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Soil biogeochemical responses to the deposition of anadromous fish carcasses in inland riparian forests of the Pacific Northwest, USA

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Abstract

The historic abundance of salmonids returning to natal streams of the inland Pacific Northwest, USA may have constituted a major nutrient influx into these commonly oligotrophic ecosystems. Inland ecosystems tend to be warmer and dryer as well as more nutrient limited than coastal ecosystems. Therefore, understanding how these inland conditions influence the soil biogeochemical responses to salmon carcass deposition in riparian forests is a vital step in appropriately mitigating for the loss of this nutrient subsidy. Deposition of salmon carcasses in central Idaho riparian forests resulted in a 480-fold increase in soil nitrogen, a seven-fold increase in dissolved organic carbon, and a four-fold increase in soil respired CO₂, with significant biogeochemical responses persisting at one year post amendment. Despite these large increases in soil N and C, estimates of soil nitrogen and carbon loading only accounted for 31% and 16% of the fish carcass nitrogen and carbon, respectively. However, the magnitude and persistence of soil biogeochemical responses to the deposition of anadromous fish carcasses in Central Idaho attests to the importance of this nutrient subsidy to inland riparian ecosystems of the Pacific Northwest.

Key words: anadromous fish, carcass decomposition, soil nitrogen, marine derived nutrients, riparian soil

Introduction

Setting ecologically defensible recovery goals for endangered species is aided by fully understanding their ecologic role within ecosystems. The 673 400 km² Columbia River Basin is believed to have once provided spawning habitat for up to 14.9 million anadromous fish annually (Gresh et al. 2000, NRC 1996), which equates to an annual influx of up to 3 million kg of nitrogen (N) and 10.5 million kg of carbon (C) to this basin (Dempson et al. 2009, Gresh et al. 2000). By contrast, the current anadromous fish returns to the Columbia River Basin, including hatchery fish, is less than 5% of historic abundance (FPS 2014). Recovery goals for several salmonid species have been set within the Columbia River Basin (Perry et al. 2003), yet little is known about the ecologic influence of salmonids in this largely semi-arid region (Koyama et al. 2005, Hilderbrand et al. 1996). This is a large knowledge gap considering that semi-arid inland ecosystems encompass over 80% of the historic natal range of anadromous fish in the Pacific Northwest, USA (NRC 1996).

Understanding the ecologic role of returning salmonids within riparian forests of the Columbia River Basin is particularly challenging. The use of stable N isotopes has affirmed the presence and utilization of salmonid derived N by vegetation in inland Columbia River Basin forests (Koyama et al. 2005) and resident grizzly bears (Hildebrand et al. 1996). Yet, relatively little is known about the effects of anadromous fish carcass deposition on soil N and C biogeochemistry outside of maritime coastal ecosystems (e.g. Holtgrieve et al. 2009, Gende et al. 2007, Drake et al. 2005). Applying the primary findings from these coastal investigations to inland semi-arid ecosystems would require the assumption that the climatic differences between these ecosystems would minimally influence biogeochemical processes. However, the observation of fish carcass decomposition in as little as 16 days in a semi-arid inland

environment relative to 76 days in a maritime coastal environment does not support this assumption (Wheeler et al 2014, Drake et al. 2005). The slower rate of carcass decomposition in coastal ecosystems and thus slower nutrient release rate could allow uptake by vegetation (Drake et al. 2006) and soil microbes (Kaye and Hart 1997) as well as nutrient losses through denitrification (Holtgrieve et al. 2009), leaching (Pinay et al. 2003), and possibly volatilization (Wheeler et al. 2014) to reduce the amplitude of coastal soil biogeochemical responses relative to inland ecosystems. Therefore, the application of coastal soil biogeochemical responses to inland ecosystems may under estimate the ecologic role of anadromous fish carcass deposition within inland semi-arid riparian ecosystems.

For these commonly nutrient limited inland ecosystems of the Pacific Northwest, the deposition of anadromous fish carcasses represents a substantial infusion of vital nutrients. These inland forests soils have very low concentrations of inorganic N, which limits vegetative (Moore and Mika 1991) and soil microbial productivity (Koyama et al. 2012, 2010, 2005; Stephan et al. 2012). There is further evidence that soil microbial productivity in this region is also co-limited by the availability of mineralizable C (Koyama et al. 2010). The deposition of anadromous fish carcasses laden with labile C in these inland forests could therefore be expected to stimulate measurable increases in microbial productivity. Further, the increased availability of labile C can stimulate or "prime" soil microbes into accessing otherwise recalcitrant soil N and C reserves (Blagodatskaya and Kuzyakov 2008). Nutrient poor soils are the most likely to experience soil microbial priming (Fontaine et al. 2011). Therefore, the deposition of anadromous fish carcasses in inland riparian forests could be expected to result in the increased mineralization of soil N and C reserves.

To better understand the soil biogeochemical responses of anadromous fish carcass deposition within semi-arid inland riparian forests of the Pacific Northwest the objective of this investigation was to measure the influences of anadromous fish carcass deposition on soil microbial productivity and the abundance of soil nitrogen and carbon. Specifically: 1) What influence does fish carcass deposition have on the abundance and form of N and C in the soil directly beneath the carcasses? 2) What influence does the carcass amendment have on soil microbial productivity? 3) Does the addition of organic C from the carcasses result in a priming of soil microbial productivity and the release of sequestered soil N? 4) How much of the N and C contained within the fish carcasses makes it into the soil where it is available for plant and microbial utilization?

Methods

This investigation was one of several collaborative research efforts within central Idaho, USA to quantify the ecosystem wide biogeochemical responses to salmon nutrient deposition within the oligotrophic North Fork Boise River watershed (Collins et al. 2016, Collins and Baxter 2014, Marcarelli et al. 2014, Wheeler et al. 2014). This 980 km² drainage ranges in elevation from 1060 to 2990 m a.s.l and resides on a large geologic formation referred to as the Idaho Batholith, which is predominantly granitic in composition and limits the availability of geologic nutrients (Collins et al. 2016, Marcarelli et al. 2014). Within this drainage, a 500 m study reach comprised of 500 m of contiguous stream channel and the adjacent riparian corridor was established along nine 1st – 3rd order tributaries which had been selected based on typical stream characteristic for the region (e.g. lithology, geomorphology, vegetation; Collins et al. 2016). These streams were randomly assigned as treatment or control streams. A subset of these streams were selected for

the riparian soil investigation based on similar geomorphic characteristics and established vegetative communities (Table 1).

Riparian study design

The riparian area was defined as the area from mean high water to where the vegetative community indicated a transitioned from hydric to semi-arid soil conditions (~10 - 50 m from the stream edge), typically in association with the toe slope of the valley or a substantial terrace. The study areas were characterized by type C4 streams within type II valleys (Rosgen and Silvey 1996). The riparian soils predominantly exhibited a shallow (0-4 cm) surface horizon of partially decomposed organic material dominated by a gravelly sandy loam with a subsoil layer to 38 cm of gravelly sandy loam (Wendt et al. 1975, 1973). Cobble and boulder sized granitic parent materials were often present within the shallow soil profile and visible across the landscape. The soil litter layer was notably minimal (< 1cm) to absent in all study reaches except Beaver Cr. which had localized areas of pine needle accumulation up to 5 cm deep. The riparian vegetative communities were open mixed conifer forests comprised of willows (*Salix* spp), dogwood (*Cornus* spp.), and tall grasses (*Festuca* spp.) (Marcarelli et al. 2014) residing under predominantly Douglas-fir (*Pseudotsuga menziesii*) and lodgepole pine (*Pinus contorta*) canopies. Regional precipitation and temperature data were acquired from Natural Resource Conservation Service (NRCS) SNOTEL climate stations at Graham Guard Station to the north of the study sites and Mores Creek summit to the west. All study areas were within 25 km of both climate stations. Additionally, each stream reach was instrumented with an air temperature sensor (HOBO Pro, Onset Computer Corporation, USA).

For this investigation, a series of 1 m² riparian soil plots were established along two streams targeted for anadromous fish carcass amendment (Little Beaver Cr/Trail Cr) and two

streams designated as reference streams (Banner Cr/Beaver Cr). The plots were established throughout the length of each study reach in association with conifer seedlings (*Pseudotsuga menziesii* and *Pinus contorta*) and an herbaceous perennial (*Equisetum arvense*) as part of a companion investigation. The riparian area along reference streams contained four plots while treatment streams contained four randomly assigned control (reference) and four treatment plots. This was a multi-year investigation which required the selection of new plots each treatment year to avoid cumulative disturbance and nutrient addition effects.

Hatchery spawned Steelhead (*Oncorhynchus mykiss*) carcasses were obtained from the Dworshak National Fish Hatchery in Ahsahka, Idaho, USA. The potential of transferring pathogens or disease between watersheds mandated the pasteurization or heating of the carcasses until the cranial cavity exceeded 60° C for 20 minutes (Marcarelli et al. 2014, Wheeler et al. 2014). For logistical and disease mitigation purposes the carcasses were also stored frozen both before and after treatment. These treatments have been reported to slightly influence initial carcass fluid loss and decomposition rates but no overall differences in decomposition timing or soil nutrient contributions were detected (Wheeler et al. 2014).

In 2008, an initial investigation into the attenuation of soil N with soil depth following the addition of fish carcass tissue was conducted. For this investigation, an amendment of pureed fish carcasses was applied to the soil plots in early August at a rate of 75 g N per plot or roughly 1.5 kg of carcass. The resultant area of applied slurry was approximately 30 cm x 30 cm to a thickness of approximately 2 cm. The carcasses were pureed to prevent scavenger removal. The blending process required the addition of deionized (DI) water, therefore an equivalent amount of DI water (1 liter) was added to the control and reference plots.

In 2010, following a lack of scavenger disturbance in previous plots, intact fish carcasses were applied at a rate of ~328 g N and ~1340 g C per plot (~164 g N and 670 g C per carcass). The carcasses (~3.2 kg each) were placed two per plot, parallel to each other, centered 30 cm apart and staked to hinder their removal by scavengers. Only one of the carcasses was removed by scavengers. This was a substantial increase in carcass deposition rate over the 2008 amendment but was considered reflective of potential historic bear deposition behavior based on observations near salmon streams in Alaska (Quinn et al. 2009, 2003; Holtgrieve et al. 2009; Gende et al. 2004). The control and reference plots received an application of 2 liters of DI water to control for any soil biogeochemical responses due to the moisture in the carcasses.

Soil sampling

To assess the change in soil N and C content, one 5 x 10 cm core was collected, using an AMS soil core sampler designed to minimize compaction (AMS, Inc., USA), from the periphery of each 1 m² plot one week prior to the fish carcass amendment and then directly beneath the amendment at two weeks, four weeks, and then approximately every four weeks until winter conditions inhibited access to the sites. Sampling resumed in the spring once all field sites were accessible. The soil cores collected from control and reference plots were randomly distributed within the plots. The soil cores in 2008 were separated into depths of 0-1 cm, 1-3 cm, and 3-8 cm and each portion sieved to 2 mm and homogenized with the similar portion of an adjacent plot prior to analysis for extractable inorganic N. The soil cores in 2010 were sieved to 2 mm and homogenized as a whole for extractable inorganic N, dissolved total nitrogen (DTN), total soil N and C as well as $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, dissolved organic carbon (DOC), acid hydrolysable C, and microbial biomass N and C. The release of soil respired CO₂ from the 2010 plots was also quantified.

176 *Soil N and C biogeochemical analysis*

177 Inorganic N (NH_4^+ and NO_3^-) was assessed by agitating 15 g of each sieved (2 mm) soil
178 sample in 50 ml 2M KCl for one hour. The solutions were then vacuum filtered to 0.45 μm and
179 stored at -20°C until analysis. Determination of inorganic N concentration for the 2008 samples
180 was conducted colorimetrically using a FIALab 2500, at the College of Agricultural and Life
181 Sciences, University of Idaho, Moscow, Idaho, USA. The 2010 samples were analyzed on a
182 Quickchem 8000, at the Lachat/TruSpec Service Center, Washington State University, Pullman,
183 Washington, USA. The soil dry mass of each extracted sample was determined by oven drying a
184 separate portion of each sieved sample at 70°C for 72 hours and back calculating based on
185 gravimetric moisture content.

186 Soil DTN and DOC as well as soil microbial biomass N and C were assessed using
187 modified procedures from Horwath and Paul (1994). To determine DTN and DOC, a 10 g
188 portion of each freshly sieved soil sample was agitated in 150 ml of 0.5 M K_2SO_4 for one hour
189 then vacuum filtered to 0.45 μm . This K_2SO_4 extractant to soil ratio (15:1) was higher than the
190 standard protocol (5:1) as it was anticipated that the nutrient concentrations in the treated soil
191 samples would exceed the ion exchange capacity of the K_2SO_4 using the established protocol
192 (Needleman et al. 2001). Microbial biomass N and C were assessed using the chloroform
193 fumigation-extraction method (Horwath and Paul 1994). In short, a 10 g portion of sieved soil
194 was fumigated with chloroform under vacuum for 5 days. The soils were then purged and
195 extracted as previously described. The microbial biomass N and C are reported as the difference
196 between fumigated and unfumigated concentrations. The extracts were stored at -20°C until
197 analysis. The soil dry mass of each extracted sample was determined as described for inorganic
198 N. The extracts were analyzed via combustion catalytic oxidation on a TOC-VCSH coupled with

a TNM-1 analyzer, (Shimadzu Scientific Instruments, USA) at the Land Management and Water Conservation Research Lab, USDA-ARS, Pullman, Washington, USA. Soil extractable organic N was calculated as the difference between DTN and inorganic N.

A portion of each fresh soil sample was freeze dried, ground in a ball mill, and combusted for total soil N and C as well as $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. Additionally, the acid hydrolysable portion of total soil C was assessed following Silbeira et al. (2008) and McLauchlan and Hobbie (2004). The relative proportion of the total C pool that was acid hydrolysable was calculated as:

(1) Relative hydrolysable C (%) = $(1 - \text{recalcitrant C} / \text{total C}) \times 100$.

The use of a linear two-member isotopic mixing model to estimate the proportion of salmon nutrient contribution to target nutrient pools (e.g. Gende et al. 2007, Koyama et al. 2005) requires the $\delta^{15}\text{N}$ of a marine end member. For the purposes of this investigation the mixing model relationship of:

(2) %MDN = $((\text{TEM} - \text{SEM}) / (\text{MEM} - \text{SEM})) \times 100$

was defined so that the treatment end member (TEM) is the $\delta^{15}\text{N}$ observed in the treatment soils, the soil end member (SEM) is the $\delta^{15}\text{N}$ observed in the control soils, and the marine end member (MEM) is the mean $\delta^{15}\text{N}$ of the fluids that were released from the salmon carcasses to the soil (Wheeler et al. 2014). The $\delta^{15}\text{N}$ values of whole treated steelhead carcasses as well as the fluids exiting the decomposing carcasses to the soil were determined following Wheeler et al. (2014).

All isotope analysis was conducted on a Finnigan MAT Delta plus Isotope Ratio Mass Spectrometer (Finnigan MAT GmbH, Germany) in the Idaho Stable Isotopes Laboratory, Moscow, Idaho, USA. The isotope values are reported versus the standards of atmospheric air and Vienna PeeDee belomnite (VPDB). The precision of the measurements was $\pm 0.2\text{‰}$ for $\delta^{15}\text{N}$ and $\pm 0.15\text{‰}$ for $\delta^{13}\text{C}$.

In 2010, soil respired CO₂ was measured using a Li-COR 6400 infrared gas analyzer (Li-COR, Lincoln, Nebraska, USA) prior to treatment and again at 33, 96, 300, and 361 days post treatment. To minimize soil disturbance and preferential path flow, two 10 cm x 5 cm sections of Polyvinyl chloride (PVC) pipe were installed two weeks prior to the start of the investigation in both the treatment and reference plots to a depth of 2cm. Within the treatment plots, one of the two anadromous fish carcasses was placed directly over the PVC collars. No soil cores were collected beneath these carcasses to avoid soil disturbance effects on respiration rate.

Statistical analysis

To test for the effect of anadromous fish carcass nutrients on the target soil biogeochemical properties, a mixed effects repeated measures analysis of variance (ANOVA) was utilized. The fixed variables were treatment and time, while the random variable was the individual plots through time. Time x treatment interactions were also assessed as a fixed variable. Time and time x treatment interactions were removed from the model if not significant. The model was nullified if the time x treatment interaction was significant. Significant overall differences between treatments and controls were investigated further using one-tailed t-tests at each sampling interval. All statistical conclusions were based on an a priori $\alpha = 0.05$ significance level. The data were assessed for assumptions of normality and homogeneity of variance using residual plots and Box-Cox transformed as necessary prior to analysis. Analysis was conducted using R version 2.10.0 (R Foundation for Statistical Computing, Austria). Additionally, the mean isotopic composition of fish carcass fluids entering the soil was tested against the whole reference carcasses using the summary statistics functionality of Student's t-tests in Minitab 16 (Minitab Inc., USA). The mean isotopic composition of the fluids was determined as:

244 (3) $\overline{\delta^{15}N} = \frac{\bar{x}_1 + \bar{x}_2 \dots \bar{x}_n}{n}$, where $\bar{x}$ is the mean of daily observations and n is the number of days
245 observed.

246 *Soil N and C loading estimates*

247 The instantaneous loading of soil N per fish carcass at each sampling interval was
248 estimated utilizing the mean observed soil total N concentration, following Gende et al. 2007.
249 These estimates were calculated utilizing the mean values for all parameters in order to eliminate
250 the complexity of compounding variance.

251 The instantaneous N loading was estimated from the difference between pre and post
252 treatment soil N concentrations observed directly beneath the decomposing carcasses and scaled
253 to the estimated influenced soil mass. This soil mass was comprised of the soil directly beneath
254 the carcasses and the soil 10 cm horizontally from the edge of the carcasses, both to a depth of 10
255 cm. The depth of 10 cm was derived from the observed inorganic N concentrations with depth
256 following the 2008 nutrient amendment (see results). The extent of lateral N movement in
257 coastal soils has been reported at 20-40 cm from the edge of the carcass (Gende et al. 2007,
258 Drake et al. 2005). However, it was assumed that the gradient of lateral nutrient loading was
259 similar to vertical loading based on the apparent retentiveness of the study soils and 10 cm
260 therefore conservatively adopted as the horizontal extent of N accumulation.

261 The area of the carcasses (770 (164 stdev) cm²) was determined by importing field
262 images of the carcasses into Image J 1.47 (National Institute of Health, USA). Applying the soil
263 vertical and horizontal assumptions to the surface area occupied by the carcasses yielded a soil
264 volume below the carcasses of 7 700 cm³ and a horizontal soil volume of 17 800 cm³. The bulk
265 density of the soil (1.22 (0.2 SE) g cm⁻³) was determined by collecting intact 5x10 cm cores from
266 three plots in each treatment stream and drying them at 70° C for 72 hours. These parameters

yielded a soil mass below the carcasses of 9.4 kg ($7\,700\text{ cm}^3 \times 1.22\text{ g cm}^{-3}$) and a soil mass horizontal from the carcasses of 21.7 kg ($17\,800\text{ cm}^3 \times 1.22\text{ g cm}^{-3}$). This soil mass was then multiplied by the observed or assumed soil N concentration, with the soils horizontal from the carcasses assumed to reach a N concentration of approximately 50% of that observed directly beneath the carcasses based on the attenuation of inorganic N concentration with depth observed following the 2008 nutrient amendment. Instantaneous soil C concentrations were also estimated using the assumptions developed for the N loading estimates.

The microbial mineralization and consumption of fish carcass N within the soil was further explored utilizing reported relationships for soil microbial mineralization and ammonium consumption by Booth et al. (2005) where:

$$(4) \log \text{min (mg N kg}^{-1} \text{ dry soil d}^{-1}) = 0.540 \times \log (\text{microbial biomass N in g kg}^{-1}) + 1.08$$

$$(5) \log \text{NH}_4^+ \text{ cons (mg N kg}^{-1} \text{ dry soil d}^{-1}) = 0.48 \times \log (\text{microbial biomass N in g kg}^{-1}) + 1.102.$$

It is recognized that these estimates of N loading as well as gross soil microbial mineralization and consumption are based on a number of assumptions and simplifications. However, the findings offer valuable insight into the potential soil biogeochemical responses following anadromous fish carcasses deposition in riparian forests.

Results

In the two weeks following the 2008 treatment the average daily temperature at Mores Creek summit was 18.7° C (daily range 6.5 – 30.0) with 0.0 cm of precipitation reported and the average daily temperature at Graham Guard Station was 15.5° C (daily range 0.3 – 31.0) with 1.0 cm of precipitation reported (Table 2) (NRCS 2012). During the two weeks following the 2010 treatment the average daily temperature at Mores Creek summit was 16.6° C (daily range 3.1 – 29.7) with 1.8 cm of precipitation reported and the average daily temperature at Graham Guard

Station was 14.9° C (daily range 1.0 – 31.1) with 2.3 cm of precipitation reported (Table 2) (NRCS 2012). The climatic conditions following the 2010 treatments led to the rapid decomposition of the fish carcasses. At 11 days post treatment all that remained of the carcasses were skeletal and skin fragments.

2008 Soil N responses

The 2008 investigation provided insight into the N retentiveness of these inland riparian soils as well as the depth of soil inorganic N accumulation. The soils in this region appear to quickly attenuate inorganic N concentrations, with the peak concentrations of inorganic N at 3-8 cm depth only reaching 25% of the peak concentrations in the soil 0-1 cm deep. The peak in inorganic N concentration across all depths occurred at approximately 30 days post treatment. Plotting the net increases from pretreatment concentrations of inorganic N at 30 days post treatment for all three depths as a function of the average soil depth revealed a surprisingly linear decline in inorganic N concentrations with depth, suggesting that the extent of detectable inorganic N accumulation terminated at around 8.7 cm (Figure 1).

2010 Soil N and C responses

The 2010 placement of fish carcasses in these riparian forests yielded significant overall increases in soil extractable and total N (Table 3). By 33 days post treatment, inorganic N had increased by 480 fold with a net increase from pretreatment concentrations of 916 (219 SE) mg N kg⁻¹ dry soil for a total concentration of 918 (218 SE) mg N kg⁻¹ dry soil (Figure 2A). By 60 days post treatment, total extractable N (DTN) and bulk soil N had increased by 1025 (313 SE) and 1556 (649 SE) mg N kg⁻¹ dry soil respectively, with respective total soil concentrations of 1035 (313 SE) and 3634 (751 SE) mg N kg⁻¹ dry soil (Figures 2A and B). Soil N had begun to decrease prior to the onset of winter but did not change substantially between the November and

May samplings and all three N pools remained significantly elevated at one-year post treatment (Table 3). Soil inorganic N was predominantly ammonium-N. Nitrate-N was not significantly elevated until 300 days post treatment when it reached (24.2 (4.2 SE) mg kg⁻¹ dry soil) and remained at this level at one year post treatment.

The soil total C pool (38 (3.5 SE) g kg⁻¹ dry soil) was not significantly influenced by the treatment. However, separating this large soil C pool into major fractions of recalcitrant, slow, and active C pools revealed that the fish carcass C contribution did significantly influence the most labile active pool, with DOC increasing nearly 7 fold from 119 (39 SE) to 820 (228 SE) mg C kg⁻¹ dry soil by 60 days post treatment (Figure 3, Table 3). Treatment soil DOC concentrations had decreased substantially to 209 (98 SE) mg C kg⁻¹ dry soil by the onset of winter but remained significantly elevated over control soils through one year post treatment (Table 3). The total soil C:N ratio within treatment soils significantly decreased from 21.0 (1.3 SE) prior to treatment to 14.6 (0.8 SE) at 13 days post treatment and remained at this level through one year post treatment (Table 3).

Soil microbial responses

The increases in soil N and C resulted in significant soil microbial responses. These results are interpreted cautiously as random fumigation failures resulted in instances of irrefutable microbial consumption rather than liberation of N and C, thus leading to the removal of 17 samples from the microbial biomass N analysis and 4 samples from the microbial biomass C analysis. However, the magnitude of the microbial biomass N increases following the treatment lends to confidence in the conclusions. Within these constraints, the soil microbes were showing indications of N accumulation at 13 days post treatment and microbial biomass N had significantly increased by 33 days post treatment (Figure 4, Table 3). Additional evidence of

increased soil microbial activity was derived from the evolution of CO₂ from the soil, which had increased 4 fold by 33 days post treatment and remained elevated near the onset of snow pack (Figure 5, Table3). Interestingly, there were no differences detected in microbial biomass C between the treated and control soils, potentially due to substantial temporal variability (± 100 mg C kg⁻¹ dry soil) between sampling intervals within both treatment and control plots. However, the soil microbial biomass C:N ratio decreased from 5.4 (0.6 SE) pretreatment to 3.6 (0.7 SE) by 300 days post treatment suggesting that the soil microbial biomass was accumulating N disproportionately to C.

Soil and fish carcass isotopes

Soil $\delta^{15}\text{N}$ increased substantially following the carcass addition from 2.4‰ (0.9 SE) pretreatment to 6.7‰ (2.4 SE) at 13 days post treatment and a maximum of 9.2‰ (3.4 SE) at 96 days post treatment (Figure 6, Table 3). The treatment soil $\delta^{15}\text{N}$ remained significantly elevated (7.4‰ (1.3 SE)) relative to control soils (2.8‰ (0.8 SE)) at one year post treatment. Soil $\delta^{13}\text{C}$ (-26.3‰ (0.5 SE)) was not influenced by the carcass addition.

The $\delta^{15}\text{N}$ of the carcass fluids entering the soil were significantly elevated (df=9, t=-2.15, $P=0.03$) relative to whole carcasses. The whole fish carcasses had a $\delta^{15}\text{N}$ of 10.4‰ (0.2 SE) while the mean $\delta^{15}\text{N}$ of the carcass fluids entering the soil was 11.9‰ (0.6 SE). The C:N ratio of the whole fish was 4.08 (0.07 SE).

Soil N and C loading estimates

The estimates of instantaneous soil N loading beneath and surrounding the carcasses revealed a N loading of 11.5 g N, 25.9 g N, and 31.5 g N per fish at 13, 33, and 60 days post treatment, respectively (Table 4). The estimates of soil N loading peaked at the 60 day post treatment observation.

By comparison, utilizing the isotopic mixing model (equation 2), where the marine end member (MEM) is the mean $\delta^{15}\text{N}$ of the fluids (11.9 ‰) that were released from the salmon carcasses to the soil, suggests that the proportion of total soil N comprised of anadromous fish N at 60 days post treatment was 69%. Applying this proportion to the total soil N at 60 days post treatment of 3.6 (0.7 SE) g N kg⁻¹ dry soil and the estimate of influenced soil volume as done previously, the mixing model suggests a mean soil N loading of 50.9 g N per fish at 60 days post treatment (Table 4).

Expanding upon the instantaneous N loading rates utilizing the relationships of Booth et al. (2005) yielded an average daily mineralization rate (equation 4) over the first 13 days of 2.1 (1.7 – 2.5) mg N kg⁻¹ dry soil d⁻¹ (Table 5). This estimate would yield a gross mineralization of N within the projected carcass influenced soil volume of 2 974 mg N in the 13 days following carcass deposition. The peak gross mineralization rate based on this relationship was 3.7 mg N kg⁻¹ dry soil d⁻¹ at 96 days post treatment (Table 5). During the same time period (0-13 days post treatment) the estimate of average ammonium consumption (equation 5) was 2.6 (2.2 – 3.0) mg N kg⁻¹ dry soil d⁻¹ or 3 806 mg N by day 13. The estimated peak in soil microbial ammonium consumption reaching 4.5 mg N kg⁻¹ dry soil d⁻¹ at 96 days post treatment (Table 5).

Calculating the net extractable C loading, as done with total N, suggests instantaneous C loadings of 5.8, 11.6, and 14.2 g C per fish at 13, 33, and 60 days post treatment, respectively (Table 4). Using the C:N of the whole fish carcasses (4.08) and the total soil N loading estimates, C loadings of roughly 46.9, 105.6, and 128.5 g C per fish would have been expected in the absence of C consumption at 13, 33, and 60 days post treatment, respectively. Extrapolating the observed peak soil respiration rate (10.59 $\mu\text{mol m}^{-2} \text{s}^{-1}$) to the surface area of the carcasses (770 cm²) and the 10 cm surrounding the carcasses (1780 cm²) with the assumptions that soil

respiration was limited by microbial biomass rather than nutrient concentrations and environmental conditions, the soil respiratory loss of C could account for up to 2.8 g C d⁻¹ or up to roughly 92.5 g C by 33 days post treatment.

Discussion

The magnitude and persistence of soil biogeochemical responses to the deposition of anadromous fish carcasses in Central Idaho far exceeded reported responses in coastal ecosystems of the Pacific Northwest (e.g. Holtgrieve et al. 2009, Gende et al. 2007, Drake et al. 2005). The dramatic 480-fold increase in inorganic N and nearly two-fold increase in total soil N as well as the persistence of these nutrients in the soil for at least a year attests to both the nutrient limitations of these riparian forests as well as the potential ecologic importance of this nutrient subsidy. The changes in soil C concentrations were not as dramatic as the changes in soil N, with only dissolved organic C substantially increasing roughly seven-fold. However, the organic nutrient amendment did stimulate soil microbial productivity for at least three months as is evident from the four-fold increase in soil respiration and the shift in the microbial biomass C:N ratio from 5.4 (0.6 SE) to 3.6 (0.9 SE). Unfortunately, the persistence of elevated N and C levels through the duration of the observations left us unable to confirm the liberation of sequestered soil nutrients through soil microbial priming. Surprisingly, the estimates of the N and C contributed to the soil in the immediate vicinity of the carcasses at most only accounted for 31% and 16% of the total N (~164 g N) and C (~670 g C) that was available in the fish carcasses, respectively.

The observations of soil inorganic N accumulation as well as the estimated rates of soil microbial N mineralization and consumption elucidate the biogeochemical responses during riparian fish carcass decomposition. At 13 days post treatment (two days following carcass

skeletonization), total soil N had increased by 569.8 (330 SE) mg N kg⁻¹ dry soil (Figure 2B), which was primarily attributed to an increase in inorganic N concentrations of 561.3 (109 SE) mg N kg⁻¹ dry soil (Figures 2A). This increase in total soil N yielded an estimate of soil N loading at 13 days post treatment of 11.5 g N per fish carcass. Surprisingly, the estimate of gross soil N mineralization during this time period (Day 0-13) only accounted for a cumulative mineralization of 2.97 g N, which would have been cancelled out by an estimated gross consumption of 3.8 g N (equations 4 and 5, Table 5). These estimates suggest that soil microbial mineralization was not responsible for the abundance of mineralized N within the soil and that the vast majority of N released from the carcasses was already in a mineralized form. This initial flush of mineralized N can likely be attributed to the accumulation of metabolic wastes produced by fly larvae and microbes during the decomposition of the fish carcasses. Fly larvae in particular are reported to be ravenous consumers of fish carcass tissue (Collins and Baxter 2014, Lisi and Schindler 2011, Hocking and Reimchen 2006) and through the excretion of ammonia rich metabolic waste were likely responsible for the observed pulse of mineralized N.

It was also interesting that the level of mineralized N in the soil continued to increase to an observed peak at 33 days post treatment despite the carcasses flesh being fully decomposed at 11 days post treatment, leaving only the dried skin and skeleton. In order for the soil inorganic N to reach its peak of 918 mg N kg⁻¹ dry soil at 33 days post treatment, gross soil mineralization would have needed to exceed ammonium consumption on average by 18 mg kg⁻¹ dry soil d⁻¹ between 13 and 33 days post treatment. However, soil net mineralization rates of this magnitude were not supported by the estimated rates of soil mineralization and consumption (Table 5) or the minimal accumulation of soil organic N (Figure 2A) and soil microbial N (Figure 4) at 13 days post treatment. The substantial increase in soil inorganic N without explanatory increases in

soil mineralization rates would indicate the presence of intermediate mineralized N reservoirs which delayed entry into the soil. There are a couple of likely reservoirs for the additional observed mineralized N in the soil. The removal of the greater than 2 mm coarse soil fraction prior to soil sample analysis would have excluded surface organic material from the analysis. This surface organic material could have served as a storage medium for N rich fluids leaving the fish carcasses. The greater than 2 mm organic fraction of the soil samples was a small portion of the overall soil samples (roughly 1-5% by volume) but future efforts would be judicious to quantify the labile nutrient content of this potential nutrient reservoir.

The sieving of the soil samples also removed the nutrient laden fly larvae that had burrowed into the soil following carcass skeletonization. It is notable that the abundance of the fly larvae was quite high with a 5 x 10 cm treatment soil core generally containing a visually estimated 100 – 200 fly larvae at 13 days post treatment. The release of N from the coarse soil fraction through either leaching or decomposition as well as the release of metabolic wastes by fly larvae most likely account for the observed increase in mineralized soil N in the weeks following skeletonization. The estimated abundance of fly larvae in the soil samples declined to fewer than a dozen larvae per soil core between the samples collected at days 33 and 60. As the number of larvae declined in the soil, a significant increase in adult fly abundance was measured (Collins and Baxter 2014). These observations of fly larvae decline and adult fly abundance in conjunction with the peak in observed soil N concentrations at 60 days post treatment are suggestive of flies acting as a nutrient reservoir and distribution network. Considering the sizeable amount of N in question and the ability of flies to redistribute this nutrient broadly within the ecosystem, further understanding of this phenomenon is warranted.

In warm semi-arid inland ecosystems volatile N loss can be expected. An abundance of mineralized N at the soil surface or within the carcass cavity can result in atmospheric N loss and a change in the isotopic ratio of the N contribution leaching into the soil. Salmon typically spawn in this region during the late summer to fall when climate conditions are the driest and warmest of the year (Fulton 1968). For example, the daily maximum temperatures during the first 30 days of this investigation were frequently 30-34° C within the study areas. These hot temperatures promote the volatile loss of ammonia during and in the weeks following carcass decomposition (Miles et al. 2011, Zhang et al. 2013). The high accumulation of N near the soil surface as suggested by the 2008 investigation would further increase the potential for volatile loss of ammonia. The observed 4 ‰ $\delta^{15}\text{N}$ enrichment of the carcass fluids entering the soil, as reported by Wheeler et al. (2014) and the 1.5 ‰ $\delta^{15}\text{N}$ enrichment observed in this investigation, suggests that discriminatory loss of N is occurring to the atmosphere. However, to our knowledge the magnitude of volatile ammonia loss following anadromous fish carcass deposition has not been directly investigated.

The potential magnitude of volatile ammonia loss and the enrichment in $\delta^{15}\text{N}$ due to discrimination is particularly important when considering the use of an isotopic mixing model to estimate the utilization of anadromous fish nutrients by ecosystem components in semi-arid ecosystems. For example, utilizing the linear two-member mixing model (equation 2) with the mean $\delta^{15}\text{N}$ of the fluids (11.9 ‰) as the marine end member (Wheeler et al. 2014) yielded a soil N loading of 50.9 g N per fish at 60 days post treatment. Whereas utilizing the mean $\delta^{15}\text{N}$ of whole carcasses (10.4 ‰) would yield a soil N loading estimate of 60.5 g N per fish at 60 days post treatment. By accounting for the isotopic enrichment that is occurring during decomposition, estimated N deposition rates decline by almost 16%. It is apparent from these

estimates that additional consideration for isotopic enrichment when using isotopic mixing models to estimate anadromous fish nutrient utilization in semi-arid ecosystems would be judicious.

Clearly, the estimation methods utilized for this investigation yielded a range of soil N loading estimates (31.5-50.9 g N) for the soil in close proximity to the anadromous fish carcasses that were considerably lower than the total N contained within each fish carcass (~164 g N). However, it is emphasized that diffuse pathways of nutrient redistribution within the ecosystem as well as nutrients bound within the greater than 2 mm fraction of soil removed during sieving would not be accounted for in these estimates (Whitney and Zabowski 2004). This would include a large number of fly larvae that were found in the upper 5 cm of soil with nutrient laden tissue and digestive tracts full of nutrient rich fluids as well as nutrients removed from the sample area by fly larva migration, adult fly emergence, scavengers, and tertiary consumers. It is apparent from the findings of this investigation that future efforts to reconcile the riparian nutrient budget following anadromous fish deposition would benefit greatly from the inclusion of fly larvae influences on nutrient dynamics as well as a full accounting of soil biogeochemical properties.

Carbon loading

One of the discussions that is largely lacking from the salmon nutrient literature is that of the influences of the labile carbon contribution from anadromous fish carcasses on soil nutrient cycling. This may be due to the large size of the resident soil C pool and its ability to obscure the influence of a salmon C contribution (Holtgrieve et al. 2009, Gende et al. 2007) or due to the rapid biotic utilization of this highly labile C subsidy (Collins and Baxter 2014, Lisi and Schindler 2011, Hocking and Reimchen 2006). Within the study plots the pre-treatment total soil C was 38 (0.9 SE) g C kg⁻¹ dry soil, which equates to roughly 1,200 g C within the estimated

carcass influenced soil mass. The treatment fish carcasses contained roughly 670 g C. This makes anadromous fish a potentially sizable C subsidy to these riparian soils. However, only a small portion (up to 14.2 g C) of this contribution was observed in the soil or accounted for in the observations of soil respired CO₂ (up to 2.8 g C d⁻¹). It is likely that a sizable portion of the missing C was metabolized above ground by fly larvae and microbes during carcass decomposition (Collins and Baxter 2014, Lisi and Schindler 2011, Hocking and Reimchen 2006). The large pulse of inorganic N into the soil during decomposition would support this assertion of substantial metabolic C utilization. Our inability to detect significant contributions of fish carcass C to the larger soil C pools or microbial biomass C leaves the remaining fates of the missing C as most likely related to the removal of the greater than 2 mm soil fraction and fly larvae during sieving, as well as the diffuse movement of C during adult fly emergence (Collins and Baxter 2014). It is apparent from these observations that the C within anadromous fish carcasses is quickly utilized within these inland riparian forests.

We were unable to confirm the liberation of sequestered N or C due to soil microbial "priming". The observed increases in soil N and C were easily attributed to the liberation of N and C from the carcasses. Further, with the total soil N concentrations remaining elevated at one year post treatment, relative to pretreatment and reference concentrations, it was not possible to determine if sequestered soil N was being liberated. The size of the soil recalcitrant and slow C pools also largely limited the ability to detect C liberation from these pools. However, it is notable that DOC remained elevated at one-year post treatment while soil respiration had returned to background levels.

Implications

The elevated levels of soil N and C at one-year post treatment within these inland soils demonstrates the N retentiveness of these ecosystems as well as the long term effects a fish carcass deposition event has on the availability of soil N. This availability and persistence of N would not only benefit the vegetative species capable of capitalizing on and storing this pulse of N (Drake et al. 2006) but would also ensure that species with annual or perennial life cycles would benefit from available N during the subsequent growing season. The resultant increases in vegetative biomass production can lead to long-term increases in both above ground and below ground productivity and changes in biodiversity through biotic feedbacks (Roe et al. 2010, Bump et al. 2009, Kaye and Hart 1997, Hobbie 1992). A salmon carcass has been demonstrated to have a localized influence on soil nutrients (Gende et al. 2007, Drake et al. 2005). However, isotopic and observational investigations in spawning habitats with active bear populations suggest that salmon carcasses can be randomly deposited up to several hundred meters from the stream edge (Koyama et al. 2005, Gende et al. 2004, Gende and Quinn 2004, Bilby et al. 2003, Hilderbrand et al. 1999). This annual distribution of numerous fish carcasses as well as additional diffuse pathways of nutrient redistribution would therefore collectively benefit forested ecosystems by increasing nutrient deposition to a large spatial extent of the soil and plant community.

The historic abundance of salmonids returning to spawn along approximately 21 325 km of historically accessible stream habitat (NRC 1996) within the Columbia River Basin has been estimated at between 8 – 14.9 million fish (Gresh et al. 2000, NRC 1996). If we assume the mean riparian area of greatest deposition is within 50 m of the stream (Gende et al. 2004, Bilby et al. 2003, Hilderbrand et al. 1999) and a carcass deposition rate of 25% of the run (Quinn et al. 2009, 2003), there would have been an average heterogeneous deposition across the Columbia River Basin of 93-175 carcasses $\text{ha}^{-1} \text{yr}^{-1}$ within these riparian corridors. Using the estimated soil

contribution of 31.5 g N per fish, this equates to roughly 3-5 kg N ha⁻¹ yr⁻¹ within these riparian corridors. An additional influx of N would also be expected from metabolic wastes derived from carcasses consumed within the stream (Quinn et al. 2009, Hocking and Reimchen 2006, Hilderbrand et al. 1999). By comparison, the vast majority of the Pacific Northwest is reported to receive less than 1 kg N ha⁻¹ yr⁻¹ from atmospheric deposition with National Trends Network stations within 80 km of the study area reporting annual inorganic N deposition rates since the year 2000 of 0.39-2.17 kg N ha⁻¹ yr⁻¹ (NADP 2017, Galloway 2004). The accumulation of N in semi-arid inland forests from N-fixation is also suspected to be quite low at less than 2 kg ha⁻¹ yr⁻¹ (Jurgensen et al. 1990). This places the deposition of anadromous fish carcasses as a potential major contributor of N to these inland riparian ecosystems.

Conclusion

The results of this investigation demonstrate the magnitude of influence the deposition of salmon carcasses has on the soil biogeochemistry of inland oligotrophic forests of the Pacific Northwest. This investigation revealed a 480 fold increase in soil available N directly below anadromous fish carcasses and the elevated persistence of soil N at one year post treatment. The availability of soil N into the subsequent growing season increases the accessibility of N to vegetative species with differing phenology. We observed a dramatic increase in microbial activity as indicated by a nearly 4 fold increase in soil respiration and a 4 fold increase in microbial biomass N but we did not confirm a "priming" effect in the sense of microbial liberation of pre-existing N and C reserves. A large portion of the N and C in the carcasses was not accounted for in the soil loading estimates. An undetermined amount of this N and C may have been lost through metabolic processes and volatilization. However, a considerable amount of this N and C may have also been exported from the plots to other parts of the forest through diffuse pathways such

564 as the emigration of fly larvae and through consumption by other insects and scavengers. It is
565 apparent from these observations that mitigation efforts in inland ecosystems would benefit from
566 further investigation into the influence of salmon derived nutrients in semi-arid inland riparian
567 forests, particularly in regard to soil microbial responses following salmon carcass deposition as
568 well as the potential for volatile N loss. The deposition of a salmon carcass is a localized event
569 but through the N retention of these inland ecosystems and dispersal mechanisms salmon have
570 historically enriched the productivity of riparian forests.

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References

- Bilby, R.E., Beach, E.W., Fransen, B.R., Walter, J.K., and Bisson, P.A. 2003. Transfer of nutrients from spawning salmon to riparian vegetation in western Washington. *Transactions of the American Fisheries Society* 132(4):733-745.
- Blagodatskaya, E., and Kuzyakov, Y. 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. *Biology and Fertility of Soils* 45(2): 115-131.
- Booth, M.S., Stark, J.M., and Rastetter, E. 2005. Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. *Ecological Monographs* 75(2): 139-157.
- Bump, J.K., Peterson, R.O., and Vucetich, J.A. 2009. Wolves modulate soil nutrient heterogeneity and foliar nitrogen by configuring the distribution of ungulate carcasses. *Ecology* 90(11): 3159-3167.
- Collins, S.F., Baxter, C.V., Marcarelli, A.M., and Wipfli, M.S. 2016. Effects of experimentally added salmon subsidies on resident fishes via direct and indirect pathways. *Ecosphere* 7(3).
- Collins, S.F., and Baxter, C.V. 2014. Heterogeneity of riparian habitats mediates responses of terrestrial arthropods to a subsidy of Pacific salmon carcasses. *Ecosphere* 5(11).
- Dempson, J. B., Braithwaite, V.A., Doherty, D., and Power, M. 2009. Stable isotope analysis of marine feeding signatures of Atlantic salmon in the North Atlantic. *ICES Journal of Marine Science* 67:52-61.
- Drake, D. C., Smith, J.V., and Naiman, R.J. 2005. Salmon decay and nutrient contributions to riparian forest soils. *Northwest Science* 79(1):61-71.
- Drake, D. C., Naiman, R.J., and Bechtold, J.S. 2006. Fate of nitrogen in riparian forest soils and trees: an ^{15}N tracer study simulating salmon decay. *Ecology* 87(5):1256-1266.

- 602 Fish Passage Center (FPS). 2014. Columbia Basin Fisheries Agencies and Tribes. Annual adult
603 salmon totals. <http://www.fpc.org/>. Updated February 20, 2014. [Accessed March 26, 2014].
- 604 Fontaine, S., Henault, C., Aamor, A., Bdioui, N., Bloor, J.M.G., Maire, V., Mary, B., Revailiot,
605 S., and Maron, P.A. 2011. Fungi mediate long term sequestration of carbon and nitrogen in
606 soil through their priming effect. *Soil Biology and Biochemistry* 43: 86-96.
- 607 Fulton, L.A. 1968. Spawning areas and abundance of Chinook salmon (*Oncorhynchus*
608 *tshawytscha*) in the Columbia River Basin - past and present. United States Fish and
609 Wildlife Service Special Scientific Report - Fisheries No. 571.
- 610 Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P.,
611 Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F.,
612 Porter, J.H., Townsend, A.R., and Vorosmarty, C.J. 2004. Nitrogen cycles: past, present,
613 and future. *Biogeochemistry* 70: 153-226.
- 614 Gende, S. M., Miller, A.E., and Hood, E. 2007. The effects of salmon carcasses on soil nitrogen
615 pools in a riparian forest of southeastern Alaska. *Canadian Journal of Forest Research*
616 37:1194-1202.
- 617 Gende, S.M., Quinn, T.P., Willson, M.F., Heintz, R., and Scott, T.M. 2004. Magnitude and Fate
618 of Salmon-Derived Nutrients and Energy in a Coastal Stream Ecosystem. *Journal of Fresh*
619 *Water Ecology* 16(1): 149-160.
- 620 Gende, S.M., and Quinn, T.P. 2004. The relative importance of prey density and social
621 dominance in determining energy intake by bears feeding on Pacific salmon. *Canadian*
622 *Journal of Zoology* 82: 75-85.

- 623 Gresh T., Lichatowich, J., and Schoonmaker, P. 2000. An estimation of historic and current
624 levels of salmon production in the Northeast Pacific ecosystem: Evidence of a nutrient
625 deficit in the freshwater systems of the Pacific Northwest. *Fisheries* 25(1): 15-21.
- 626 Hilderbrand, G.V., Hanley, T.A., Robbins, C.T., and Schwartz, C.C. 1999. Role of brown bears
627 (*Ursus arctos*) in the flow of marine nitrogen into a terrestrial ecosystem. *Oecologia*
628 121(4):546-550.
- 629 Hilderbrand, G.V., Farley, S.D., Robbins, C.T., Hanley, T.A., Titus, K., and Servheen, C. 1996.
630 Use of stable isotopes to determine diets of living and extinct bears. *Canadian Journal of*
631 *Zoology* 74: 2080-2088.
- 632 Hobbie, S.E. 1992. Effects of plant species on nutrient cycling. *Tree* 7(10): 336-339.
- 633 Hocking, M.D., and Reimchen, T.E. 2006. Consumption and distribution of salmon
634 (*Oncorhynchus* spp.) nutrients and energy by terrestrial flies. *Canadian Journals of Fisheries*
635 *and Aquatic Science* 63:2076-2086,
- 636 Holtgrieve, G.W., and Schindler, D.E. 2009. Large predators and biogeochemical hotspots:
637 brown bear (*Ursus arctos*) predation on salmon alters nitrogen cycling in riparian soils.
638 *Ecological Research* 24:1125-1135.
- 639 Horwath, W.R., and Paul, E.A. 1994. Microbial biomass. P. 753-773. In Weaver, R.W., Angle,
640 J.S., and Bottomley, P.S (ed.). *Methods of Soil Analysis: part 2 microbiological and*
641 *biochemical properties*. Soil Science Society of America, Madison, Wisconsin.
- 642 Jurgensen, M.F., Tonn, J.R., Graham, R.T., Harvey, A.E., and Geier-Hayes, K. 1990. Nitrogen
643 fixation in forest soils of the inland northwest. *Symposium on management and productivity*
644 *of western-montaine forest soils*, Boise, ID, April 1990.

- 645 Kaye, J.P., and Hart, S.C. 1997. Competition for nitrogen between plants and soil
646 microorganisms. *Tree* 12(4): 139-143.
- 647 Koyama, A., Stephan, K., and Kavanagh, K.L. 2012. Fire effects on gross inorganic N
648 transformation in riparian soils in coniferous forests of central Idaho, USA: wildfires v.
649 prescribed fires. *International Journal of Wildland Fire* 21: 69-78.
- 650 Koyama, A., Kavanagh, K.L., and Stephan, K. 2010. Wildfire effects on soil gross nitrogen
651 transformation rates in coniferous forests of central Idaho, USA. *Ecosystems* 13: 1112-1126.
- 652 Koyama, A., Kavanagh, K., and Robinson, A. 2005. Marine nitrogen in central Idaho riparian
653 forests: evidence from stable isotopes. *Canadian Journal of Fisheries and Aquatic Sciences*
654 62: 518-526.
- 655 Lisi, P.J., and Schindler, D.E. 2011. Spatial variation in timing of marine subsidies influences
656 riparian phenology through a plant-pollinator mutualism. *Ecosphere* 2(9).
- 657 Marcarelli, A.M., Baxter, C.V., and Wipfli, M.S. 2014. Nutrient additions to mitigate for loss of
658 Pacific salmon: consequences for stream biofilm and nutrient dynamics. *Ecosphere* 5(6).
- 659 McLauchlan, K.K., and Hobbie, S.E. 2004. Comparison of labile soil organic matter
660 fractionation techniques. *Soil Science Society of America Journal* 68: 1616-1625.
- 661 Miles, D. M., Rowe, D.E., and Cathcart, T.C. 2011. High litter moisture content suppresses litter
662 ammonia volatilization. *Poultry Science* 90:1397-1405.
- 663 Moore, J.A., and Mika, P.G. 1991. Nitrogen fertilizer response of Rocky Mountain Douglas-fir
664 by geographic area across the inland northwest. *Western Journal of Applied Forestry* 6(4):
665 94-98.
- 666 National Atmospheric Deposition Program (NADP). 2017. National Trends Network: total
667 inorganic nitrogen deposition. <http://nadp.sws.uiuc.edu/>. [Accessed August 16, 2017].

- 668 National Research Council (NRC). 1996. Committee on Protection, and Management of Pacific
669 Northwest Anadromous Salmonids. Upstream: salmon and society in the Pacific Northwest.
670 National Academies Press, 1996.
- 671 Natural Resource Conservation Service (NRCS). 2012. National Water and Climate Center:
672 SNOTEL data. <http://www.wcc.nrcs.usda.gov/snotel/>. [Accessed March 21, 2012].
- 673 Needleman, B.A., Wander, M.M., and Shi, G.S. 2001. Organic carbon extraction efficiency in
674 chloroform fumigated and non-fumigated soils. Soil Science Society of America Journal 65:
675 1731-1733.
- 676 Perry, C.A., Kavanagh, K.L., and Scott, J.M. 2003. Pacific Salmon: Setting Ecologically
677 Defensible Recovery Goals. BioScience 53(7): 622-623.
- 678 Pinay, G., O'Keefe, T., Edwards, R., and Naiman, R.J. 2003. Potential Denitrification Activity in
679 the Landscape of a Western Alaska Drainage Basin. Ecosystems 6: 336-343.
- 680 Quinn, T. P., Carlson, S.M., Gende, S.M., and Rich, H.B. 2009. Transportation of Pacific salmon
681 carcasses from streams to riparian forests by bears. Canadian Journal of Zoology/Revue
682 Canadien de Zoologie 87(3):195-203.
- 683 Quinn, T.P., Gende, S.M., Ruggerone, G.T., and Rogers, D.E. 2003. Densit-dependent predation
684 by brown bears (*Ursus arctos*) on sockeye salmon (*Oncorhynchus nerka*). Canadian Journal
685 of Fisheries and Aquatic Science 60: 553-562.
- 686 Roe, A.M., Meyer, C.B., Nibbelink, N.P., and Ben-David, M. 2010. Differential tree and shrub
687 production in response to fertilization and disturbance by coastal river otters in Alaska.
688 Ecology 91(11): 3177-3188.
- 689 Rosgen, D.L., and Silvey, H.L. 1996. Applied River Morphology. 2nd ed. Wildland Hydrology,
690 Fort Collins, CO. Ch. 4-5.

- 691 Silbeira, M.L., Comerford, N.B., Reddy, K.R., Cooper, W.T., and El-Rifai, H. 2008.
692 Characterization of soil organic carbon pools by acid hydrolysis. *Geoderma* 144: 405-414.
- 693 Stephan, K., Kavanagh, K.L., and A. Koyama, A. 2012. Effects of spring prescribed burning and
694 wildfires on watershed nitrogen dynamics of central Idaho headwater areas. *Forest Ecology*
695 *and Management* 263: 240-252.
- 696 Wendt, G.E., Thompson, R.A., and Larson, K.N. 1975. Land systems inventory – A basic
697 inventory for planning and management. Forest Service, Intermountain Region. p.54.
- 698 Wendt, G.E., Fisher, D. and Cole, G.F. 1973. Soil-hydrologic reconnaissance survey. Idaho City
699 Ranger District, Boise National Forest. P.221 (unpublished report).
- 700 Wheeler, T.A., Kavanagh, K.L., and Daanen, S.A. 2014. Terrestrial salmon carcass
701 decomposition: nutrient and isotopic dynamics in central Idaho. *Northwest Science* 88(2):
702 106-119.
- 703 Whitney N., and Zabowski, D. 2004. Total soil nitrogen in the course fraction and at depth. *Soil*
704 *Science Society of America Journal* 68: 612-619.
- 705 Zhang, Y., He, N., Zhang, G., Huang, J., Wang, Q., Pan, Q., and Han, X. 2013. Ammonia
706 emissions from soil under sheep grazing in inner Mongolian grasslands of China. *Journal of*
707 *Arid Land* 5(2):155-165.

Table 1. Description of study sites within the North Fork Boise River watershed.

Stream	Latitude (N)	Longitude (W)	Elevation (m)
Banner Creek	43°59'14"	115°32'46"	1625
Beaver Creek	43°49'30"	115°31'08"	1311
Little Beaver Creek	43°58'07"	115°35'48"	1572
Trail Creek	43°54'00"	115°23'12"	1571

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Table 2. Measured climatic conditions within the North Fork Boise River watershed.

	Mean		Temperature Range		Precipitation (cm)	
	Temperature (°C)		min/max (°C)			
	Mores Creek	Graham Guard	Mores Creek	Graham Guard	Mores Creek	Graham Guard
20 Year average	5.7	4.8	-19.9/33.1	-29.3/34.2	117	78
Year post 2008 treatment	6.1	5.0	-18.6/31.3	-27.7/32.6	110	79
Two weeks post 2008 treatment	18.7	15.5	6.5/30.0	0.3/31.0	0.0	1.0
Year post 2010 treatment	5.2	4.5	-23.1/30.2	-29.4/31.7	146	95
Two weeks post 2010 treatment	16.6	14.9	3.1/29.7	1.0/31.1	1.8	2.3

Notes: Year post represent the climate for the 12 months following fish carcass placement and two weeks post is the climate for the two weeks following carcass placement. Climate data recorded by the Natural Resource Conservation Service SNOTEL climate stations at Mores Creek Summit to the west of the study area (43.93N, 115.67W, Elevation 1860 m) and Graham Guard Station to the north of the study area (43.95N, 115.27W, Elevation 1734 m) (NRCS 2012).

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Table 3. The results of statistical analysis using repeated measures analysis of variance (ANOVA) for overall treatment effect through time and one-tailed t-tests for differences at each observational day.

Dependent Soil Variable	Repeated Measures ANOVA	One-tailed t-tests Days since treatment					
		13	33	60	96	300	361
Inorganic N	DF = 14 T = -14.1 P < 0.01	DF = 13 T = 21.8 P < 0.01	DF = 14 T = 21.8 P < 0.01	DF = 14 T = 11.9 P < 0.01	DF = 14 T = 9.4 P < 0.01	DF = 14 T = 9.4 P < 0.01	DF = 14 T = 2.77 P < 0.01
Organic N	DF = 14 T = 3.5 P < 0.01	DF = 13 T = 1.6 P = 0.07	DF = 14 T = 2.5 P = 0.01	DF = 14 T = 2.3 P = 0.02	DF = 14 T = 3.0 P < 0.01	DF = 14 T = 2.9 P < 0.01	DF = 14 T = 2.3 P = 0.02
Total N	DF = 14 T = -2.3 P = 0.04	DF = 14 T = 1.8 P < 0.05	DF = 14 T = 2.6 P = 0.01	DF = 14 T = 2.6 P = 0.01	DF = 12 T = 2.0 P = 0.03	DF = 14 T = 2.4 P = 0.01	DF = 14 T = 2.2 P = 0.02
Total C	DF = 14 T = -0.7 P = 0.5	NA	NA	NA	NA	NA	NA
Total C:N	DF = 14 T = -4.5 P < 0.01	DF = 14 T = 5.8 P < 0.01	DF = 14 T = 3.8 P < 0.01	DF = 14 T = 6.5 P < 0.01	DF = 14 T = 6.5 P < 0.01	DF = 14 T = 3.4 P < 0.01	DF = 14 T = 3.1 P < 0.01
Relative Hydrolysable C	DF = 14 T = -0.5 P = 0.6	NA	NA	NA	NA	NA	NA
Dissolved Organic C	DF = 14 T = -4.1 P < 0.01	DF = 14 T = 5.0 P < 0.01	DF = 14 T = 3.4 P < 0.01	DF = 14 T = 4.4 P < 0.01	DF = 14 T = 3.1 P < 0.01	DF = 14 T = 2.5 P = 0.01	DF = 14 T = 3.3 P < 0.01
Soil Respired CO₂		Not measured	DF = 14 T = 3.9 P < 0.01	Not measured	DF = 14 T = 1.8 P < 0.05	DF = 14 T = 0.2 P = 0.4	DF = 14 T = 0.6 P = 0.3
Microbial Biomass N	DF = 14 T = 2.9 P = 0.01	DF = 11 T = 1.0 P = 0.2	DF = 13 T = 2.7 P = 0.01	DF = 10 T = 3.6 P < 0.01	DF = 10 T = 3.4 P < 0.01	DF = 11 T = 0.1 P = 0.4	DF = 11 T = 0.4 P = 0.4
Microbial Biomass C	DF = 14 T = 0.3 P = 0.7	NA	NA	NA	NA	NA	NA
Microbial Biomass C:N	DF = 14 T = -6.4 P < 0.01	DF = 10 T = 4.6 P < 0.01	DF = 12 T = 4.3 P < 0.01	DF = 10 T = 4.5 P < 0.01	DF = 10 T = 4.5 P < 0.01	DF = 10 T = 5.2 P < 0.01	DF = 8 T = 0.4 P = 0.4
Soil $\delta^{15}\text{N}$		DF = 14 T = 5.6 P < 0.01	DF = 14 T = 3.8 P < 0.01	DF = 14 T = 6.4 P < 0.01	DF = 12 T = 6.5 P < 0.01	DF = 14 T = 5.8 P < 0.01	DF = 14 T = 8.6 P < 0.01

Notes: The repeated measures ANOVA for soil respired CO₂ is not shown due to a significant effect of time. Not applicable (NA) entries signify that t-tests were not conducted due to a non-significance of the repeated measures ANOVA. The T statistics are derived from F statistics and are therefore absolute values of T. Bolded *P*-values are significant at $\alpha = 0.05$.

Table 4. The estimates of instantaneous soil N and C loading utilizing the change in soil total N and soil dissolved organic C from pretreatment concentrations as well as a linear two-member isotopic mixing model following the riparian deposition of anadromous fish carcasses.

DST	Change in soil total N (g/kg)	Scaled to soil under carcass ^a (g N)	Scaled to soil horizontal to carcass ^b (g N)	Total soil N loading (g)	Isotopic mixing model N loading estimate using 11.9 permil (g)
13	0.57	5.3	6.2	11.5	24.4
33	1.28	12.0	13.9	25.9	44.3
60	1.56	14.6	16.9	31.5	50.9
DST	Change in soil dissolved organic C (g/kg)	Scaled to soil under carcass ^a (g C)	Scaled to soil horizontal to carcass ^b (g C)	Total soil C loading (g)	
13	0.287	2.7	3.1	5.8	
33	0.571	5.4	6.2	11.6	
60	0.701	6.6	7.6	14.2	

Notes: DST represents days since treatment, the change in soil N or C concentrations represents the change in concentration from the previous sampling, ^a g N or C/kg dry soil x 9.4 kg dry soil, ^b g N or C/kg dry soil x 50% x 21.7 kg dry soil

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Table 5. The estimates of soil microbial nitrogen mineralization and consumption based on observed soil microbial biomass N prior to and following riparian deposition of anadromous fish carcasses.

DST	Measured Value Microbial Biomass N (g/kg)	Estimate of soil mineralization ^a (mg N/kg soil/day)	Scaled to soil under carcass ^b (mg N)	Scaled to soil horizontal to carcass ^c (mg N)	Total N mineralized (mg)	Daily average mineralized N (mg) from previous	Cumulative mineralized N (mg) from previous
0	0.0274	1.72	16.2	175.3	191.5		
13	0.0504	2.39	22.5	243.6	266.1	228.8	2973.9
33	0.0910	3.29	30.9	335.3	366.2	316.1	6322.9
60	0.0751	2.97	27.9	302.72	330.6	348.4	9407.6
DST	Measured Value Microbial Biomass N (g/kg)	Estimate of soil consumption ^d (mg N/kg soil/day)	Scaled to soil under carcass ^b (mg N)	Scaled to soil horizontal to carcass ^c (mg N)	Total N consumption (mg)	Daily average N consumption (mg) from previous	Cumulative N consumption (mg) from previous
0	0.0274	2.25	21.1	229.3	250.5		
13	0.0504	3.01	28.3	306.8	335.1	292.8	3806.0
33	0.0910	4.00	37.6	407.7	445.3	390.2	7803.5
60	0.0751	3.65	34.3	372.0	406.3	425.8	11496.5

Notes: DST represents days since treatment, ^a log min = 0.540 x log MBN + 1.08 (Booth et al. 2005), ^b mg N/kg dry soil x 9.4 kg dry soil, ^c mg N/kg dry soil x 50% x 21.7 kg dry soil, ^d log cons = 0.480 x log MBN + 1.102 (Booth et al. 2005)

Figure 1. 2008 soil inorganic N (NH₄⁺-N + NO₃⁻-N) concentrations for soils at 0-1, 1-3, and 3-8 cm in depth across n=4 carcass slurry treatment plots at 30 days post treatment when observed concentrations peaked. The linear fit (solid line) is through the maximum values in order to estimate the maximum penetration depth of the N contribution. These regressed maximum values across all three depths derive from the same set of homogenized soil samples.

215x166mm (300 x 300 DPI)

Figure 2. The mean A) Soil extractable inorganic and organic N and B) Bulk soil total N for $n=8$ soil plots in relation to days since treatment in 2010. The values to the left of zero are pretreatment values. There was an overall significant effect ($P<0.05$) of treatment on all three N concentrations (Table 3). The asterisks indicate significant ($P<0.05$) point in time differences between treated and control soils.

279x361mm (300 x 300 DPI)

Figure 3. The mean soil dissolved organic carbon (DOC) for n=8 plots in relation to days since treatment in 2010. Values to the left of zero are pretreatment concentrations. There was an overall significant effect ($P<0.05$) of treatment on DOC (Table 3). The asterisks indicate significant ($P<0.05$) point in time differences between treated and control soils.

215x166mm (300 x 300 DPI)

Figure 4. Mean soil microbial biomass N in relation to days since treatment in 2010. Values to the left of zero are pretreatment concentrations. Sample size varies from $n=5-7$. There was an overall significant effect ($P<0.05$) of treatment on microbial biomass N (Table 3). The asterisks indicate significant ($P<0.05$) point in time differences between treated and control soils.

215x166mm (300 x 300 DPI)

Figure 5. 2010 soil respired CO₂ (n=8) in relation to days since treatment. Values at zero are pretreatment concentrations. There was an overall significant effect ($P<0.05$) of treatment on soil respired CO₂ (Table 3). The asterisks indicate significant ($P<0.05$) point in time differences between treated and control soils.

215x166mm (300 x 300 DPI)

Figure 6. The soil $\delta^{15}\text{N}$ of the 2010 riparian plot soils as a function of days since treatment. Error bars are standard error of the mean. The initial values are the pretreatment values. The * indicates a significant difference at $\alpha=0.05$ in $\delta^{15}\text{N}$ between the soils of the treated and control plots. The dashed trend lines visually connect the data trends but are not empirical due to an inability to quantify soils during the snow covered winter months.

215x166mm (300 x 300 DPI)