gende et al. 2001). Bears at spawning streams spend time walking the banks and rest in beds along the shore. Day beds are several to hundreds of meters away from the rivers. Periodically bears move long distances away from, or between, spawning streams. While bears move around on land their waste products, as well as salmon carcasses carried in from feeding, provide a nutrient source to terrestrial ecosystems. Bears congregate in large numbers on salmon spawning rivers, and salmon comprise a large portion of bear diets during spawning times. Since bears move large distances along streams and between streams their dispersal of salmon-derived nutrients could be important over large areas.

Marine-derived nutrients from salmon transported by bears, may be detected by analyzing stable isotopes. Salmon have high levels of the naturally occurring, stable, heavy isotopes of nitrogen (^{15}N) and carbon (^{13}C) compared to most northwestern American inland freshwater or terrestrial systems (Ben-David et al. 1998b, Kline et al. 1990). Enrichment of the heavy isotopes of nitrogen and carbon is denoted by

comparing isotope levels in a sample to isotope levels in a standard according to the following equation:

$$\delta^{15}N \text{ or } \delta^{13}C = ((R_{sample}/R_{standard})-1)\cdot 1000$$

where $R = {}^{15}N/{}^{14}N$ or ${}^{13}C/{}^{12}C$. Delta^{15}N and δ^{13}C are expressed in parts per thousand, ‰. Standards (air for nitrogen and PeeDee Belemnite limestone for carbon) have δ^{15}N and δ^{13}C values of zero ‰ by definition ($R_{sample}/R_{standard} = 1$).

Pacific salmon have δ^{15}N in the range +11‰ to +14‰, and δ^{13}C of about -18‰ (Kline et al. 1990, 1993, Bilby et al. 1996, Ben-David et al. 1997, 1998b, Hilderbrand et al. 1999a, Kline 2001). Northwestern American freshwater primary producers and terrestrial vegetation and soil are usually lower in δ^{15}N and δ^{13}C than salmon (Mathisen et al. 1988, Kline et al. 1990, 1993, Ben-David et al. 1998a). Although it is impossible to specify generally accurate "typical" values for vegetation or soil (Handley and Scrimgeour 1997, Hogberg 1997, Ben-David et al. 1998a), studies in Alaska and Washington have found that vegetation and soil δ^{15}N values are often negative, and δ^{13}C is often near -30‰ (Bilby et al. 1996, Ben-David et al. 1997, 1998b, Hilderbrand et al. 1999a, Helfield and Naiman 2001). The difference between salmon natural isotopic abundance ($\delta^{15}N \approx +13‰$, $\delta^{13}C \approx -18‰$) and terrestrial plant and soil isotope levels (δ^{15}N often close to zero or negative, δ^{13}C often less than -25‰) suggests that stable isotopes may be used to evaluate marine contributions to terrestrial environments.

A two-source isotopic mixing model (Kline et al. 1990, 1993) has been used to estimate the proportion of N in riparian vegetation that originated from either marine or terrestrial sources. Riparian vegetation has been reported to have δ^{15}N somewhere

between the $\delta^{15}N$ of the two sources of N. The relative similarity of vegetation $\delta^{15}N$ to the $\delta^{15}N$ of either source can be used to calculate the relative quantity of N derived from the two sources based on the following equation:

$$\%MDN = (N_{veg} - N_{terr})/(N_{MDN}-N_{terr}) \cdot 100$$

where %MDN is the percent of N in vegetation that is derived from marine sources, N_{veg} is the $\delta^{15}N$ of the vegetation, N_{MDN} is the $\delta^{15}N$ that vegetation would have if marine-derived N was the only source of N, and N_{terr} is the $\delta^{15}N$ vegetation would have if terrestrially derived N was the only N source. The primary difficulty in using this approach is obtaining accurate estimates of the $\delta^{15}N$ of vegetation grown exclusively on one source or the other. All reported studies have assumed that the $\delta^{15}N$ of vegetation grown solely on MDN is the same as $\delta^{15}N$ of salmon bodies (a questionable assumption). The $\delta^{15}N$ of vegetation grown solely on terrestrially-derived N has been estimated by measuring the $\delta^{15}N$ of vegetation growing in "reference sites" which are either riparian stretches where salmon do not spawn (Bilby et al. 1996, Helfield and Naiman 2001), or sites far away from spawning rivers (Hilderbrand et al. 1999a). Assuming that $\delta^{15}N$ of terrestrial N at a reference site is the same at a spawning site is also a very questionable assumption. Estimates of %MDN made using this approach have ranged from 15.5% (Hilderbrand et al. 1999a) to 24% (Helfield and Naiman 2001) (Table 1).

Complications of quantifying MDN include variable and unpredictable isotopic fractionation as well as non-uniform microsite characteristics and nutrient processing (Handley and Scrimgeour 1997, Hogberg 1997). Evidence from other studies suggests

that $\delta^{15}N$ of salmon-derived nitrogen may not be the same in soil, vegetation, and salmon because fractionation (discrimination between heavy and light isotopes) occurs during N cycling and transfer (Handley and Scrimgeour 1997, Hogberg 1997, Kendall 1998). In addition, the $\delta^{15}N$ of terrestrially derived N may not be the same at spawning sites and reference sites because fractionation occurs differently in response to variable site conditions. Fractionation in soil and vegetation has been shown to vary with temperature, moisture, acidity, N concentration, and many other factors (Handley and Scrimgeour 1997, Hogberg 1997, Kendall 1998, Neilson et al. 1998) so that $\delta^{15}N$ signatures appear to be site specific (Handley et al. 1999, Handley and Chang 2000). Although quantification of marine-derived nutrients is problematic, the evaluation of stable N and C isotopes can still provide useful information about nutrient sources, especially when there is a large difference in isotopic signatures between sources, and when isotopic fractionation rates can be determined (Handley and Scrimgeour 1997, Hogberg 1997, Ben-David et al. 1998a, 1998b, Robinson 2001).

Based on studies prior to 1998 (Willson and Halupka 1995, Bilby et al. 1996, Hilderbrand et al. 1996, Ben-David et al. 1997) we hypothesized that brown bears transported significant amounts of marine-derived nutrients into forests near salmon spawning rivers. Brown bear behavior suggests that isotopic enrichment from salmon-derived nutrients would be greater on bear trails and beds. Brown bears feeding on spawning salmon do not preferentially defecate or urinate in certain "latrine" areas. Most bear feces are found along bear trails and near day beds where single bears or family groups (sow with cubs) rest during the day. Therefore we predicted that if bears are transporting substantial amounts of MDN into forests, soil and vegetation near bear

trails and beds would be enriched in $\delta^{15}N$ and $\delta^{13}C$ relative to soil and vegetation away from trails or in areas where bears do not consistently travel. We also predicted that this pattern would be detectable near bear trails further away from the river where bears moved between feeding areas or away from the river. To evaluate patterns of $\delta^{15}N$ and $\delta^{13}C$ relative to bear trails and beds we undertook a study at two rivers along the coast in British Columbia, Canada.

Since 1998 three other studies (Ben-David et al. 1998b, Hilderbrand et al. 1999a, Helfield and Naiman 2001) have provided additional evidence of salmon-derived nitrogen in terrestrial systems. These studies provide an interesting context for our study. Ben-David et al. (1998b), Hilderbrand et al. (1999a), and Helfield and Naiman (2001) documented increased nitrogen-15 enrichment in vegetation near salmon spawning streams compared to vegetation further away from salmon spawning rivers and compared to vegetation near streams where salmon were not spawning. Ben-David et al. (1998b) and Hilderbrand et al. (1999a) reported higher levels of ^{15}N where piscivore activity (noted as feces or by telemetry) appeared to be highest. They explained a correlation between high levels of ^{15}N and piscivore activity by suggesting that salmon predators have moved salmon into the forests, thus enriching ^{15}N in the areas where piscivores spend most time or urinate and defecate most frequently. Hilderbrand et al. (1999a) mentioned the possibility, but did not provide measurements, of localized nitrogen distribution patterns associated with patterns of concentrated bear activity.

None of the previous studies (Ben-David et al. 1998b, Hilderbrand et al. 1999a, Helfield and Naiman 2001) provided data on a scale less than 10 m. If bears comprise a major vector for salmon-derived nutrients into forests then we would expect to find

evidence of salmon-derived nutrients on a scale corresponding to areas of locally high bear activity, which appears to be along bear trails and beds in the forest. Hilderbrand et al. (1999a) pointed out that bears are not distributed evenly across the landscape, and mentioned that areas of focused activity can occur, for instance, where fishing is most profitable. We add that high activity also occurs on trails and in beds when bears move between or away from profitable fishing areas.

Ben-David et al. (1998b), Hilderbrand et al. (1999a), and Helfield and Naiman (2001), focused on large-scale patterns of $\delta^{15}N$ in vegetation so they did not include soil isotope analysis. Measuring isotopic enrichment of soil benefits isotope tracing because: (1) Two isotopes (^{15}N and ^{13}C) can be used in soil to estimate marine inputs, while only ^{15}N provides useful information in vegetation about marine inputs because plants obtain all their C from the atmosphere; (2) Soil samples can be collected at regular intervals while plants' occurrence, abundance, and rooting patterns are less uniform and less predictable; and (3) Isotopic fractionation during plant uptake or internal N translocations might help to obscure or mimic a marine salmon ^{15}N signature. Plant assimilation of nitrogen could involve at least one more fractionating process than whatever fractionation has already occurred in the soil. Reliable quantification of marine nitrogen in plants requires an estimate of the amount of fractionation between salmon-nitrogen sources, soil, and vegetation.

To investigate the pattern and magnitude of MDN distribution by brown bears in the coastal rainforest of northwestern America we compared N and C concentrations, and their isotopic enrichments, in soil and vegetation on bear trails and beds and in adjacent areas where bear activity was not as concentrated. Our objective was to

determine whether there were measurable patterns of ^{15}N and ^{13}C enrichment relative to highly localized areas of brown bear activity, and if so, to determine how much nitrogen and carbon was distributed by bears.

Methods

Site Characteristics

The Koeye River Watershed (51° 46' N 127° 53' W) is one of the least disturbed areas of coastal temperate rainforest in British Columbia, Canada. It is a biologically rich, low relief coastal watershed containing a large estuary and wetlands, and two medium sized freshwater lakes. Recently protected by federal agreement, the Koeye was described by the British Columbia Land Use Coordination Office (LUCO 1999) as having an unusually productive forest resulting in high biological diversity, grizzly bear habitat, and salmonid values. Mean annual rainfall exceeds 350 cm. The river runs from Koeye Lake (approximately 5 km inland) to Fitzhugh Sound on the Pacific Ocean. The Koeye estuary extends approximately 1000 m inland.

Western hemlock (*Tsuga heterophylla*), coastal Douglas fir (*Pseudotsuga menziesii*), western redcedar (*Thuja plicata*), Sitka spruce (*Picea sitchensis*) and yellow-cedar (*Chamaecyparis nootkatensis*) predominate in the forest, with an understory of salal (*Gaultheria shallon*), salmonberry (*Rubus spectabilis)*, and fern (*Blechnum splicant*). Alder (*Aldus rubra*) was not common on the lower Koeye River where we established transects, although it can be found in the estuary. Moss (probably *Rhytidiadephus loreus, Hylocomium splendens,* and/or *Kindbergia oregana)* was common on the forest floor and on many structures throughout the study area.

The Koeye has supported runs of pink (*Oncorhynchus gorbuscha*), chum(*Oncorhynchus keta*), sockeye (*Oncorhynchus nerka*), and coho salmon (*Oncorhynchus kisutch*). The mean reported annual number of spawning fish from 1950 to 1998 was 23,000 +/- 6500 salmon (MFO 1998). Twenty thousand pink and a few hundred chum salmon returned to the Koeye River in 1998 when we collected our data. The salmon run began in August and continued until at least mid-November. Anecdotal evidence suggests that moderate numbers of bears, including sows with cubs, occur along the Koeye River during all periods of the year. There were at least three and probably not more than 15 brown bears along the stretches (approximately 2 km) of river where we established transects. Evidence of bears fishing included fresh scats, tracks, and torn salmon carcasses.

The inland end of the estuary is shallow-- less than 50 cm deep at low tide-- and multi-channeled (Fig. 7). Eastward, upstream, there are two islands on the north. Bears have created a trail parallel to the Koeye River at the east end of the second island, and on the mainland north of the river. The shallow end of the estuary provides easy access to salmon. Short spur trails connect day beds in the forest to the main trail, which parallels the river. Less distinct trails are located in some areas on the second island and the mainland.

We established 10 transects across bear trails or beds. We located one transect across a day bed (B1) and one across the main trail (T1) on the second island, as well as five transects across the main trail on the mainland and three more across beds on the mainland. Transects were chosen so that there was minimal dip along the transect line or perpendicular to transects. To the north of the estuary was wooded forest similar to

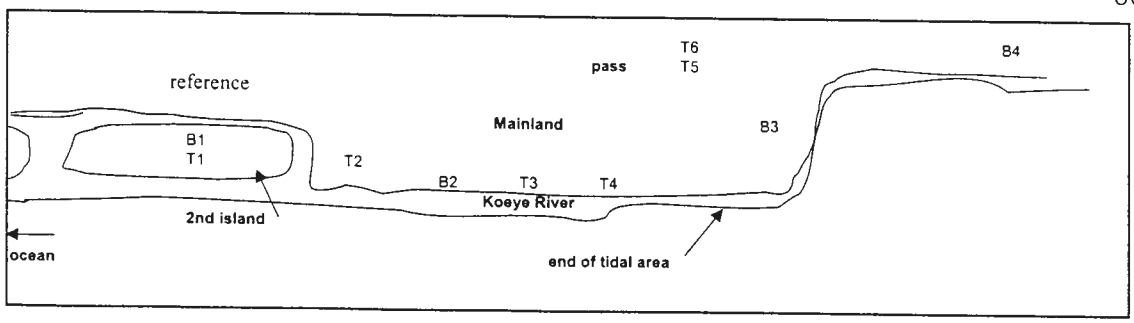

FIG. 7. Map of Koeye River with transects labeled.
Transects are labeled "T" across trails, and "B" through beds. All transects except B2 and B3 were established perpendicular to the nearest free water, and samples were collected at 0, 0.5, 1, 2, 4, 6, and 10 m away from the trail on both sides, or up to 4 m away from beds.

the mainland along the Koeye River, but there were no apparent bear trails or areas of bear activity. Because of similarities in topography and vegetation we thought this area came as close as possible to reflecting the Koeye River area in the absence of bears. Therefore we used this area as a reference area. We established one transect in this reference area on level ground approximately 20 m from water. Two of the transects (T5 and T6) were located at either side of a small pass where bears had created a trail while traveling from one area of the Koeye River to another around steep rapids. This pass was approximately 500 m away from the river and 100 m higher than the river. The ridgeline dipped from both directions to the trail in the pass, and the trail climbed steep slopes toward the pass from both sides. At the top of the pass the trail was level for about 20 m and water puddled in several locations there. The transects we established in the pass maintained a constant elevation to nearly 10 m on both sides of the trail by running along the sidehills.

During a related study (see previous chapter) we collected soil samples from two transects along the Glendale River, near Knight Inlet, British Columbia. The Glendale River is approximately 500 km south of the Koeye River, on the Canadian mainland. Glendale River's forest is similar to the Koeye River forest except that there has been a greater human presence around Knight Inlet, including logging, tourism, and commercial fishing. The Glendale River now supports runs of coho, chum, and pink salmon. Brown bears feed on salmon in the river, and on pink salmon that spawn in an artificial spawning channel near the river's mouth. We collected samples from two level transects within 10 m of the river approximately one and 2 km upstream from the artificial spawning channel.

Sample Collection

Soil was collected along transects from 0.5, 1, 2, 4, 6, and 10 m on either side of trails and beds by driving a 5-cm diameter stainless steel corer into the soil to a depth of 15 cm. Moss and litter (F and L layers) were removed prior to sampling. Roots from salmonberry and false lily of the valley grew densely at the bottom of the moss layer where organic soil was apparent. We removed the moss down to this layer. Soil samples from trails, beds, and surrounding forest were very organic and uniformly dark reddish to black. No consistent difference in soil color or density was observed between on-trail and off-trail samples. Beds all showed evidence of excavation and were noticeably drier than surrounding soil. Soil collected during spring sampling was placed in a cooler prior to transport, then frozen at the USU lab. Soil from fall sampling was frozen within 24 hr of collection. Prior to analysis soil samples were thawed at 5° C for

approximately 24 hr then homogenized. A subsample of the homogenized sample was transferred to a beaker or grinding jar, then dried at 70° C for 5 to 9 d. The dried samples were then crushed by grinding with roller bars for at least 8 hr. Dried samples were transferred from grinder jars to plastic (snap cap) vials for storage until subsamples could be weighed into tin capsules for analysis of N and C by direct combustion and mass spectrometry. Portions of some soil samples were extracted in 0.5 M K_2SO_4 for mineral N analysis, and some were weighed before and after drying to determine water content.

We collected whole or half young leaves or parts of leaves punched out with a sharpened section of 15-mm diameter aluminum tube. We collected samples from plants (false lily of the valley [*Maianthemum dilatatum*], bunchberry [*Cornus canadensis*], salal [*Gaultheria shallon*] and salmonberry [*Rubus spectabilis*]) nearest to the transect point from which soil was sampled. We attempted to collect parts of at least two species from each point, and to sample the same species at all points, but that was not always possible due to limited distribution of some species along the transects. In the spring, false lily of the valley was nearly ubiquitous, so we sampled it predominantly. In the fall, false lily of the valley was uncommon, so we sampled mostly bunchberry, which grew in more locations. Plant samples were folded into Whatman #1 filter paper and placed in plastic slide sheets. In the spring, when freezing facilities were unavailable, we placed samples in the consistently cool hold of the boat that we used as a base-camp. During transport out of the Koeye River study area, back to Utah State University, the plastic sheets containing plant samples were kept in a plastic cooler. In the fall, we froze all vegetation samples within 24 hr of collection.

In the laboratory at USU, unfrozen leaf samples were removed from the plastic pouches or bags and dried at 70° C for 5 to 8 d. Frozen samples were freeze-dried for at least 48 hr. Dried samples were placed in plastic bags which were then placed in a dessicator containing concentrated H_2SO_4, or sealed into 1-gallon zip-shut bags containing $CaCl_2$, as a desiccant, for storage.

Sample Analysis

We measured total N, $\delta^{15}N$, total C, and $\delta^{13}C$ in soil samples and most vegetation samples (some vegetation samples were only analyzed for total N and $\delta^{15}N$) by continuous-flow direct combustion and mass spectrometry using a Europa Scientific SL2020 system (PDZ Europa, Cheshire, UK).

Latex gloves were worn when handling all materials. Dried, ground samples were weighed into 8-mm by 5-mm tin (Sn) capsules or disks. Empty tin disks or capsules matching those containing samples were used as blanks. The instrument used blanks to automatically blank-correct ^{15}N enrichments. Reference standards of known N and C content, and known $\delta^{15}N$ and $\delta^{13}C$ were analyzed at the beginning, end, and throughout each run, generally after every 12 samples. Reference standards consisted of Gold Medal® all-purpose, bleached, wheat flour, apple leaf, or soil/flour mixture crushed in 8-mm x 5-mm tin capsules. For five transects, only soil from transect endpoint and center samples was analyzed due to funding constraints. Soil from some or all internal points as well as endpoints and center points were analyzed for the other five transects and the reference transect.

Eighteen soil samples (five from a bed transect, the rest from transects across trails) were analyzed for NO_3^- and NH_4^+. Subsamples (10-20 g) of feces or homogenized soil were extracted in plastic specimen cups containing 100 ml 0.5 M K_2SO_4 and 0.5 ml $CHCl_3$. Extraction in the spring was performed in the field. All extraction in the fall was performed in the laboratory. Extractions were shaken for at least 30 min at 180 rpm with an orbit shaker or moderately with a horizontal shaker, and allowed to settle (at 5° C) undisturbed for 6 to 8 hr. The supernatant was filtered through pre-rinsed Whatman #4 filter paper. The extract solution was then frozen. To prepare for further analysis, filtered extractions were thawed and crystals were re-dissolved by shaking with an orbit shaker (>200 rpm). A few samples required heating to 50° C in a water bath before the crystals dissolved. Concentrations of NO_3^- and NH_4^+ were determined colorimetrically using a flow-injection autoanalyzer (Lachat Instruments, Milwaukee, Wisconsin, USA).

Statistical Analysis

We used paired *t*-tests to determine whether spring and fall soil samples from the same points were statistically different. We also used a K-nearest neighbor randomization test (Rosing et al. 1998) with $\delta^{15}N$ and $\delta^{13}C$ data to compare the spring soil samples to fall soil samples in two isotopic dimensions $\delta^{15}N$ and $\delta^{13}C$. Paired *t*-tests were done using EXCEL.

We used paired *t*-tests to determine whether $\delta^{15}N$, $\delta^{13}C$, total N, and total C in soil measured at the centers of transects (on bear trails or beds) were different from values measured at ends of those transects (furthest sampled points away from bear trails

or beds) using data from all nine transects for which endpoints were sampled (B3 was not included because only one endpoint was measured at that transect). Pairwise comparisons were performed between the endpoint away from the river, the midpoint, and the endpoint away from the river. Endpoints were 4 m away from beds and 6 or 10 m away from trails. To evaluate the overall significance of $\delta^{15}N$ variation between transect centers and endpoints we used a single factor ANOVA with data standardized to zero at transect centers, followed by a Fisher's least-significant-difference test for pairwise differences. Data was standardized prior to ANOVA analysis by subtracting $\delta^{15}N$ of the central sample from $\delta^{15}N$ of the endpoints for each transect. Computations were done using EXCEL, SAS/STAT, and SYSTAT.

To evaluate soil trends in all variables relative to distance from the river, we used linear regression on samples from the centers of transects. Data from transect centers were used rather than transect averages because variance was high for whole transects. Transect centers provided a means of comparing marine inputs to transects at different distances from the river because transect centers were on bear trails or beds, and trails or beds were the areas of expected highest marine inputs for each transect. Although it is unlikely that $\delta^{15}N$ truly decreased linearly with distance from the river, a linear model provided a rough estimate of trend that is consistent with the observed data. Computations were done using PROC REG in SAS/STAT.

To evaluate soil trends relative to position along transects we used linear regression on data standardized to the center transect value. Sample N or C data were standardized by subtracting the center (trail or bed) value from every data point, by transect, so that the center value was zero and every other point's value represented

some difference from the center. Regression was done with data averaged for all transects by distance from the center of the transect (0.5, 1, 2, 4, 6, 10 m). For each distance there were two average values, one on each side of the transect. Average values at each distance did not include data from every transect because samples were not analyzed for every distance along every transect. Computations were done using PROC REG in SAS/STAT.

Due to large differences between leaf $\delta^{15}N$ values and the small number of samples of any one species at any given distance, general statistical analysis (such as ANOVA or multivariate regression) were not appropriate for vegetation data. We did not have data from all species on all transects. Nor did we have data from the same species at every point along many transects. Paired comparisons (*t*-tests) were used to compare transect centers to endpoints for species on transects where central and endpoint samples were available. For a species' transect center data we averaged data from within 0.5 m on both sides of the trail or bed and used that as the center. Computations were done using EXCEL.

The relationship between soil $\delta^{15}N$ and leaf $\delta^{15}N$, and differences in this relationship among species, were assessed using a general linear model of leaf $\delta^{15}N$, including soil $\delta^{15}N$ on a continuous scale, species on a categorical scale, and the interaction of these two factors as explanatory variables. Essentially, the statistical model fit a separate regression line for each species, and permitted statistical comparison of regression coefficient estimates among species. Pairwise comparisons of slope estimates were made using contrasts within the full model. Computations were done using PROC MIXED in SAS/STAT.

Marine-Derived Nitrogen Calculations

We used the same type of linear two-source mixing model to calculate MDN as used by Bilby et al. (1996), Hilderbrand et al. (1999a), and Helfield and Naiman (2001) after Kline et al. (1990, 1993). To estimate MDN in soil we used $\delta^{15}N$ of salmon tissue as $\delta^{15}N$ of the marine source in soil (salmon tissue $\delta^{15}N = +12.40‰$). The terrestrial source $\delta^{15}N$ signature in soil was assumed to be equal to $\delta^{15}N$ measured in soil on the reference transect (reference soil $\delta^{15}N = -0.07‰$).

To estimate MDN in vegetation we used separate calculations for each species. Since we could not directly measure $\delta^{15}N$ of 100% MDN in vegetation, we calculated $\delta^{15}N$ for each plant species as if it were growing in soil with 100% MDN. That means we used the regression relationships between vegetation and soil to calculate $\delta^{15}N$ of each species as if it were growing on soil with $\delta^{15}N$ of salmon tissue. Delta^{15}N of the terrestrial N source as it appeared in vegetation was represented either by $\delta^{15}N$ of vegetation we sampled at the reference area, or by calculating $\delta^{15}N$ for the species we could not sample at the reference site, as if they were grown on soil from the reference area. For both soil and vegetation we performed MDN calculations using reference data from the season in which a sample was collected.

We estimated MDN at different locations on the bear trail and at different beds by calculating MDN for soil samples collected at transect centers. We assessed the variability of MDN estimates for whole transects by comparing MDN estimates between sample types averaged by transect. We also assessed the variability of MDN estimates between sample types by comparing MDN estimates for soil and vegetation collected at

the same time from the same points. Statistical comparison of MDN for different sample types was performed pairwise using paired *t*-tests with EXCEL.

Results

Soil $\delta^{15}N$ and $\delta^{13}C$: Spring and Fall

Soil samples collected in the fall and spring agreed closely for both $\delta^{15}N$ and $\delta^{13}C$. Since there was no significant difference between spring and fall samples ($P > 0.40$ paired *t*-test, $P > 0.10$ K-nearest neighbor randomization test) they were averaged when both were available for the same point.

Soil $\delta^{15}N$ and $\delta^{13}C$: Patterns Within Transects

Samples from the center of trails and beds had slightly, but significantly, higher $\delta^{15}N$ than samples from transect endpoints ($P < 0.028$, SE = 0.453, $n = 9$, ANOVA). Delta^{15}N in soil from transect centers was significantly higher than $\delta^{15}N$ in soil at the ends of transects away from the river ($P < 0.01$) (Fig. 8). Soil from the centers of transects also appeared to have higher $\delta^{15}N$ than samples from the ends of transects closest to the river in general (in six of nine transects; data was only available from one endpoint on the 10th transect, B3) but the difference was not significant ($P = 0.33$). Soil from the ends of transects furthest from the river showed significantly lower $\delta^{15}N$ than the ends closest to the river. When mean $\delta^{15}N$ at all transect positions was evaluated by simple linear regression, $\delta^{15}N$ was shown to decline slightly for points further from the center of transects (R = -0.59, $n = 11$, $P = 0.06$; Fig. 9). There was high $\delta^{15}N$

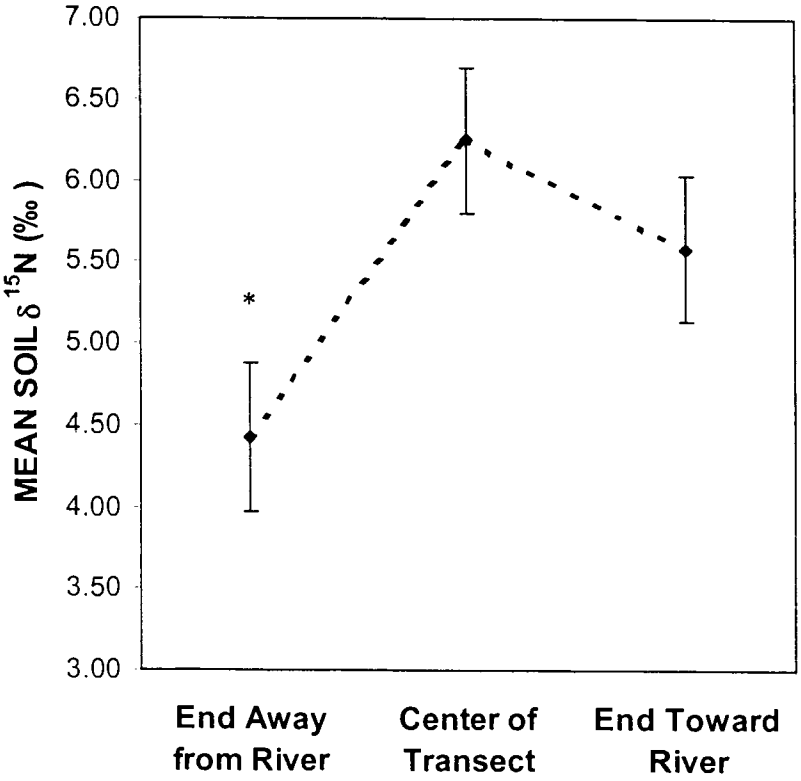

FIG. 8. Soil $\delta^{15}N$ transect centers versus endpoints.
This chart shows data averaged for nine transects. Transects were centered on bear trails and beds. Endpoints were labeled relative to the river for convenience, although only eight of the transects were perpendicular to the river. Transect B3 is not included because data were only available from one endpoint on that transect. * indicates significant ($P < 0.01$) difference between endpoint and center. Error bars represent standard error.

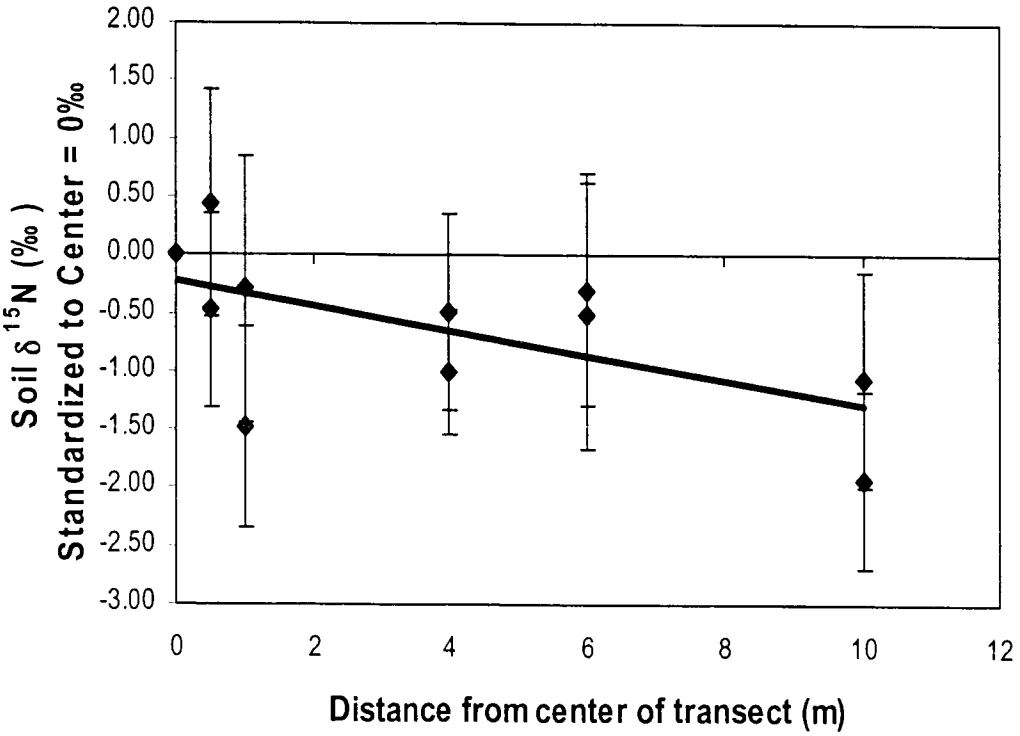

FIG. 9. Mean soil $\delta^{15}N$ trend within transects relative to center.
Soil $\delta^{15}N = -0.23 - (\text{m from center}) \cdot 0.11$, $R^2 = 0.35$, $n = 11$, $P = 0.06$. Data were averaged by distance from the center of the transect and side, so two points at each distance represent means from opposite sides of the transects. Error bars are standard error.

variation between individual points on transects (even adjacent points) nearest trails and beds, but the overall mean $\delta^{15}N$ at every distance from the center was less than $\delta^{15}N$ at the center of the transects, except for 0.5 m from center toward the river. Delta^{13}C was not significantly different between either of the endpoints and the center or each other ($P > 0.50$). There was no correlation between either total N or total C with distance from the center of the transects, and no significant difference in total N, or total C between samples from the centers and endpoints of transects (Mean total N = 0.01 g N/g dry soil, SD = 0.003, $n = 82$; Mean Total C = 0.42 g C/ g dry soil, SD = 0.12, $n = 82$; Mean $\delta^{13}C$ = -26.39‰, SD = 0.56, $n = 93$). Although differences in $\delta^{13}C$ were very small, the highest $\delta^{13}C$ in soil occurred at 1 m or less from the center of trails or beds in all 10 transects.

We found no correlation between NO_3^- or NH_4^+ and soil $\delta^{15}N$ although nitrate levels did correlate linearly with ammonium levels (R = -0.82, log(NH_4^+) vs. NO_3^-). There was also no correlation between $\delta^{15}N$ and total N in soil samples.

Overall, $\delta^{15}N$ appeared to decrease further away from the river. However, linear regression with data from transect centers indicated no significant $\delta^{15}N$ trend with distance from the river (soil $\delta^{15}N = 7.03 - 0.01 \cdot$ m to river's edge, $P = 0.24$, $R^2 = 0.17$). Total soil N was significantly correlated ($P = 0.02$, $R^2 = 0.51$) with distance from the river, but slope of the correlation line was very small (slope = + 0.000018 g N/g dry soil/ m). Excluding transects T3 (base of hill) and B3 (bed on steep hill), which were both geographically unusual in addition to distance from the river, $\delta^{15}N$ decreased significantly with increasing distance from the river but total soil N did not

(soil $\delta^{15}N = 6.40 - 0.01 \cdot$m from river's edge, $P = 0.02$, $R^2 = 0.60$). Delta^{13}C and total soil C were not correlated with distance from the river ($P > 0.75$).

Transects at Glendale River were similar to the Koeye River except that variation within transects was higher at Glendale River. We found that the pattern of $\delta^{15}N$ enrichment was not smooth from endpoint to endpoint across the trail, but that that soil from the trails had higher $\delta^{15}N$ than soil at transect endpoints away from the river. Soil from the trails also had, however, lower $\delta^{15}N$ than soil at the endpoints towards Glendale River.

Soil $\delta^{15}N$ and $\delta^{13}C$: Comparisons Among Transects

A K-nearest neighbor randomization test (Rosing et al. 1998), which compared transects in 2-dimensional space defined by $\delta^{15}N$ as one axis and $\delta^{13}C$ as the other axis, indicated a significant difference ($P < 0.05$) between the reference transect and all other transects at the Koeye River except two: a transect on the mainland closest to the beginning of the trail in the estuary (T2) and across a bed on a steep slope above a rapid section of the river (B3). A transect at the bottom of a small hill where water pooled in the trail (T3) had ^{15}N enrichment significantly higher than any other transect. We discerned no pattern of inter-transect dissimilarity relative to position along river, proximity to river, proximity to good fishing locations, or elevation.

With regard to single elements, the reference transect showed the lowest $\delta^{15}N$ of any transect we sampled ($\delta^{15}N = -0.07$, SE = 0.37‰, $n = 6$) (Fig. 10). Both transects (T5 and T6) from the high pass between feeding areas had low $\delta^{15}N$ values similar to the

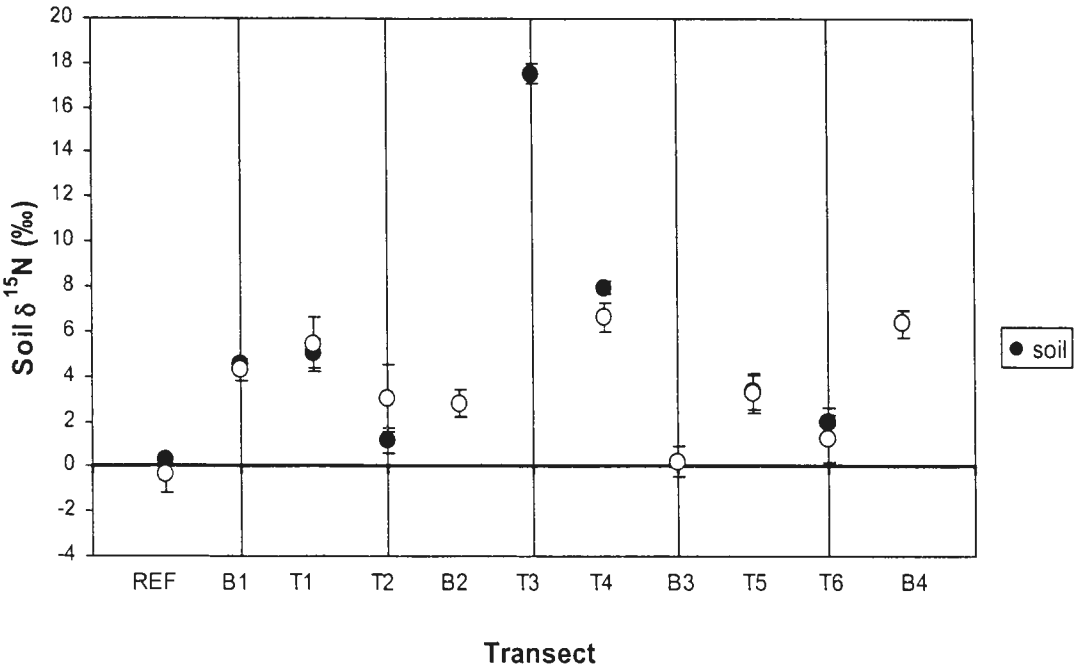

FIG. 10. Mean soil $\delta^{15}N$ for each transect during spring and fall.
Closed circles are from samples collected in the spring, open circles are for samples collected in the fall. Error bars are standard error.

reference transect. Delta^{15}N was less than 1‰ for most samples from the reference transect and both transects in the pass, although mean $\delta^{15}N$ in the pass was greater than 1‰ due to two unusually ^{15}N-enriched samples close to the trail, one from each transect. Other transects from the 2nd Island and the mainland had $\delta^{15}N$ values generally greater than 2‰. The transect across a bed on a steep slope (B3) also had $\delta^{15}N$ values less than 1‰. Transect T3, where water puddled in the trail, had remarkably high $\delta^{15}N$ values (+17.53, SE = 0.43‰). Delta^{13}C did not differ substantially between transects (Fig. 11). As with the dual isotope analysis, we could determine no pattern of inter-transect dissimilarity, except for those mentioned, relative to position along river or any other observed factors for either $\delta^{15}N$ or $\delta^{13}C$.

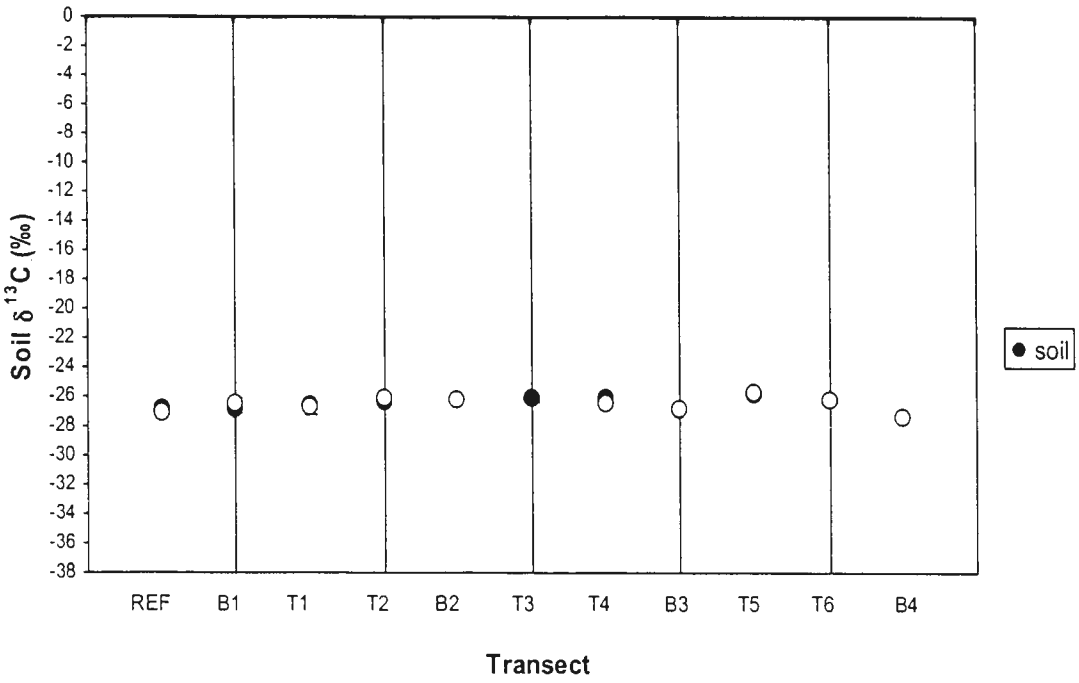

FIG. 11. Mean soil $\delta^{13}C$ for each transect during spring and fall.
Closed circles are from samples collected in the spring, open circles are for samples collected in the fall. Error bars are standard error.

Vegetation $\delta^{15}N$ and $\delta^{13}C$: Spring and Fall

Paired spring and fall samples (the same species from the same point on the same transect) were significantly lower for total N, total C, and $\delta^{13}C$ in fall samples (for N and C, $P < 0.01$; for $\delta^{13}C$, $P = 0.04$). Delta^{15}N of leaf samples collected in the fall averaged 1.57‰ (SE = 0.37) lower than $\delta^{15}N$ of leaf samples collected in the spring, although the difference was not significant ($P = 0.14$). Fall leaf samples also contained less total nitrogen, less total C, and lower $\delta^{13}C$ than spring samples. Since N and C data were so different from spring and fall samples, they were not pooled for evaluation.

Vegetation and Soil Correlation

Generally, leaf $\delta^{15}N$ was less than soil $\delta^{15}N$. The magnitude of the difference depended on species and on the level of soil $\delta^{15}N$. Delta15N of vegetation increased as $\delta^{15}N$ of total soil N increased for all species ($P < 0.001$). However, leaf $\delta^{15}N$ was generally less than soil $\delta^{15}N$, and the magnitude of the difference depended on species (Fig. 4). The slope of the linear relationship between leaf $\delta^{15}N$ and soil $\delta^{15}N$ differed among species (test of interaction between soil $\delta^{15}N$ and species, $P = 0.015$). Pairwise comparisons of slopes among species indicated no apparent difference between salmonberry and false lily of the valley, and no apparent differences among salal, bunchberry, and salmonberry. However, the slope for false lily of the valley was greater than slopes for salal and bunchberry.

Patterns of $\delta^{15}N$ variation within transects and between transects seemed to reflect soil $\delta^{15}N$ patterns. Leaf $\delta^{15}N$ was elevated near trails in bunchberry, false lily of the valley and salmonberry, but not in salal. Bunchberry leaves had lowest $\delta^{15}N$ (less than -2‰) at the reference transect and from the pass (T5 and T6). Bunchberry from other transects was greater than 0‰ (up to +8.5‰). A similar pattern (lowest at the reference transect and at T5 and T6) existed for other species (Fig. 12). Mean false lily of the valley $\delta^{15}N$ was lower than mean $\delta^{15}N$ for every other species by transect.

Delta^{13}C, which was essentially the same in all vegetation samples, did not correlate with $\delta^{13}C$ of the soil. Delta^{13}C did not differ significantly by species along transects ($P > 0.05$) or between transects (Fig. 13).

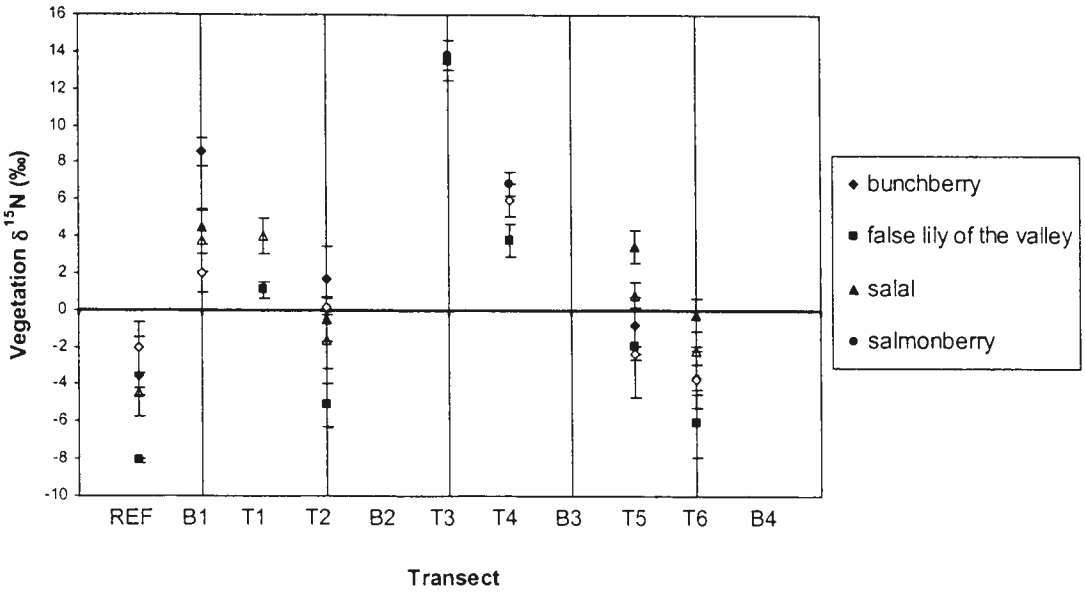

FIG. 12. Mean vegetation $\delta^{15}N$ for each transect during spring and fall. Closed circles are from samples collected in the spring, open circles are for samples collected in the fall. Error bars are standard error. No vegetation samples were collected at B2, B3, or B4.

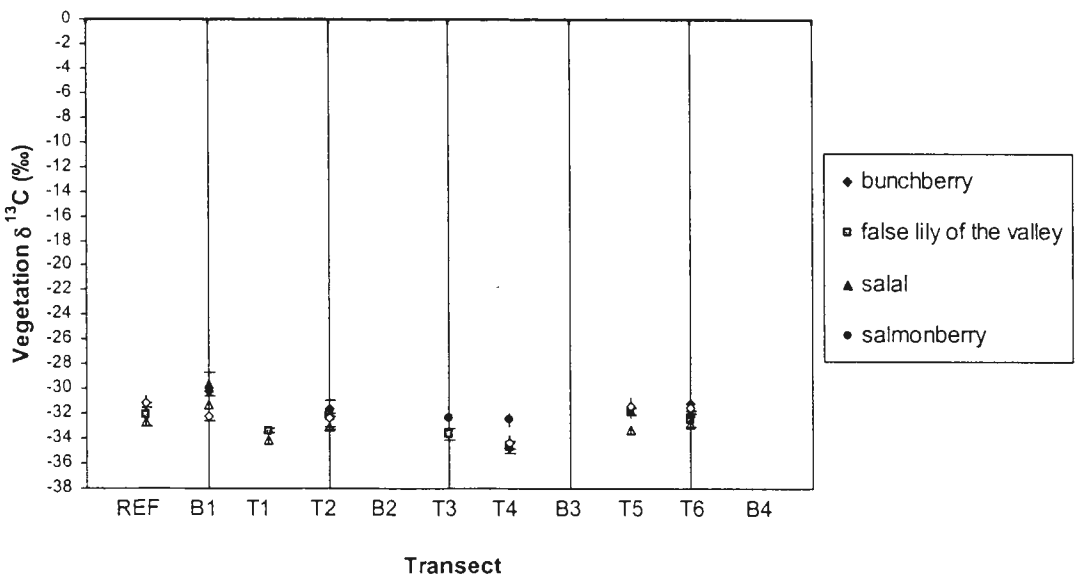

FIG. 13. Mean vegetation $\delta^{13}C$ for each transect during spring and fall. Closed circles are from samples collected in the spring, open circles are for samples collected in the fall. Error bars are standard error. No vegetation samples were collected at B2, B3, or B4.

Marine-Derived Nitrogen Calculations

Based on soil data from the centers of bear trails and beds, we calculated MDN ranging from 9% to 140% (or 5% to 142% when non-center points were included). Estimated MDN in vegetation, based on the calculated marine $\delta^{15}N$ signature for each species, varied more than 50% between some species averaged by transect, and did not always agree very closely with MDN estimates from mean soil $\delta^{15}N$ (Table 4). Samples of different types from the same point, collected at the same time, differed by 9% (soil vs. false lily of the valley) to 25% (bunchberry vs. false lily of the valley), although the paired difference was only significant ($P < 0.05$) for MDN estimates in soil compared to false lily of the valley (Table 5). Since the relationship between the $\delta^{15}N$ of salal and soil was not significant, we did not include salal in our MDN calculations.

TABLE 4. Mean estimated marine-derived nitrogen by transect at Koeye River.

	Soil[1]			Bunchberry			False lily of the valley			Salmonberry		
	MDN	SD	*n*	MDN	SD	*n*	MDN	SD	*n*	MDN	SD	*n*
T1	40%	0.12	6				57%	0.06	6			
T2	11%	0.15	6	49%	0.32	7	18%	0.14	4			
T3	142%	0.09	7				133%	0.15	5	138%	0.19	8
T4	56%	0.12	11	110%	0.30	6	73%	0.13	6	71%	0.11	3
T5	25%	0.16	9	9%	0.22	8	38%	0.29	3			
T6	13%	0.16	9	-13%	0.31	9	12%	0.19	3			
B1	35%	0.06	7	97%	0.50	4						
B2	25%	0.11	5									
B3	5%	0.09	3									
B4	53%	0.09	4									

[1]Spring and fall MDN estimates in soil were averaged prior to MDN computation whenever both existed at the same point.

TABLE 5. Mean difference between marine-derived nitrogen estimates for different types of samples collected at the same time at the same points (<1 m^2).

Comparison	MDN difference	SD	*n*	*P*
Soil - bunchberry	-17%	0.52	26	0.11
Soil - false lily of the valley	-9%	0.14	19	0.01
Soil - salmonberry	-15%	0.47	8	0.40
Bunchberry - false lily of the valley	25%	0.40	6	0.19
Salmonberry - false lily of the valley	-1%	0.12	8	0.80

Discussion

The patterns of $\delta^{15}N$ enrichment that we observed are consistent with the hypothesis that dispersal of marine-derived nitrogen by bears led to elevated $\delta^{15}N$ close to trails and beds. As expected, we measured an inverse relationship between $\delta^{15}N$ and distance from the centers of bear activity (trails and beds). Samples from the centers of transects across bears' trails and beds showed higher $\delta^{15}N$ than samples at the endpoints of transects (points furthest away from the trails or beds), and mean $\delta^{15}N$ declined at all distances away from trails and beds. Although $\delta^{15}N$ variation was high among individual points along transects, when the data from all transects were combined it revealed a general trend of slight, but significant $\delta^{15}N$ elevation (less than 2‰) near trails and beds. Whole transect differences in ^{15}N enrichment reflected MDN inputs by bears. Samples from the reference area, where bear activity was lowest, had the lowest $\delta^{15}N$. Samples from transects in the pass (T5 and T6) also had low $\delta^{15}N$ compared to other transects with high bear activity since bear activity is lower in the pass than closer

to fishing areas. Transects in the pass had higher $\delta^{15}N$ than the reference transect, suggesting dispersal of MDN by brown bears.

Soil and vegetation nearer the river generally had higher $\delta^{15}N$, corroborating research from Alaska (Ben-David et al. 1998b, Hilderbrand et al. 1999a, Helfield and Naiman 2001). Increased ^{15}N enrichment closer to Glendale River supports the generalization that soil and vegetation are ^{15}N enriched near salmon spawning rivers, possibly indicating nutrient transport from the river into the forest via methods not necessarily constrained to bear trails or beds. Higher $\delta^{15}N$ near rivers does not always indicate the presence of marine-derived N. A decrease in $\delta^{15}N$ with increasing distance away from rivers has also been documented in other ecosystems where there were no salmon, and has been attributed to soil fractionation processes probably associated with increasing dryness (Garten 1993, Nadelhoffer et al. 1996). If non-bear vectors were the main dispersers of MDN, or if geographic variations due to fractionation were the main causes of elevated $\delta^{15}N$ near the Koeye River, we would expect $\delta^{15}N$ from transect centers (bear trails or beds) to follow the same general pattern of higher $\delta^{15}N$ close to the river, declining with distance away. However, $\delta^{15}N$ from transect centers did not correlate significantly with distance from the river, suggesting that salmon-N had been moved onto bear trails and beds.

We designed this study to focus on bear trails and beds, thus minimizing our measurements of MDN contributions by other animals. Evidence of salmon-derived nitrogen on bear trails reflects bears' dispersal of MDN, although other animals use bear trails. Feces and tracks on bear trails close to the river were predominantly from bears.

We probably would not have noticed most of the sign from smaller animals like weasels or martens, but we would not expect their activity to be as localized to bear trails. Wolves use bear trails, but they also travel off trail throughout very large ranges (Darimont and Paquet 2000, Darimont personal communication). The total N input from wolves would not be as large or as concentrated as that from bears along the trails we studied.

The relatively small difference we measured in $\delta^{15}N$ between ends of transects and centers is consistent with a diffuse salmon-derived N signature, expected where more than one MDN vector is important, and where there has been a long history of MDN inputs. Avian piscivores would create a pattern of MDN dispersal that was diffuse across the landscape, or concentrated on areas other than bear trails. Abiotic transport vectors (flooding, hyporrheic flow, or wind) would also follow different landscape patterns than bear trails or beds. Secondary nutrient transport such as uptake by plants and redistribution of MDN in litter would further blur patterns of salmon-nutrient deposition, especially as N recycling and consequent movement away from bear trails has occurred over a long period of time. Since there are so many MDN vectors besides bears, the fact that we have documented any pattern associated with bear trails and beds supports the hypothesis that bears' MDN contribute MDN to forests.

The high $\delta^{15}N$ variability we measured in soil and vegetation along our transects, especially close to bear trails and beds, gives an idea of the small-scale on which differences can occur in $\delta^{15}N$. Localized differences in pH, temperature, moisture, and microbial communities, which affect $\delta^{15}N$ signatures (Handley and Scrimgeour 1997, Hogberg 1997, Kendall 1998, Neilson et al. 1998) occur along bear trails and in the

surrounding forest as a result of variable forest structure, although the scale and magnitude of these differences has not been measured. Marine-derived nutrient inputs themselves might actually increase $\delta^{15}N$ variability in vegetation growing in the same soil. Ben-David et al. (1998a) suggested that otter fertilization of latrine sites in Alaska might have led to non-uniform N pools and less competition for available N, giving rise to the unusually high variability of $\delta^{15}N$ in vegetation that they sampled on otter latrine sites.

In contrast, $\delta^{13}C$ we measured was not significantly higher closer to beds or trails, contrary to our expectations. The lack of correlation between patterns of $\delta^{13}C$ and $\delta^{15}N$ enrichment can be explained by the large contribution of terrestrial plant C to the terrestrial C budget compared to marine-C inputs. Net primary production in similar ecosystems of the northwest is approximately 6500 kg C/ha/yr (Gholz 1982). The large amount of C fixed by terrestrial plants would likely obscure the $\delta^{13}C$ signature of a much smaller marine-derived C input (atmospheric $\delta^{13}C$ is much lower than marine $\delta^{13}C$). In comparison, the contribution of marine-derived nitrogen would not need to be very great to form a large percentage of the total N inputs. Non-marine N inputs at the Koeye River probably occur as N fixation and wet deposition, neither of which is thought to be very large compared to potential marine-N inputs. Nitrogen fixation probably was not a large source of non-marine N inputs to the soil near our transects since we established transects where no alder was found. Wet deposition of N at similar northwestern sites was only on the order of 2 kg N per hectare per year (Heaton 1986, Garten 1992, Lajtha and Marshall 1994, Handley et al. 1999, Hilderbrand et al. 1999a, Nadelhoffer et al.

1999). A model that we used to evaluate fractionation in another study (see previous chapter) shows that MDN inputs of even less than one kg N/ha can substantially affect $\delta^{15}N$ of soil under an N budget similar to the Koeye River's. Marine contributions would also be more likely to elevate $\delta^{15}N$ than $\delta^{13}C$ if the primary input from bears is in the form of isotopically enriched urine (Hilderbrand et al. 1999a) since urea contains two atoms of nitrogen for every carbon.

In addition, there is evidence for high fractionation between C in bears' feces and C in the soil. Carbon-13 fractionation during fecal decomposition was shown in a companion study (see previous chapter) to leave soil about 6‰ $\delta^{13}C$ depleted relative to pink salmon tissue. Fractionation between feces and soil indicates that fecal contributions to bear trails and beds would lead to little or no ^{13}C enrichment.

Based on ^{15}N enrichment, we estimated MDN using a two-source linear mixing model in the same way that researchers have estimated MDN for other systems (Kline et al. 1990, 1993, Bilby et al. 1996, Hilderbrand et al. 1999a, Helfield and Naiman 2001). For our marine source $\delta^{15}N$ signature in soil we assumed that $\delta^{15}N$ of total N in soil was the same as $\delta^{15}N$ of salmon tissue. However, rather than assuming that $\delta^{15}N$ of marine-N in plants equaled $\delta^{15}N$ of salmon tissue, as other researchers have done, we used the regression relationships between $\delta^{15}N$ of total soil N and plant N to calculate $\delta^{15}N$ of salmon-N as it would appear in vegetation. As a result our MDN estimates in vegetation were relatively high, compared to $\delta^{15}N$ that would have been calculated with salmon $\delta^{15}N$ as the marine-N source signature. Our method was an improvement on previous

MDN calculations since we took into account isotopic changes between salmon tissue and vegetation. Two problems with accurate quantification of MDN were apparent.

First, unpredictable $\delta^{15}N$ variation resulted in low correlation between $\delta^{15}N$ of soil and $\delta^{15}N$ of some vegetation species. For example, $\delta^{15}N$ of salal was not significantly correlated with soil $\delta^{15}N$ so it could not be included in our MDN calculations. Similarly, although it was significant, the correlation between bunchberry and soil $\delta^{15}N$ was so low that the relationship did not provide very much predictive power. Low predictability of plant $\delta^{15}N$ based on soil total N $\delta^{15}N$ is one reason why our MDN estimates varied widely between sample types when we calculated MDN for several species of vegetation and soil at the same points. Ben-David et al. (1998b) also documented different $\delta^{15}N$ signatures in different species of vegetation growing in the same areas of Alaska, suggesting that vegetation utilized different N pools. Ben-David et al. (1998b) wrote that some plants appeared to utilize MDN while others did not. We add that fractionation of soil or plant N pools could have changed the MDN $\delta^{15}N$ signature enough for it to be mistakenly attributed to different MDN levels in some vegetation.

Secondly, the assumption that $\delta^{15}N$ of 100% MDN in the soil matched $\delta^{15}N$ of salmon tissue was probably not accurate, as evidenced by very high $\delta^{15}N$ in some samples. For example, at transect T3, $\delta^{15}N$ of soil and plants exceeded $\delta^{15}N$ of salmon. Since $\delta^{15}N$ of samples from T3 exceeded $\delta^{15}N$ of the marine-N source, MDN estimates for that site exceeded 100%. Any marine-derived nitrogen present in samples from T3 had either been fractionated so much that MDN had $\delta^{15}N$ greater than fresh salmon $\delta^{15}N$,

or the marine-derived N fraction was masked by some other N pool with a very high $\delta^{15}N$ signature. Similar fractionating or mixing processes could have occurred at lower levels in other samples without being recognized. Processes leading to elevated $\delta^{15}N$ could be mistakenly attributed solely to the presence of salmon-N if they did not cause $\delta^{15}N$ in samples to be higher than the assumed marine-N source signature.

It appears that ^{15}N fractionation and mixing, evident as high $\delta^{15}N$ variability, were large enough to prevent the reliable quantification of MDN. Therefore, the numerical results of our mixing model calculations are probably not individually accurate measures of MDN. We agree with Ben-David et al. (1998b) and Ben-David and Schell (2001) that calculations of marine derived nitrogen are best used as a relative index of marine-derived nutrients. However, given the context of our study, which includes several other studies documenting apparent MDN near other spawning streams, the numbers of salmon that spawned in the Koeye River, the presence of brown bears whose scraps and feces were visual signs of their salmon dispersal, and our data which showed a measurable elevation in $\delta^{15}N$ associated with bear trails and beds, we consider it reasonable to assert that MDN levels along bear trails and beds were in the range indicated by soil from most of our transects (5% to 56%, median = 25%, n = 9). Transect (T3), which had unusually high $\delta^{15}N$, was an outlier for which MDN estimates are unacceptable, probably due to ^{15}N elevation resulting from denitrification or other fractionating losses from water puddled on the trail at that transect. The assertion of about 25% MDN along bear trails and beds is subject to all of the errors in mixing model calculations described above and in a companion study (see previous chapter), and is not

reliable as a single, absolute measure of MDN on every bear trail or bed. However, our estimate is reasonable for the amount of MDN distributed onto bear trails within our study site. Our MDN estimate (and all other researchers' estimates) depend on the assumption that 100% MDN in the soil had $\delta^{15}N$ very similar to $\delta^{15}N$ of salmon tissue consumed by bears, and that $\delta^{15}N$ of soil at the reference transect was very similar to $\delta^{15}N$ of terrestrial N at all the other transects. Although both of those assumptions are questionable, the first is acceptable as long as isotope changes between urine, feces, and soil at the Koeye River are similar to isotope changes between feces and soil at Glendale River (see previous chapter). The second assumption, that terrestrial $\delta^{15}N$ in soil from our reference transect represents a constant terrestrial $\delta^{15}N$ along the Koeye River, is acceptable if terrestrial $\delta^{15}N$ did not differ substantially between most transects and the reference area. A second estimate of MDN (14%) near bear trails and beds, based on the difference in ^{15}N between the reference and spawning sites at the Koeye River (see previous chapter), supports the conclusion that overall rates of MDN input were within the range computed from $\delta^{15}N$ using the two-source mixing model (5% to 56%).

Conclusion

The results of this study are consistent with the hypothesis that brown bears transported substantial amounts of MDN into forests near salmon spawning rivers. Delta^{15}N at bear trails and beds was higher than $\delta^{15}N$ in the same type of sample from several meters away on both sides, and compared to $\delta^{15}N$ of a reference transect, supporting the assertion that bears have been significant vectors of MDN. In contrast,

$\delta^{13}C$ was not elevated near bear trails or beds, probably because C inputs from terrestrial vegetation were very large compared to marine-C inputs.

Although isotopic data from our transects qualitatively supports brown bear MDN inputs, quantification of MDN near bear trails and beds proved to be questionable based on $\delta^{15}N$ of soil or vegetation that we measured. For example, very high levels of $\delta^{15}N$ at some points indicated that N fractionation or N pool mixing led to $\delta^{15}N$ levels unacceptable for mixing model predictions using $\delta^{15}N$ of salmon tissue as the marine source $\delta^{15}N$ signature. We believe the linear two-source mixing model currently provides only limited quantitative information when used to calculate MDN on land. However, we attempted to estimate MDN by using mixing-model methods that included improvements on the methods of other researchers. Specifically, we improved previous estimates of mixing model endmembers by measuring N and $\delta^{15}N$ in soil, and including the possibility for changes in $\delta^{15}N$ of MDN between soil and plants. Relative to our reference soil $\delta^{15}N$, we calculated between 5% and 56% MDN along bear trails and beds, with a median of 25%. This estimate is reasonable for MDN in soil within 10 m of bear trails and beds, and is supported by an estimate of 14% MDN we computed from the total ^{15}N difference between reference and spawning sites at the Koeye River.

References

Allendorf, F. W., D. Bayles, and D. L. Bottom. 1997. Prioritizing pacific salmon stocks. Conservation Biology **11**:140-152.

Ben-David, M., R. T. Bowyer, L. K. Duffy, D. D. Roby, and D. M. Schell. 1998b. Social behavior and ecosystem processes: river otter latrines and nutrient dynamics of terrestrial vegetation. Ecology **79**:2567-2571.

Ben-David, M., T. A. Hanley, D. R. Klein, D. M. Schell. 1997. Seasonal changes in diets of coastal and riverine mink: the role of spawning Pacific salmon. Canadian Journal of Zoology **75**:803-811.

Ben-David, M., T. A. Hanley, and D. M. Schell. 1998a. Fertilization of terrestrial vegetation by spawning pacific salmon: the role of flooding and predator activity. Oikos **83**:47-55.

Ben-David, M., and D. M. Schell. 2001. Mixing models in analyses of diet using multiple stable isotopes: a response. Oecologia **127**:180-184.

Bilby, R. E., B. R. Fransen, and P. A. Bisson. 1996. Incorporation of nitrogen and carbon from spawning coho salmon into the trophic system of small streams: evidence from stable isotopes. Canadian Journal of Fisheries and Aquatic Science **53**:164-173.

Butler, D. R. 1995. Zoogeomorphology: animals as geomorphic agents. Cambridge University Press, Cambridge, Massachusetts, USA.

Cederholm, C. J., D. B. Houston, D. L. Cole, and W. J. Scarlett. 1989. Fate of coho salmon (*Oncorhynchus kisutch*) carcasses in spawning streams. Canadian Journal of Fisheries and Aquatic Science **46**:1347-1355.

Cederholm, C. J., D. H. Johnson, R. E. Bilby, L. G. Dominguez, A. M. Garrett, W. H. Graeber, E. L. Greda, M. D. Kunze, B. G. Marcot, J. F. Palmisano, R. W. Plotnikoff, W. G. Pearcy, C. A. Simenstad, and P. C. Trotter. 2000. Pacific

salmon and wildlife-ecological contexts, relationships, and implications for management. Special edition technical report prepared for D. H. Johnson and T. A. O'Neil (managment directors), wildlife-habitat relationships in Oregon and Washington. Washington Department of Fish and Wildlife, Olympia, Washington, USA.

Cederholm, C. J., M. D. Kunze, T. Murota, and A. Sibatani. 1999. Pacific salmon carcasses: essential contributions of nutrient and energy for aquatic and terrestrial ecosystems. Fishery Bulletin **24**:6-15.

Darimont, C. T. and P. C. Paquet. 2000. The Gray Wolves (*Canis lupus*) of British Columbia's Coastal Rainforests. Pages 1-62 *in* Findings from year 2000 pilot study and conservation assessment prepared for the Raincoast Conservation Society. Victoria, British Columbia, Canada.

Garten, C. T., Jr. 1992. Nitrogen isotope composition of ammonium and nitrate in bulk precipitation and forest throughfall. International Journal of Environmental and Analytical Chemistry **47**:33-45.

Garten, C. T., Jr. 1993. Variation in foliar ^{15}N abundance and the availability of soil nitrogen on Walker Branch watershed. Ecology **74**:2098-2113.

Gende, S. M., T. P. Quinn, and M. F. Willson. 2001. Consumption choice by bears feeding on salmon. Oecologia **127**:372-382.

Gholz, H. L. 1982. Environmental limits on aboveground net primary production, leaf area, and biomass in vegetation zones of the Pacific northwest. Ecology **63**:469-481.

Gilbert, B. K., and R. M. Lanner. 1995. Energy, diet selection and restoration of brown bear populations. Proceedings of a symposium: management and restoration of small and relictual bear populations presented at the ninth international conference on bear research and management, Grenoble, France, 19-22 October 1992. Museum of Natural History. Grenoble, France.

Handley, L. L., A. T. Austin, D. Robinson, C. M. Scrimgeour, J. A. Raven, T. H. E. Heaton, S. Schmidt, and G. R. Stewart. 1999. The ^{15}N natural abundance ($\delta^{15}N$) of ecosystem samples reflects water availability. Australian Journal of Plant Physiology **26**:135-210.

Handley, L. L., and S. X. Chang. 2000. Site history affects soil and plant ^{15}N natural abundance ($\delta^{15}N$) in forests of northern Vancouver Island, British Columbia. Functional Ecology **14**:273-280.

Handley, L. L., and C. M. Scrimgeour. 1997. Terrestrial plant ecology and ^{15}N natural abundance: the present limits to interpretation for uncultivated systems with original data from a Scottish old field. Advances in Ecological Research **27**:133-212.

Heaton, T. H. E. 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. Chemical Geology (Isotope Geoscience Section) **59**:87-102.

Helfield, J. M., and R. J. Naiman. 2001. Effects of salmon-derived nitrogen on riparian forest growth and implications for stream productivity. Ecology **82**:2403-2409.

Hildebrand, G. V., S. D. Farley, C. T. Robbins, T. A. Hanley, K. Titus, and C. Servheen. 1996. Use of stable isotopes to determine diets of living and extinct bears. Canadian Journal of Zoology **74**:2080-2088.

Hilderbrand, G. V, T. A. Hanley, C. T. Robbins, and C. C. Schwartz. 1999a. Role of Brown Bears (*Ursus arctos*) in the flow of marine nitrogen into a terrestrial ecosystem. Oecologia **121**:546-550.

Hilderbrand, G. V., S. G. Jenkins, T. A. Hanley, C. T. Robbins, and C. C. Schwartz. 1999b. Effect of seasonal differences in dietary meat intake on changes in body mass and composition in wild and captive brown bears. Canadian Journal of Zoology **77**:1623-1630.

Hilderbrand, G. V., C. C. Schwartz, C. T. Robbins, M. E. Jacoby, T. A. Hanley, S. M. Arthur, and C. Servheen. 1999c. The importance of meat, particularly salmon, to body size, population productivity, and conservation of North American brown bears. Canadian Journal of Zoology **77**:132-138.

Hogberg, P. 1997. Tansley Review No. 95 ^{15}N natural abundance in soil-plant systems. New Phytologist **137**:179-203.

Kendall, C. 1998. Tracing nitrogen sources and cycling in catchments. Pages 519-576 *in* C. Kendall and J. J. McDonnell, editors. Isotope tracers in catchment hydrology. Elsevier Science B. V., Amsterdam, Netherlands.

Kline, T. C., Jr. 2001. Assessing the role of salmon carcasses in the nitrogen budget of sockeye salmon (*Onchorhyncus nerka*) nursery lakes using stable isotopes. Abstract *in* American Fisheries Society 2001 International Conference April 24-26: Restoring nutrients to salmonid ecosystems. Eugene, Oregon, USA. http://

www.gpafs.org/confnutr/

Kline, T. C., Jr., J. J. Goering, O. A. Mathisen, P. H. Poe, and P. L. Parker. 1990. Recycling of elements transported upstream by runs of pacific salmon: I. $\delta^{15}N$ and $\delta^{13}C$ evidence in Sashin Creek, southeastern Alaska. Canadian Journal of Fisheries and Aquatic Science **47**:136-144.

Kline, T. C., Jr., J. J. Goering, O. A. Mathisen, P. H. Poe, P. L. Parker, and R. S. Scalan. 1993. Recycling of elements transported upstream by runs of pacific salmon: II. $\delta^{15}N$ and $\delta^{13}C$ evidence in the Kvichak river watershed, Bristol Bay, southwestern Alaska. Canadian Journal of Fisheries and Aquatic Science **50**:2350-2365.

Kline, T. C., Jr, J. J. Goering, and R. J. Piorkowski. 1997. The effects of salmon carcasses on Alaskan freshwaters. Pages 179-203 *in* A. M. Milner and M. W. Oswood, editors. Freshwaters of Alaska: ecological synthesis. Springer-Verlag, New York, New York, USA.

Lajtha, K., and J. D. Marshall. 1994. Sources of variation in the stable isotope composition of plants. Pages 1-21 *in* K. Lajtha and R. Michener, editors. Stable Isotopes in ecology and environmental science. Blackwell Science, Oxford, UK.

LUCO (British Columbia Land Use Coordination Office). 1999. Appendix V: revised study areas descriptions. Koeye. *In* Central Coast Land and Resource Management Plan: The Central Coast Protected Area Strategy (PAS) Report. http://www.luco.gov.bc.ca/lrmp/cencoast/reports/ccpasrpt/ccpasap5.htm#KOEYE

Mathisen, O. A., P. L. Parker, J. J. Goering, T. C. Kline Jr., P. H. Poe, and R. S. Scalan. 1988. Recycling of marine elements transported into freshwater by anadromous

salmon. Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie **23**:2249-2258.

MFO (British Columbia Ministery of Fisheries and Oceans) 1998. Statistical area 8: north coast salmon escapement. British Columbia, Canada. http://www.pac.dfo-mpo.gc.ca/ops/northfm/Areas/Area8/Default.htm

Nadelhoffer, K. J., B. A. Emmett, P. Gundersen, O. J. Kjonaas, C. J. Koopmans, P. Schleppi, A. Teitema, and R. F. Wright. 1999. Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests. Nature **398**:145-148.

Nadelhoffer, K., G. Shaver, B. Fry, A. Giblin, L. Johnson, and R. McKane. 1996. ^{15}N natural abundances and N use by tundra plants. Oecologia **107**:386-394.

Neilson, R., D. Hamilton, J. Wishart, C. A. Marriott, B. Boag, L. L. Handley, C. M. Scrimgeour, J. W. McNicol, and D. Robinson. 1998. Stable isotope natural abundances of soil, plants and soil invertebrates in an upland pasture. Soil Biology and Biochemistry **30**:1773-1782.

Olson, T. L., B. K. Gilbert, and R. C. Squibb, 1997. The effects of increasing human activity on brown bear use of an Alaskan river. Biological Conservation **82**:95-99.

Piorkowski, R. J. 1995. Ecological effects of spawning salmon on several south-central Alaskan streams. PhD Thesis. University of Alaska, Fairbanks, Alaska, USA.

Quinn, T. P., and M. T. Kinnison. 1999. Size-selective and sex-selective predation by brown bears on sockeye salmon. Oecologia **121**:273-282.

Robinson, D. 2001. $\delta^{15}N$ as an integrator of the nitrogen cycle. Trends in Ecology & Evolution **16**:153-162.

Rosing, M. N., M. Ben-David, and R. P. Barry. 1998. Analysis of stable isotope data: a K nearest-neighbors randomization test. Journal of Wildlife Management **62**:380-388.

Ruggerone, G. T., R. Hanson, and D. E. Rogers. 2000. Selective predation by brown bears (*Ursus arctos*) foraging on spawning sockeye salmon (*Oncorhynchus nerka*). Canadian Journal of Zoology **78**:974-981.

Tardiff, S. E., and J. A. Stanford. 1998. Grizzly bear digging effects on subalpine meadow plants in relation to mineral nitrogen availability. Ecology **79**:2219-2228.

Willson, M. F., and K. C. Halupka. 1995. Anadromous fish as keystone species in vertebrate communities. Conservation Biology **9**:489-497.

CHAPTER 4

CONCLUSION

In the nitrogen limited northwestern temperate rainforest (Chabot and Mooney 1985, Kimmins 1997, Vitousek et al. 1997, Handley et al. 1999), brown bears appear to move substantial amounts of marine-derived nitrogen onto their trails and beds, thus introducing nitrogen into the forest. We found increased $\delta^{15}N$ in soil and vegetation associated with bear trails and beds along the Koeye River in British Columbia, which agrees with the hypothesis that bears transport marine-derived N away from salmon spawning rivers. Not only do bears transport marine-derived nitrogen as they carry salmon products into the forest, but they also transport all the other nutrients found in salmon, such as carbon, potassium, phosphorous, and calcium, which cannot currently be traced from marine to terrestrial ecosystems using isotopic methods. In consequence of the transport of marine-derived nutrients by brown bears, terrestrial systems receive extra nutrient inputs. The spatial extent of N contribution by bears, based on transport along trails and beds, is unknown at a landscape scale. However, if brown bears moved all of the N we measured at bear trails and beds relative to the reference level, then bears probably added 5% to 56% MDN to the soil where they were active.

Since bears transport nutrients from salmon into the forest they link natural systems that have often been considered separate (Pringle 2001). Nitrogen transported by brown bears is evidence that marine nutrients flow against gravity through the land and water interface. A large-scale salmon ecosystem has been recognized that includes aquatic and terrestrial systems (Willson and Halupka 1995, Bilby et al. 1996, Ben-David

et al. 1998, Willson et al. 1998, Cederholm et al. 1999, 2000, Naiman et al. 2000, Reimchen 2000), but the role of brown bears as a link between the different components is just beginning to be established (Hilderbrand et al. 1999). The salmon ecosystem may include the activities of brown bears as an element in perpetuating nutrient transfers. Since bears are one of the factors linking rivers and forests, understanding brown bears, forests, or salmon rivers means considering a larger system that includes all three components.

Salmon, as the marine component of a larger system, provide nutrients to forests, but their interaction with other animals, such as brown bears, also has the potential to influence the forest in more ways than simply the introduction of nutrients. For example, in addition to fertilizing the forest, salmon bodies provide a focal point for predator and microbial activity (Piorkowski 1995, Willson and Halupka 1995, Cederholm et al. 2000). One result of brown bears' focus on spawning salmon is that the bears establish and fertilize a network of trails and beds along salmon spawning rivers and into nearby forests. Salmon-derived nutrient fertilizer distributed along the bear-trail network may affect microbial and vegetation diversity by providing a patchy resource base. The effects and extents of non-uniform dispersal of marine-derived nutrients by bears and other salmon predators remain to be studied.

Recognizing the effects of marine-derived nutrients along bear trails and beds depends on the amount of marine-derived nitrogen and other nutrients distributed by bears. While observation suggests that bears move large amounts of marine nutrients into forests, and nitrogen isotope evidence is also consistent with the idea that brown bears convey substantial salmon-N into the forest, actually quantifying those inputs

continues to pose problems. Using nitrogen isotope techniques to quantify marine inputs to terrestrial forests has a set of unique problems associated with isotopic fractionation. Net nitrogen isotope fractionation is so unpredictable in soil or vegetation samples that, although nitrogen isotopes indicate large-scale patterns of N source differences, quantitative precision is difficult. We showed, for instance, that a range of marine-derived nitrogen (MDN) estimates (5% to 142%) was possible for soil and plants from the same site. In this case MDN estimates were questionable since estimates of greater than 100% MDN indicated that fractionating processes had substantially altered $\delta^{15}N$ of salmon N. Similar alteration could have occurred in other samples without increasing MDN estimates above 100%, so the problem would not have been recognized, and higher $\delta^{15}N$ was attributed only to salmon-N. A simulation model further indicated that fractionation of N outputs, even at low levels, could substantially change MDN estimates (by more than 70%) while N inputs remained constant. Due to the low predictability of N isotope fractionation and the high potential for error resulting from N isotope fractionation in soil or plants, MDN estimates are considered indicative of relative amounts of marine-N rather than accurate quantities. At best, MDN estimates provide a range of possible MDN values. Along the Koeye and Glendale Rivers in British Columbia, elevated $\delta^{15}N$ can be interpreted as evidence that bears have transported MDN, and, although quantitative estimates of 5% to 56% MDN appear reasonable, they are subject to sampling errors and errors associated with isotopic fractionation. Even though we strongly suggest that MDN estimates cannot be precise given current knowledge and methods, we found that calculating MDN from the difference in N pools between spawning and reference sites provided an additional

quantitative check on MDN levels. Based on the difference in total ^{15}N pools, we estimated 14% MDN in the soil near bear trails and beds at the Koeye River, which was in the range of MDN values estimated from our $\delta^{15}N$ values. These values are also reasonably consistent with MDN estimates from isotopic studies by Hilderbrand et al. (15.5% to 17.8% in spruce within 500 m of spawning streams in Alaska; 1999), Helfield and Naiman (12% to 32% in riparian plants in Alaska; 2001), and Bilby et al. (17.5% in riparian plants in Washington; 1996). These MDN values (which were all computed from $\delta^{15}N$ data using a linear two-source mixing model) are also consistent with an estimate by Hilderbrand et al. (10% to 25% MDN input to the total riparian N budget; 1999) based on nitrogen budgets and the spatial distribution of bears. All of these estimates are based on coarse assumptions, but their general agreement supports the assertion that MDN provides a sizable portion of terrestrial N budgets. Our study specifically helps to verify that brown bears transport a considerable amount of MDN as they move salmon byproducts along trails and beds away from spawning rivers.

Suggestions for Further Study

The effects of the introduction of marine-derived nitrogen on forests are still not largely understood, so studies are needed to determine whether increased plant, animal, or microbial productivity and/or diversity results from MDN inputs. Since marine-N inputs could increase productivity of terrestrial vegetation and microbial communities, research is needed to test the hypothesis that vegetation and microbial communities are more productive near bear trails and beds where elevated $\delta^{15}N$ indicates higher levels of MDN. In addition, since bears transport N along their trails and at their beds, MDN

distribution by bears is patchy and might further enhance small-scale community differences within the forest. Higher diversity might be expected where patchy MDN inputs exist. Further study is needed to test the hypothesis that plant, animal, and/or microbial communities are more diverse where elevated $\delta^{15}N$ in bear trails and beds indicates inputs of MDN.

Further study is also needed to evaluate N isotope fractionation in soil and vegetation under conditions of the coastal rainforest so that N isotope information can be accurately evaluated quantitatively. Quantifying MDN in soil or vegetation under field conditions remains problematic because general patterns of N isotope fractionation have not been recognized, if they exist. Additional studies should include analysis of N isotopes in both soil and vegetation so that the N isotope relationship between soil and plants may be better determined. Researchers who undertake N isotope studies in the forests of salmon ecosystems should also consider sampling more than one type of vegetation since it has been documented that some plants appear to utilize MDN while others do not, and it is unknown whether patterns of MDN use remain constant for a species in different areas (Ben-David et al. 1998). In addition, development of methods for quantitatively tracing other marine-derived nutrients would provide another check on MDN estimates.

References

Ben-David, M., T. A. Hanley, and D. M. Schell. 1998. Fertilization of terrestrial vegetation by spawning pacific salmon: the role of flooding and predator activity. Oikos **83**:47-55.

Bilby, R. E., B. R. Fransen, and P. A. Bisson. 1996. Incorporation of nitrogen and carbon from spawning coho salmon into the trophic system of small streams: evidence from stable isotopes. Canadian Journal of Fisheries and Aquatic Science **53**:164-173.

Cederholm, C. J., D. H. Johnson, R. E. Bilby, L. G. Dominguez, A. M. Garrett, W. H. Graeber, E. L. Greda, M. D. Kunze, B. G. Marcot, J. F. Palmisano, R. W. Plotnikoff, W. G. Pearcy, C. A. Simenstad, and P. C. Trotter. 2000. Pacific salmon and wildlife-ecological contexts, relationships, and implications for management. Special edition technical report prepared for D. H. Johnson and T. A. O'Neil (managment directors), wildlife-habitat relationships in Oregon and Washington. Washington Department of Fish and Wildlife, Olympia, Washington, USA.

Cederholm, C. J., M. D. Kunze, T. Murota, and A. Sibatani. 1999. Pacific salmon carcasses: essential contributions of nutrient and energy for aquatic and terrestrial ecosystems. Fishery Bulletin **24**:6-15.

Chabot, B. F., and H. A. Mooney. 1985. Physiological ecology of North American plant communities. Chapman and Hall, New York, New York, USA.

Handley, L. L., A. T. Austin, D. Robinson, C. M. Scrimgeour, J. A. Raven, T. H. E. Heaton, S. Schmidt, and G. R. Stewart. 1999. The ^{15}N natural abundance ($\delta^{15}N$) of ecosystem samples reflects water availability. Australian Journal of Plant Physiology **26**:135-210.

Helfield, J. M., and R. J. Naiman. 2001. Effects of salmon-derived nitrogen on riparian forest growth and implications for stream productivity. Ecology **82**:2403-2409.

Hilderbrand, G. V., T. A. Hanley, C. T. Robbins, and C. C. Schwartz. 1999. Role of brown bears (*Ursus arctos*) in the flow of marine nitrogen into a terrestrial ecosystem. Oecologia **121**:546-550.

Kimmins, J.P. 1997. Forest ecology: a foundation for sustainable management. Prentice Hall, Englewood Cliffs, New Jersey, USA.

Naiman, R. J., R. E. Bilby, and P. A. Bisson. 2000. Riparian ecology and management in the Pacific coastal rainforest. BioScience **50**:996-1011.

Piorkowski, R. J. 1995. Ecological effects of spawning salmon on several south-central Alaskan streams. PhD Thesis. University of Alaska, Fairbanks, Alaska, USA.

Pringle, C. M. 2001. Hydrologic connectivity and the management of biological reserves: a global perspective. Ecological Applications **11**:981-998.

Reimchen, T. E. 2000. Some ecological and evolutionary aspects of bear-salmon interactions in coastal British Columbia. Canadian Journal of Zoology **78**:448-457.

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and D. G. Tilman. 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecological Applications **7**:737-750.

Willson, M. F., S. M. Gende, and B. H. Marston. 1998. Fishes and the forest. Bioscience **48**:455-462.

Willson, M. F., and K. C. Halupka. 1995. Anadromous fish as keystone species in vertebrate communities. Conservation Biology **9**:489-497.