The Biogeochemical Responses of Central Idaho Riparian Forests to the Deposition of Salmon Carcasses

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Authorization to Submit Dissertation

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Abstract

Pacific salmonids are thought to provide a vital nutrient link between the ocean and their natal streams. However, most of what is known about the ecologic role of salmonids in their natal ecosystems is derived from coastal maritime investigations. Appropriately mitigating for the decline of returning salmonids to inland ecosystems requires understanding the biogeochemical responses of these ecosystems to the deposition of salmon born nutrients, particularly within riparian ecosystems where the semi-arid climatic conditions of this region may have the greatest influence on these biogeochemical responses.

The terrestrial decomposition of salmon carcasses and the subsequent release of carbon and nitrogen in an inland semi-arid environment as well as the influences of freezing and heat treating salmon carcasses for disease mitigation was evaluated. Decomposition proceeded rapidly in this semi-arid climate overriding any overall influences of the disease mitigation methods on decomposition time or soil carbon and nitrogen accumulation. A notable finding was a 4 ‰ $\delta^{15}N$ enrichment of the carcass fluids entering the soil, relative to whole carcasses. This alteration of isotopic ratios has direct implications on isotopic mixing model estimates of soil and vegetative marine derived nutrient composition.

The soil biogeochemical responses to the deposition of anadromous fish carcasses in riparian forests of central Idaho were investigated and the soil loading of carbon and nitrogen per fish carcass estimated. This investigation revealed dramatic increases in soil carbon and nitrogen chemistry that far exceeded and persisted longer than reported responses in coastal ecosystems. However, the carbon and nitrogen loading estimates were only able to account for a small portion of the carbon and nitrogen in the fish carcasses.

The annual and inter-annual responses of riparian vegetation to the deposition of anadromous fish carcasses in riparian forests of central Idaho was investigated. The investigation revealed that herbaceous species can quickly utilize carcass nutrient subsidies but that the nutrients are depleted within two to three years. Conifer seedlings appear to have a much higher resource acquisition and storage capacity as foliage produced three years after carcass nutrient additions contained large quantities of marine derived nitrogen.

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Chapter 1

Introduction to the Dissertation

Biotic influences on ecosystems

Organisms are capable of directly and indirectly influencing the productivity of their environment. Examples of this influence include increases in grassland productivity through herbivory driven soil nutrient processes (Singer and Schoenecker 2003) and predator-prey induced soil nutrient hotspots leading to increased vegetative productivity and changes in biodiversity (Bump et al. 2009, Towne 2000). Pacific salmonids are thought to be particularly influential due to their ability to transfer large quantities of nutrients over considerable geographic distances and across multiple ecosystem boundaries (Gresh et al. 2000), for example, from aquatic to terrestrial riparian ecosystems (Koyama et al. 2005, Helfield and Naiman 2006). However, nearly all of what is understood about the ecosystem responses to salmon nutrient subsidies derives from investigations in coastal maritime ecosystems where salmon abundance remains relatively robust (NRC 1996) and the ecosystems highly productive (Grier 1979). By contrast, salmonids have historically returned to vastly distant inland streams (NRC 1996) where the climate and productivity of these ecosystems can be drastically different (Koyama et al. 2012, 2010, 2005; Stephan et al. 2012; Moore and Mika 1991). This prompts the question of how these semi-arid inland riparian ecosystems respond to salmon nutrient subsidies.

Pacific salmonids in the Pacific Northwestern USA, British Columbia, and Alaska

Pacific salmonids are thought to be a substantial nutrient vector between the ocean and inland ecosystems (Gresh et al. 2000). For example, salmonids of the Pacific Northwest have historically migrated up to a 1000 km from their natal streams to the ocean (NRC 1996) where they gain 90% or more of their adult body mass (Gende et al.2004, 2002) before returning to their natal streams to spawn and contribute their nutrient laden carcasses to these ecosystems (Cederholm et al. 1999). In coastal ecosystems, salmonids returning to their natal streams are preyed upon in great numbers by bears (Quinn et al. 2009, Quinn et al. 2003) as well as by wolves (Adams et al. 2010) and other scavengers (Cederholm et al. 1989). The fate of the nutrients from these salmon may be particularly important for the productivity of riparian ecosystems. The proportion of the salmon run being predated upon by bears alone has

been reported to range from 40% to over 80% of the population (Quinn et al. 2009), with up to 80% of the salmon preyed upon by bears potentially transported into the riparian forest (Gende et al. 2004, Quinn et al 2009). These carcasses are often only partially consumed (Gende 2004, 2001), leaving the remainder of the carcass to decompose and release their nutrients stores. The transfer of nutrients through the metabolic wastes of predators and scavengers (Cedarholm et al. 1989, Hilderbrand et al. 1999) as well as through hyporheic flow (Pinay et el. 2009, O'Keefe and Edwards 2003) have also been reported as pathways of nutrient transfer into riparian forests. Together these nutrient transfer pathways are thought to create spatially heterogeneous gradients of soil nutrient availability reaching hundreds of meters into the riparian forest (Bartz and Naiman 2005, Drake et al. 2005, Gende et al. 2007, Gende et al. 2004, Hilderbrand et al. 1999).

The transfer of salmon nutrient into coastal riparian forests has been associated with several soil biogeochemical responses (Figure 1). The most dramatic of these responses are associated with the deposition of salmon carcass and include an increase in soil inorganic nitrogen (N) by up to 60 fold within 8 weeks of carcass deposition before declining to reference conditions by one year following deposition (Drake et al. 2005, Gende et al. 2007). A significant Increase in other soil nutrients such as potassium, sulfur, and phosphorus has also been associated with salmon carcass deposition in riparian forests (Drake et al. 2005). Further, the deposition of a highly labile organic resource such as salmon carcasses would be expected to elicit an increase in soil microbial productivity (Barton et al. 2013). Reports of soil microbial processes near salmon carcasses are largely lacking. However, Holtgrieve et al. (2009) reported increases in soil emissions of CO_2 and N_2O in conjunction with increased bear activity near salmon streams that would suggest an increase in soil microbial productivity in association with the annual return of spawning salmonids. It is evident that there is room for additional insight into the soil biogeochemical responses to salmon nutrient subsidies in coastal riparian forests. However, there are a couple of particularly important findings discussed thus far. The first of these is the potential magnitude of nutrient transfer into the riparian forest by the deposition of metabolic wastes (Hilderbrand et al. 1999) and salmon carcasses (Quinn et al. 2009, 2003). The second is the less than one year duration of elevated soil N concentrations associated with this nutrient subsidy (Drake et al. 2005, Gende et al. 2007). It is possible that some of this N is lost through leaching and denitrification (O'Keefe

and Edwards 2003; Pinay et al. 2009, 2003) but the high productivity of these forests (Grier 1979) suggests that vegetative utilization may also be a prominent pathway of soil N removal (Drake et al. 2006, Pinay et al. 2009).

A large majority of what is known about the role of salmon derived nutrients in coastal riparian ecosystems derives from stable isotope evidence of vegetative N utilization (e.g. Ben-David et al. 1998, Bilby et al. 2003, Hilderbrand et al. 1999, Hocking and Reynolds 2011). The oceanic environment where salmonids gain the vast majority of their adult biomass has a higher concentration of the less abundant ^{15}N isotope relative to inland ecosystems (Fry 2006). The ratio of $^{15}N/^{14}N$ standardized by the ratio of these isotopes in the atmosphere and expressed as a delta (δ) ratio in parts per thousand (‰) can enable the tracking of N sources within ecosystems utilizing isotopic mixing models (Fry 2006). There have been some concerns raised about the possibility of soil microbial processes leading to alterations of the soil N isotope ratio and the possibility of this influence propagating into errors within the isotopic mixing model estimates of marine derived nitrogen (MDN) utilization by vegetation (Morris et al. 2005, Pinay et al. 2003). However, this error appears to be relatively small at less the 7% (Morris et al. 2005).

Despite concerns of error propagation, these estimates provide correlative evidence of salmon N utilization by riparian vegetation (e.g. Bilby et al. 2003, Hilderbrand et al. 1999, Hocking and Reynolds 2011, Nagasaka et al. 2006). The level of $\delta^{15}N$ enrichment in riparian vegetation has been positively correlated with the prevalence of bear activity near salmon bearing streams, with the $\delta^{15}N$ enrichment of riparian vegetation being greatest near the stream where bear presence was greatest and declining exponentially within 500 meters of the stream (Hilderbrand et al. 1999). The pathways of nutrient transfer in several other investigations are less defined but the results are generally similar to Hilderbrand et al. (1999) with the greatest level of vegetative $\delta^{15}N$ enrichment being found near the stream and declining with distance from the stream (Ben-David et al. 1998, Bilby et al. 2003) and with decreasing salmon density (Nagasaka et al. 2006). This use of stable N isotopes has also been utilized to correlate the presence and abundance of spawning salmon with changes in vegetative biodiversity (Bilby et al. 2003, Hocking and Reynolds 2011) and increases in productivity (Helfield and Naiman 2001). In general, the presence of salmon in coastal ecosystems appears to lead to decreases in plant community diversity and a shift in riparian

plant communities towards the dominance of nutrient rich species (Hocking and Reynolds 2011). However, there is evidence that the magnitude and direction of these biodiversity responses may vary between watersheds (Bilby et al. 2003, Hocking and Reynolds 2011).

These coastal investigations demonstrate the potential influences of salmon nutrient subsidies to riparian ecosystems. However, these investigations also illustrate that ecosystem responses are mediated by biotic factors such as salmon abundance (Hocking and Reynolds 2011, Nagasaka et al. 2006) and predator activity (e.g. Hilderbrand et al. 1999, Quinn et al. 2009) as well as by the physical constraints of the environment such as geography and background N levels (Hocking and Reynolds 2011, Nagasaka et al. 2006). Thus prompting the question of how the riparian ecosystem responses to salmon nutrient subsidies would differ with a dramatic shift in the abiotic environment from maritime coastal ecosystems to the inland semi-arid riparian ecosystems of the Pacific Northwest.

Salmonids in the inland Pacific Northwest

Approximately 80% or more of natal salmon habitat in the Pacific Northwest is found within the Columbia River Basin (NRC 1996). This 673,400 km^2 basin is thought to have historically included over 20,000 km of salmonid accessible stream habitat (NRC 1996) and supported annual runs of up to 14.9 million returning adult salmonids (Gresh et al. 2000, NRC 1996). This has been reported to equate to the annual influx of up to 3 million kg N to this region (Gresh et al. 2000). Yet relatively little is known about the ecosystem responses to this nutrient subsidy within these inland ecosystems, particularly within riparian ecosystems. There is evidence that salmonids of the Columbia River Basin historically constituted roughly 58% of the diet of grizzly bears that once inhabited this region (Hilderbrand et al. 1996). This evidence in conjunction with the persistence of isotopic evidence of salmon N utilization by conifer trees even after decades of salmon extirpation (Koyama et al. 2005) suggest that salmon nutrients may have historically played an important role in these inland riparian ecosystems.

Inland ecosystems

Inland riparian ecosystems of the Pacific Northwest differ from coastal riparian ecosystems in several substantial ways. The first is the differences in climate. These coastal ecosystems have a maritime climate characterized by mild daily and annual temperature extremes and heavy

rainfall whereas inland ecosystems of this region are characterized by a semi-arid climate punctuated by hot, dry summers and cold, moist winters (WRCC 2011). For example, the Boise River watershed in central Idaho experiences daily diurnal temperature swings of up to 30˚ C and annual temperature extremes that range from -24˚ C to 32˚ C (NRCS 2012). This watershed is also seasonally very dry with the average annual precipitation in the Boise River watershed of central Idaho only receiving 77-117 cm of precipitation annually, predominantly as snow (NRCS 2012).

The second difference between coastal and inland ecosystems of the Pacific Northwest is the availability of N. Despite the high productivity of coastal forests (Grier 1979), the Pacific Northwest is broadly considered N limited for forest productivity (Chapin et al. 1986, ESMP 2013, Henderson et al. 1978, Moore and Mika 1991). This N limitation is thought to be particularly severe within the further inland reaches of the Columbia River Basin (Koyama et al. 2012, 2010, 2005; Stephan et al. 2012; Moore and Mika 1991) and has been associated with the internal recycling of salmon derived N in conifers even after decades of salmonid extirpation (Koyama et al. 2005). However, this recycling of N has the resultant nutrient cycling consequence of increasing the C:N of the forest litter pool (Heath et al. 1988, Valachovic et al. 2004) and thus exasperating soil nutrient availability through the suppression of soil microbial mineralization rates, as is characteristic of this region (Hart et al. 1997, Henderson et al. 1978, Koyama et al. 2012, Koyama et al. 2010, Stark and Hart 1997). The N limitations within this region can be largely attributed to a lack of atmospheric N deposition and biotic N-fixation in this region, which are collectively estimated to be less than 3 kg N ha^{-1} yr^{-1} (Jurgensen et al. 1990, NADP 2014) as well as nutrient poor granitic parent geology within some sub-basins that contribute little to the N availability in these ecosystems (Henderseon et al. 1978). By comparison, the prevalence of N fixing *Alnus* spp. in coastal ecosystems has been reported to fix in excess of 100 kg N ha^{-1} yr^{-1} (Jurgensen et al. 1990). This would make the annual influx of salmon nutrients a potentially significant source of nutrient enrichment to this inland region.

Based on the biotic and abiotic characteristics of inland ecosystems of the Pacific Northwest, the ecosystem responses to the deposition of salmonids in this inland region might be amplified considerably from coastal ecosystem responses. The rate of nutrients release from fish carcasses deposited in the riparian forest is largely a function of environmental

temperature with higher temperature promoting an increase in decomposition rate, as demonstrated for human remains (Vass et al. 1992, Megyesi et al. 2005). Based on this evidence, it is expected that the decomposition of fish carcasses would occur faster than the 76 calendar days observed in a coastal riparian ecosystem (Drake et al. 2005), thus releasing nutrients faster and leading to higher soil N concentrations than observed in coastal investigations (Drake et al. 2005, Gende et al. 2007). Microbial productivity is also thermally regulated and would therefore be expected to increase with higher temperature (Zak et al. 1999), especially with the contribution of a highly labile, moisture laden, organic substrate such as a salmon carcass (Barton et al. 2013). Finally, the availability of soil N in these N limited ecosystems would be expected to stimulate vegetative productivity and potentially lead to increases in biomass production (Gough et al. 2004, Helfield and Naiman 2001, Moore and Mika 1991). However, there may be other co-limiting resources in this harsh semi-arid environment that limit the full potential of these responses. These assumptions and predictions are explored in the subsequent chapters of this dissertation.

Scope of this dissertation

In this dissertation, I present three investigations into the biogeochemical responses of anadromous fish carcass deposition in riparian forests of central Idaho, USA. Chapter 2, entitled "Terrestrial salmon carcass decomposition: nutrient and isotopic dynamics in central Idaho," investigated the rate of salmon carcass decompostion under semi-arid climatic conditions during the month of August when salmonids typically spawn in this region. This investigation also evaluated the timing and magnitude of soil carbon (C) and nitrogen (N) increases within the soil as well as the isotopic C and N composition of the fluids being released from the carcasses to the soil as a validation of the marine end member value utilized in isotopic mixing models. One caveat of conducting these investigations was a need to store the carcasses frozen and to mitigate for pathogenic risks by heat treating the carcasses. The potential influences of these treatments on the aforementioned parameters were thus included in this initial investigation.

In chapter 3, entitle "Soil biogeochemical responses to the deposition of anadromous fish carcasses in inland riparian forests of the Pacific Northwest, USA," the responses of exchangeable and non-exchangeable soil C and N pools were investigated following the deposition of fish carcasses in riparian forests of the North Fork Boise River watershed in

central Idaho. The responses of soil C following salmon carcass deposition is largely lacking from the scientific literature, thus this chapter includes insight into the magnitude and the duration of the soil C and N responses as well as insight into the responses of the soil microbial community to this highly labile organic resource. These responses as well as soil isotopic N evidence are then utilized to explore the C and N contributions of anadromous fish carcasses to riparian soils of this region at multiple spatial scales.

Chapter 4 is entitled "Marine derived nutrients and understory vegetation in oligotrophic inland riparian forests of the Pacific Northwest, USA." This chapter explores the utilization of marine derived nitrogen (MDN) by common horsetail (*Equisetum arvense*) through the nutrient pathways of aquatic transfer from carcass treated streams and from the terrestrial deposition of anadromous fish carcasses. The utilization of MDN from terrestrial carcass deposition by conifer seedlings (*Pseudotsuga menziesii* and *Pinus contorta*) was also assessed. The utilization of MDN by these species was determined through changes in foliar N isotopes. Potential increases in productivity were assessed for all species using changes in foliar C isotopes as an indicator of changes in photosynthetic capacity. Additionally, potential increases in above ground biomass production in conifers was assessed. The foliar N isotope values were then applied to allometric relationships developed for the conifer seedlings in this region to estimate the amount of MDN sequestered in these seedlings following the terrestrial deposition of fish carcasses.

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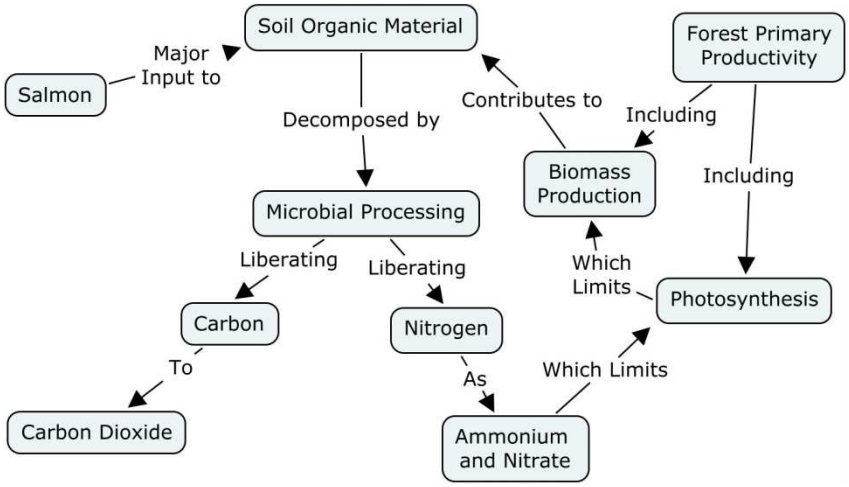
Figure 1. A simple schematic of the expected flow of salmon carcass C and N through the soil and vegetative components of a riparian ecosystem.



Chapter 2

Terrestrial salmon carcass decomposition: nutrient and isotopic dynamics in central Idaho

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

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Abstract

Terrestrial salmon carcass decomposition and nutrient dynamics have been reported for coastal marine ecosystems of the Pacific Northwest but are lacking for semi-arid inland ecosystems of this region. Understanding these processes is a critical step in accurately understanding the biochemical responses to natural salmon deposition and appropriately mitigating for its loss. Additionally, the movement of carcasses for nutrient enhancement poses the risk of spreading pathogens and disease. Freezing and pasteurization of the carcasses reduces this risk. However, the effect of these treatments on decomposition processes is unknown. Decomposition rate and nutrient dynamics were investigated in semi-arid central Idaho by decomposing salmon carcasses in soil-filled microcosms. Rapid thermal accumulation in this semi-arid climate resulted in completion of soft tissue decomposition (skeletonization) in 16 days. Soil dissolved organic C (DOC) and dissolved total N (DTN) increased dramatically with respective increases from pretreatment concentrations of 7 and 48 fold by the time skeletonization occurred. Isotopic analysis of fluids beneath the carcasses revealed up to a 6 ‰ change in fluid $\delta^{13}C$ and $\delta^{15}N$ during decomposition as well as an overall 4 ‰ enrichment in mean fluid $\delta^{15}N$, relative to whole reference carcasses. Freezing and pasteurization of carcasses only yielded differences during the first few days of decomposition relative to the fresh carcasses. These results suggest that decomposition of carcasses in inland riparian forests proceeds very rapidly and that treated carcasses are suitable surrogates for fresh carcasses in semi-arid regions of central Idaho.

Introduction

The dispersal of hatchery spawned salmon carcasses to supplement nutrient loss from natal salmon streams is being considered for mitigation across the Pacific Northwest of North America (Amos and Thomas 2002, Compton et al. 2006, Strobel et al. 2009). Improving aquatic productivity is a major focus, but salmon influence on terrestrial ecosystems is also recognized (Ben-David et al. 1998; Helfield and Naiman 2001, 2006; Bilby et al. 2003; Drake et al. 2006; Hocking and Reynolds 2011). Bears in particular are known to transport large quantities of nutrient rich salmon carcasses into terrestrial ecosystems (Cederholm et al. 1989, Gende and Quinn 2004, Quinn et al. 2009) and to redistribute salmon derived nutrients through metabolic wastes (Hilderbrand et al. 1999). Soil nutrients have been found to increase markedly in the immediate vicinity of decaying salmon carcasses in coastal rainforests (Holtgrieve et al. 2009) and remain elevated for at least 154 days (Drake et al. 2005, Gende et al. 2007). The decomposition of salmon carcasses and the subsequent release of nutrients in a coastal riparian forest have been documented (Drake et al. 2005). However, to our knowledge, no systematic study has been conducted to examine the process of salmon carcass decomposition and the subsequent release of nutrients in inland semi-arid terrestrial ecosystems.

Salmonids historically spawned in large numbers within the headwaters of the Columbia River basin, including central Idaho (Fulton 1968, Gresh et al. 2000). This region is both drier and warmer than the marine west coast climate where all of the previous studies were conducted. For example, during the spring Chinook spawning season (July-September), central Idaho experiences mean monthly temperatures that are as much as 7˚ C warmer than coastal Washington while also receiving roughly one-third of the average monthly precipitation (WRCC 2011). These climatic differences can have a strong influence on carcass decomposition.

The biophysical processes influencing decomposition include nutrient content, moisture availability, and temperature. The carbon to nitrogen ratio (C:N) of Atlantic salmon carcasses has been reported to range from 3.5:1 to 9:1 (Dempson et al. 2009), which promotes rapid decomposition (Swift et al. 1979). Moisture is generally not limiting since salmon carcasses are 60-70 percent water (Ofstad et al. 1996a, Wold and Isaksson 1997). Therefore, neither of these factors is expected to cause a change in salmon decomposition rate in interior

semi-arid forests relative to marine coastal ecosystems. However, higher temperatures were found to promote decomposition of human remains especially in the presence of ample moisture (Vass et al. 1992, Megyesi et al. 2005). Therefore, with a favorable C:N and a high moisture content it was hypothesized that decomposition of salmon carcasses and the release of nutrient laden fluids would be strongly influenced by temperature and accumulation of degree days. Understanding these processes is a critical step in accurately understanding the biochemical responses to natural salmon deposition and appropriately mitigating for its loss.

Stable isotopes are often used to track the fate of marine-derived nutrients from salmon carcasses yet to our knowledge no one has investigated the isotopic composition of the dissolved organic carbon (DOC) and dissolved total nitrogen (DTN) released during decomposition. Several biochemical processes may influence the isotopic composition of the fluids released from the fish carcasses. In particular, lighter isotopes are often more chemically reactive, more readily volatilized, and may be differentially metabolized resulting in enrichment of the remaining pool (Fry 2006). The loss of volatile organic C and N compounds during decomposition has been documented for human cadavers (Hoffman et al. 2009) and thus likely occurs for other taxa as well. Determining potential ^{15}N enrichment during decomposition is essential to define since the $\delta^{15}N$ of carcasses tissue has been utilized in mixing models to estimate the percentage of foliar N derived from salmon carcasses (e.g. Helfield and Naiman 2001, Bilby et al. 2003).

One of the major concerns with using salmon carcasses for nutrient mitigation is that carcasses pose the risk of spreading pathogens and disease (Compton et al. 2006, Pearsons et al. 2007). Therefore, it has been suggested that freezing (Hedrick et al. 2008) and pasteurization (Hoffman and Markiw 1977,Wolf and Markiw 1982, Mulcahy et al. 1984) may be necessary to reduce the risk of spreading fish borne pathogens. This practice has not been widely adopted (CCFEG 2011) but may increase in the future if salmon carcasses are seen as a viable restoration tool in areas where salmon have been extirpated.

Several biophysical processes controlling decomposition may be impacted by pasteurization and freezing. The freezing of fish carcasses results in the formation of cellular and intercellular ice crystals (Ayala et al. 2005). These crystals in turn lead to the destruction of cell membranes and the release of cellular fluid post-thaw (Ayala et al. 2005). Similarly, heating of the carcass in excess of 60˚C as suggested (Noga 2000) exceeds temperatures

known to denature cellular structures in fish (Ofstad et al. 1996a,b) and result in premature cellular fluid loss. Based on this evidence we hypothesized that treating anadromous fish carcasses with pasteurization and/or freezing would significantly increase decomposition rate and decrease the time to skeletonization in a terrestrial environment, relative to untreated carcasses. This would in turn result in earlier increases in soil DOC and DTN, relative to untreated carcasses. In addition, heating the carcasses during pasteurization may result in loss of volatile organic compounds and potential enrichment of ^{13}C or ^{15}N in the remaining bodily fluids.

The primary objectives of this study were to determine: (1) the rate of salmon carcass decomposition, (2) the timing and amount of dissolved organic carbon (DOC) and dissolved total nitrogen (DTN) input into the soil, (3) the stable isotope ratios of the carbon and nitrogen laden fluids released from the carcasses, and (4) the effects of pasteurization and freezing on these parameters in a semi-arid riparian environment common to spawning reaches in central Idaho, eastern Washington, and eastern Oregon.

Methods

Location

This study was conducted at the University of Idaho Parker Research Farm near Moscow, Idaho, USA in early August 2009. Three 1.0 m x 1.2 m x 0.6 m high fenced enclosures were built to inhibit disturbance of the experiment by mammalian or avian scavengers. Insect access was not restricted as fly larvae in particular are known to play a large role in the progression of decomposition (Hocking and Reimchen 2006). The enclosures were placed 50 m from a perennial pond and wetland in an open grass meadow bordered by conifer and deciduous trees and covered with plywood to prevent precipitation from influencing carcass weight. Microcosms constructed of plastic lined cardboard wet-lock fish boxes measuring 70 cm x 30 cm were filled to a depth of 9 cm with well mixed local topsoil. The initial soil DOC and DTN concentrations were 348 mg C kg^{-1} dry soil ± 8 SE and 39 mg N kg^{-1} dry soil ± 1.6 SE. Four microcosms were placed within each of the three enclosures. Air temperature sensors (HOBO Pro, Onset Computer Corporation, USA) were placed in the center of each enclosure 20 cm below the plywood coverings, which effectively shielded the sensors from direct solar radiation. Temperature was recorded every 30 minutes. The chemical and biologic decomposition of flesh is regulated by thermal input (Vass et al. 1992). Therefore, the results

of this study are expressed in terms of accumulated degree days (ADD), the cumulative mean 24 hour temperature above 0˚C.

Treatments

Fresh unspawned male Chinook salmon (*Oncorhynchus tshawytscha*) carcasses of similar length (49 ± 2 cm) and physical characteristics were collected from the same population on two occasions from the Dworshak National Fish Hatchery in Ahsahka, Idaho, USA. Initially, nine carcasses that were destined for three disease-prevention treatments and two carcasses used to determine isotopic composition of whole carcasses were randomly selected. Two weeks later three fresh (untreated) carcasses were randomly selected. The carcasses were weighed within two hours of mortality. The weight of the carcasses ranged from 1.69 to 2.53 kg (mean 2.08 ± 0.07 SE). Six of the treatment carcasses and the two isotopic reference carcasses were immediately frozen at -20˚C. Pasteurization of the three remaining carcasses was accomplished by heating the carcasses in a convection oven until the cranial cavity exceeded 60˚C for 20 minutes followed by freezing at 20˚C for two weeks. The cranial temperature during pasteurization was measured by placing the probe of a digital cooking thermometer (Taylor Precision Products, Las Cruces, NM) through the olfactory and into the cranium. During pasteurization the carcasses were placed in individual parchment paper troughs. The convection oven was maintained at 100˚C until the cranial cavity reached 60˚C then the oven temperature was reduced to 70˚C to minimize cooking. Five days after the initial carcass collection, three of the immediately frozen carcasses were removed from the freezer, placed in the oven, and pasteurized then returned to the freezer for the remainder of the two weeks.

Experiment

The treated salmon carcasses were removed from the freezer twelve hours prior to the arrival of fresh carcasses and thawed in closed wet-lock fish boxes. The fresh carcasses were transported from the hatchery to the research site in a cooler and weighed within 2 hours. A total of twelve carcasses (n=3 for each treatment) were placed in individual nylon stockings to facilitate daily weighing and one carcass from each treatment was randomly placed in each enclosure. Based on the correlative relationship between rabbit carcass decomposition and relative weight loss described by Adlam and Simmons (2007), the rate of salmon

decomposition was assessed through daily weighing of the salmon carcasses until soft tissue weight loss ceased. The weight of the carcasses was obtained by carefully removing each carcass from its microcosm using a rigid plastic tray. The carcasses were weighed on a mechanical platform scale and then returned to their original position and orientation in the microcosms, this took less than 3 minutes for each carcass. For analysis purposes, carcass weights were standardized into relative proportions of the weight at time of placement in the microcosms by dividing the observed weight by the weight of the carcass at ADD = 0.

To assess the change in soil C and N content, two 2.5 cm soil cores to the full depth of the microcosm (9 cm) were collected beneath each salmon carcass, homogenized, and sieved to 2 mm. Fly larvae in the soil samples were removed during sieving and were not included in soil analyses. Soil samples were collected prior to carcass placement and 2, 5, 9, and 16 days post placement. Soil DOC and DTN were assessed following Horwath and Paul (1994). Briefly, a 10 g portion of each freshly sieved soil sample was agitated in 50 ml of 0.5 M K_2SO_4 for one hour then vacuum filtered to 0.45 μm. The extracts were stored at -20˚C until analysis. The soil dry mass of each extracted sample was determined by oven drying a separate portion of each sieved sample at 70˚C for 72 hours and back calculating based on gravimetric moisture content. 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.

Separately homogenizing two frozen Chinook salmon carcasses and randomly sampling each mixture with replication established whole fish reference C and N isotope ratios. To compare our results to published literature, the muscle tissue from each carcass was separated from the reminder of carcass and the muscle tissue initially homogenized separately from the remainder of the carcass. Approximately 2.5% of the total carcass weight (50g) of pureed muscle tissue was randomly removed from the slurry for analysis. The remaining muscle slurry was then thoroughly homogenized with the rest of the carcass and sampled in a similar manner to assess the isotopic composition of the carcass in its entirety. The fish reference samples were stored at 20˚C then freeze-dried and ground with a mortar and pestle prior to combustion for $\delta^{13}C$ and $\delta^{15}N$ on a Finnigan MAT Delta plus Isotope Ratio Mass Spectrometer (Finnigan MAT Gmbh, Germany) in the Idaho Stable Isotopes Laboratory,

Moscow, Idaho, USA. The C and N isotope ratios of these frozen carcasses were considered representative of fresh fish carcasses as C and N isotope ratios have been found to remain stable when preserved with freezing (Kaehler and Pakhomov 2001, Barrow et al. 2008).

To determine the C and N isotopic composition of nutrients released from the salmon carcasses to the soil, glass fiber filters were placed between each carcass and the soil to collect bodily fluids. Millipore AP40 glass fiber filters of 47 mm diameter were cut into fourths and one portion placed under each carcass mid-section. A 5x8 cm barrier of cellophane tape was placed between the soil and glass fiber filter to prevent adsorption from the soil. The glass fiber filters were replaced daily until soft tissue decomposition was complete. The glass fiber filters were stored at -20˚C then freeze-dried and combusted for $\delta^{13}C$ and $\delta^{15}N$ on a Finnigan MAT Delta plus Isotope Ratio Mass Spectrometer (Finnigan MAT Gmbh, Germany) in the Idaho Stable Isotopes Laboratory, Moscow, Idaho, USA.

Statistics

The relative carcass weights and the soil DOC and DTN for each treatment type were compared against that of the fresh carcasses for effects of treatment through time and time x treatment interactions using mixed effects repeated measures analysis of variance (ANOVA). If a time x treatment interaction was found then that treatment was removed from the model prior to analysis for treatment effects. Potential differences were investigated further using one-way ANOVA and Tukey's Honestly Significant Difference (HSD) at each sampling interval. Non-linearity in the C and N isotope values with time prevented model convergence so they were only analyzed with one-way ANOVA and Tukey's HSD 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 natural log transformed as necessary prior to analysis. Analysis was conducted using R version 2.10.0 (R Foundation for Statistical Computing, Austria). In addition, mean isotopic composition of fish carcass fluids weighted by daily relative weight lost was tested between treatments and against the whole reference carcasses using the summary statistics functionality of Student's t-tests in Minitab 16 (Minitab Inc., USA). Weighted means were calculated using the formula:

$$\bar{x} = \frac{\sum_{i=1}^{n} w_i x_i}{\sum_{i=1}^{n} w_i}$$

where w_i is the proportion of daily weight loss and x_i is the isotopic value for each observation. The variance of the weighted means was calculated using the formula:

$s^2 = \frac{V_1}{V_1^2 - V_2} \sum_{i=1}^{N} w_i (x_i - \bar{x})^2$ Where: $V_1 = \sum_{i=1}^{n} w_i$ and $V_2 = \sum_{i=1}^{n} w_i^2$.

The inferences that were made in this study were around the overall means of the population of sample means (the mean of the sampling distribution of sample means). Therefore, the standard error (SE) was used as the estimate of spread (the standard deviation of the sample means).

Results

Decomposition

Treating the carcasses by freezing only and the combinations of freezing and pasteurization resulted in a significant loss of carcass weight before deployment (P<0.05). Carcasses treated with freezing only, pasteurization then freezing, and freezing then pasteurization lost a mean 3.2% ± 0.6 SE, 9.2% ± 0.9 SE, and 16.8% ± 0.6 SE of their initial fresh weight, respectively.

The air temperature within the enclosures during the study ranged from 2˚ to 37˚ C with a 24 hour mean of 18.6˚C ± 0.7 SE resulting in the accumulation of 300 degree days in approximately 16 calendar days (Figure 1). The fresh carcasses reached skeletonization (termination of soft tissue decomposition) at 342 ± 29 SE ADD while the frozen only, pasteurized then frozen, and frozen then pasteurized carcasses reached skeletonization at 269 ± 37 SE, 274 ± 30 SE, and 310 ± 6 SE ADD, respectively. These end points were not significantly different and were therefore pooled, resulting in a mean time to skeletonization of 299 ± 15 SE ADD.

Though decomposition of all carcasses terminated at the same time, some early differences in decomposition rate due to treatment were detected (Table 1). Treating carcasses with freezing and pasteurization resulted in a higher relative loss of weight in the first 18 ADD (Figure 2A) and thus lower (P<0.05) relative weights compared to the fresh carcasses (Figure 2B). The relative daily weight loss (Figure 2A) did not differ between the treated and fresh carcasses for the remainder of decomposition and the relative weights of all treatments converged by 127 ADD (Figure 2B). However, this initial divergence of weight loss resulted

in a difference (P=0.01) in the overall decomposition rate for the pasteurized then frozen treatment relative to the fresh carcasses (Table 2).

Soil DOC and DTN

The timing of soil DOC and DTN accumulation closely tracked the carcass decomposition rates. Large increases in DOC and DTN were measured at 191 ADD (Figures 3A and B), which coincides with the period of greatest relative daily weight loss (Figure 2A). The mean cumulative increase, in soil DOC and DTN was 1057 mg C kg^{-1} dry soil ± 159 SE and 885 mg N kg^{-1} dry soil ± 115 SE per kg of pretreatment carcass mass at the termination of decomposition (Figures 3A and 3B).

The combined treatments of freezing and pasteurization resulted in higher (P<0.05) soil DOC and DTN concentrations relative to the fresh and frozen treatments but only at 40 ADD (Table 1). The nutrient increases at 40 ADD for both pasteurized treatments were similar at 154 mg C kg^{-1} dry soil ± 32 SE and 165 mg N kg^{-1} dry soil ± 28 SE per kg of pretreatment carcass mass. There were no further differences in soil DOC or DTN detected during the remainder of decomposition (Figures 3A and 3B). Our ability to test treatment effects on soil DOC and DTN through time were limited due to a large number of time x treatment interactions (Table 2). However, at skeletonization there were no detectable differences in soil DOC or DTN between treated and fresh carcasses (Figures 3A and 3B). The results at skeletonization for all carcasses were therefore pooled.

A substantial shift in the extractable soil C:N ratio from pretreatment to carcass skeletonization (298 ADD) was observed across all treatments. The soil prior to carcass placement had an extractable C:N ratio of 9:1. At skeletonization, the extractable soil C:N was 1.4:1. The C:N ratio of the reference salmon carcasses based on combustion analysis was 6:1 (data not shown).

Isotopes

The isotopic value of salmon muscle tissue is a good proxy for the entire carcass isotopic value as there were no significant differences in $\delta^{15}N$ and $\delta^{13}C$ between the muscle only and whole carcass samples of the reference carcasses. The $\delta^{15}N$ of the entire carcass and the muscle tissue was 13.8 ‰ ± 0.4 SE and 13.8 ‰ ± 0.3 SE, respectively. The $\delta^{13}C$ of the entire carcass and muscle tissue was -22.0 ‰ ± 0.1 SE and -21.2 ‰ ± 0.3 SE, respectively.

The carcasses released sufficient fluids for isotopic analysis for 227 ADD (11 days). During this time period, there were large shifts in the $\delta^{15}N$ of the fluids being released. Initially, the fluids were depleted relative to the mean fluid $\delta^{15}N$. This was followed by a period of highly enriched fluids being released starting at approx 130 ADD (Figure 4A). The mean $\delta^{15}N$ of the fluids, proportionally weighted by relative daily weight loss, was not different among the decomposing carcasses (Table 3). Thus, the weighted means were pooled across all treatments, resulting in an average $\delta^{15}N$ of the bodily fluids at the carcass-soil interface of 17.8 ‰ ± 1.5 SE (Figure 4A). This was a 4 ‰ enrichment in ^{15}N ($P<0.01$) relative to the whole isotopic reference carcasses (13.8 ‰ ± 0.4 SE). The weighted mean values were not significantly different from the straight mean values (17.4 ‰ ± 0.2 SE for $\delta^{15}N$ and -20.6 ‰ ± 0.2 SE for $\delta^{13}C$) but the straight mean reduces the estimate of the variance among the observations.

Among treatments, the $\delta^{15}N$ of the fluids released from the fresh carcasses were significantly enriched ($P<0.05$) relative to the treated carcasses through 40 ADD and remained enriched ($P<0.05$) relative to the frozen then pasteurized treatment through 65 ADD (Figure 4A). There were no differences in $\delta^{15}N$ due to treatment beyond 65 ADD

The $\delta^{13}C$ of the fluids at the soil-carcass interface exhibited a lot of variation during decomposition, both through time and among treatments. Initially, the $\delta^{13}C$ of the fluids was 1.5-2 ‰ enriched relative to the weighted mean fluid $\delta^{13}C$. The exuded fluid $\delta^{13}C$ became roughly 3 ‰ depleted relative to the weighted mean by 170 ADD then enriched back towards the weighted mean value by the time of carcass skeletonization (Figure 4B). The weighted mean $\delta^{13}C$ of the bodily fluids (-20.5 ‰ ± 1.7 SE) at the carcass soil interface for all treatments combined was not different from the isotopic reference carcasses as a whole (-22.0 ‰ ± 0.1 SE). Among treatments, the $\delta^{13}C$ of the fluids released from the pasteurized carcasses were notably depleted ($p<0.05$) relative to the fresh carcass fluids at 106 ADD and the frozen then pasteurized carcass fluids remained depleted through 127 ADD (Figure 4B).

Discussion

The decomposition of salmon carcasses progressed rapidly under the semi-arid climatic conditions in central Idaho. We observed skeletonization in 299 ADD, which agrees with a similar investigation in western Washington in late fall that reported skeletonization in 270 ADD (Drake et al 2005). However, it took only16 calendar days to accumulate the required

degree days for decomposition in Idaho compared to 76 calendar days to accumulate sufficient degree days for decomposition in western Washington. The timing of soil DOC and DTN increases closely tracked decomposition rate with the greatest increases observed during periods of rapid decomposition. The C and N isotope ratios of the fluids released during decomposition varied by up to 6 ‰ from start to finish with the weighted mean discharge of N being enriched 4‰ relative to the whole isotopic reference carcasses. We observed some initial differences in decomposition rate and subsequent nutrient release rates when salmon carcasses were treated with freezing and pasteurization prior to use for nutrient enhancement. However, given the lack of differences due to treatment beyond the first few days, we will focus our discussion on the ecological processes rather than the treatments.

Carcass decomposition rate

The rate of decomposition or relative daily weight loss was highly variable both through time and between carcasses (Figure 2A). The period of decomposition between 127 and 227 ADD was particularly dynamic with large losses in relative daily weight but also large differences in the relative daily weight loss between carcasses, as represented by the large standard error bars in figure 2A. We hypothesize that this period of high relative daily weight loss is a function of both fluid loss and outmigration of fly larvae. The variability between carcasses may be the result of larvae exiting the carcasses at different rates and times. We did not quantify the weight loss due to fluid loss or larvae emigration. However, it was noted, that the carcasses were heavily infested by 127 ADD with far fewer larvae noted by 227 ADD.

We were concerned that the use of nylon stockings to facilitate weighing influenced our observations of the decomposition process by limiting larval outmigration. The colonization of fly larvae was not impacted as flies readily laid their eggs through the mesh stocking and egg presence on the salmon carcasses was noted on the second calendar day (40ADD). Typically, fly larvae exit a carcass and burrow into the soil to pupate (Norris, 1965). The fine mesh of the nylon stockings may have hindered movement through the soil side of the stocking. However, larval outmigration was facilitated by seven 1cm^2 holes created while collecting small tissue samples during the first 127 ADD. The lack of larvae in the carcasses during the later stages of decomposition and the large number of larvae in the soil samples during the final sampling period indicates that the larvae eventually exited. However, since we did not have any carcasses without a mesh stocking, we are unable to quantify if the

mesh covering slowed down the outmigration of the larvae to the soil. If anything, this potential delay in outmigration made our estimates of decomposition rates slightly longer than the actual values.

The rapid progression of decomposition in this semi-arid environment relative to cooler coastal sites suggests that additional insight into the thermal regulation of salmon carcass decomposition is needed. Seasonal or regional differences in decomposition rate can play a large role in the dispersion of carcass nutrients within the riparian community. For instance, the number of calendar days spanned during decomposition could impact the amount of time that a carcass is available for scavengers to consume and redistribute carcass tissues and nutrients. Further, the rate of nutrient release impacts not only the availability of nutrients to the broader microbial and plant community but also the susceptibility of these nutrients to leaching and gaseous loss.

Soil DOC and DTN

Our findings suggest that the release of carcass nutrients to the soil predominantly occurs after decomposition has progressed roughly half way to skeletonization. We observed a respective 6 fold and 40 fold increase, relative to pretreatment values, in soil DOC and DTN (data not shown) between 106 and 298 ADD (Figure 3A and B). This rapid increase in soil DOC and DTN coincided with the period of rapid carcass weight loss or decomposition (Figure 2A and B) and was most likely due to fluid discharge from the carcasses. Fly larvae migrating from the carcasses into the soil may also serve as a reservoir for carcass nutrients (Levenbook 1971, Chapman 1998, Tibbets et al. 2008). However, since the adult flies migrate from the soil, the fly larvae were sieved from the soil prior to analysis and not included in the estimates of soil DOC and DTN.

The inputs of salmon carcass C and N to the soil altered the C:N ratio of the soils consistent with the increasing availability of N. We observed a shift in the soil C:N ratio from 9:1 pretreatment to 1.4:1 by 298 ADD. This low C:N ratio was particularly surprising given that the whole reference carcasses had a C:N of 6:1. This substantial shift in the soil C:N ratio below that of the carcasses themselves suggests a high level of C immobilization or respiratory loss by microbes during carcass decomposition relative to N (Cleveland and Liptzin 2007, Hartman and Richardson 2013). As organic compounds are consumed and the C metabolized, excess N is mineralized and excreted into the soil. Thus, a pulse of a C and N

laden substrate, such as salmon carcasses, can lead to the rapid microbial liberation of inorganic N. In addition, fly larva consumption of carcass tissue can also lead to the metabolic loss of C and the liberation of N in excreted waste (Green and Popa 2012). This rapid turnover of organic material and release of inorganic N is consistent with the large increases in soil inorganic N frequently observed in riparian areas following salmon carcasses deposition (e.g. Drake et al. 2005, Gende et al. 2007, Holtgrieve et al. 2009). The end result is a large increase in soil inorganic N that becomes available for plant and further microbial utilization.

Isotopes

The C and N isotope ratios of the fluids exuded from the carcasses varied by as much as 6 ‰ over the duration of decomposition (Figures 4A and B). However, the directions of the isotopic shifts were not consistent. Initially, the $\delta^{15}N$ of the fluids were depleted relative to the overall mean discharge but became more enriched just before skeletonization occurred. This pattern resulted in an overall mean fluid $\delta^{15}N$ that was enriched 4 ‰, relative to the whole reference carcasses. In contrast, the $\delta^{13}C$ of the fluids was enriched at the beginning of decomposition and became more depleted just before skeletonization occurred. Interestingly, the temporal shifts in both isotopic ratios coincide with an increase in the decomposition rate and a rapid increase in soil C and N concentrations. These temporal patterns in fluid $\delta^{15}N$ and $\delta^{13}C$ suggest two different isotopic behaviors.

The isotopic enrichment of the N compounds released during decomposition is consistent with processes leading to the preferential volatilization of the lighter N isotope (Fry 2006) and thus enrichment of the remaining fluids. The metabolism of organic materials by microbes and insects results in the accumulation of ammonia (NH_3) and ammonium (NH_4^+) (Carter et al. 2007, Green and Popa 2013). The accumulation of these compounds within the carcass or at the soil-carcass interface would lead to the potential for a high rate of volatile loss. This is supported by the observed increase in $\delta^{15}N$ (Figure 4A) starting at approximately 70 ADD prior to the onset of rapid carcass weight loss at 127 ADD (Figure 2A), indicating the fluids were accumulating in the carcass and rapid gaseous loss to the atmosphere was possible. We did not quantify the amount of N lost through volatilization. However, the warm temperatures of this semi-arid region would increase the likelihood of volatile ammonia loss (Miles et al. 2011, Zhang et al. 2013)

In contrast, the depletion of $\delta^{13}C$ during decomposition may be due to the timing of lipid decomposition. Pacific salmon tissue is roughly 6-16 % lipids (Crossin and Hinch 2005, Hamilton et al. 2005) which are substantially depleted in $\delta^{13}C$ relative to proteins and carbohydrates (Deniro and Epstein 1977). As summarized by Vass et al. (2002) and Carter et al. (2007), the initial phase of decomposition largely consists of the enzymatic destruction of cell walls and the release of cellular fluids, followed by microbial proliferation and the onset of carbohydrate, lipid, and protein destruction. This pattern of decomposition is consistent with the observed C isotopic trend (Figure 4B). The first three days (65 ADD) of decomposition produced exudates from the carcasses with a steady but higher than average $\delta^{13}C$, possibly derived from the release of cellular fluids. After 75 ADD the $\delta^{13}C$ of the exudates quickly became depleted relative to the weighted mean with the most negative values occurring during the period of greatest relative daily weight loss (Figure 2A). It is not readily apparent how much of this depletion is due to the liberation of C from lipids alone as we did not isolate the lipids from other C sources. However, the change in fluid $\delta^{13}C$ over the course of decomposition is consistent with the progression of decomposition and the breakdown of lipids.

Conclusion

Regional and seasonal temperatures appear to play a major role in the rate of salmon carcass decomposition. The rate at which carcasses decompose may influence not only the amount of time a carcass is available for scavenging and redistribution but also the rate and concentration at which carcass nutrients become available to the biotic community. We observed decomposition and nutrient release rates in a semi-arid environment that far exceeded those reported in coastal Pacific Northwest investigations. We also observed temporal patterns in the C and N isotopes of the exuded carcass fluids that varied as much as 6 ‰ over the period of decomposition with a mean $\delta^{15}N$ of the exuded fluids that was 4 ‰ enriched relative to the whole reference carcasses. Our observations indicate that it is not enough to measure the isotopic composition of the entire carcass or to assume that the isotopic values of the exuded nutrients remain static through time. These isotopic patterns have direct relevance on both trophic and mixing model investigations, possibly explaining variability in trophic observations and improving estimates of nutrient utilization by vegetation. We did observe some influences of freezing and pasteurization on salmon carcass decomposition and

nutrient release rates but these differences were constrained to the first few days of decomposition. This suggests that carcasses treated with freezing and pasteurization are suitable surrogates for fresh carcasses under semi-arid conditions.

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Table 1. Results of significant (α=0.05) differences between treated and fresh carcasses for relative daily weight loss, relative weight, soil nutrients, and the isotopic ratios of exuded carcasses fluids using ANOVA and Tukey's HSD at quantified accumulated degree day intervals.

ADD	ANOVA	Tukey's HSD	ANOVA	Tukey's HSD
	Relative daily weight loss		**Relative weight**	
18	$F_{3,8}$=43.2, *P*<0.01	PasFro, FroPas *P*<0.01	$F_{3,8}$=44.7, *P*<0.01	PasFro, FroPas *P*<0.01
40			$F_{3,8}$=38.2, *P*<0.01	PasFro, FroPas *P*<0.01
65			$F_{3,8}$=26.0, *P*<0.01	PasFro, FroPas *P*<0.01
85			$F_{3,8}$=15.1, *P*<0.01	PasFro *P*<0.01
106			$F_{3,8}$=6.3, *P*=0.02	PasFro *P*<0.01
	Soil dissolved organic carbon		**Soil dissolved total nitrogen**	
40	$F_{3,8}$=8.08, *P*<0.01	PasFro, FroPas *P*=0.02	$F_{3,8}$=45.4, *P*<0.01	PasFro, FroPas *P*<0.01
	Fluid δ^{13}C		**Fluid δ^{15}N**	
18			$F_{3,8}$=44.5, *P*<0.01	PasFro, FroPas *P*<0.01
40			$F_{3,8}$=19.7, *P*<0.01	PasFro, FroPas *P*<0.01
65			$F_{3,8}$=9.8, *P*<0.01	FroPas *P*=0.01
106	$F_{3,8}$=16.6, *P*<0.01	PasFro, FroPas *P*<0.01		
127	$F_{3,8}$=4.7, *P*=0.04	FroPas *P*<0.05		

Notes: PasFro and FroPas refer to carcasses treated with pasteurization then freezing and freezing then pasteurization, respectively.

Table 2. Results of repeated measures ANOVA comparing carcasses that had been frozen, pasteurized then frozen (PasFro), and frozen then pasteurized (FroPas) against fresh carcasses for treatment effects on the overall rate of relative carcass weight loss and the accumulation of soil dissolved organic C and dissolved total N.

		Treatment			Time x Treatment		
		d.f.	***T*-value**	***P*-value**	**d.f.**	***T*-value**	***P*-value**
Relative weight loss	Frozen	8	-0.56	0.59	200	-1.46	0.14
	PasFro	8	-3.27	0.01*	200	0.13	0.89
	FroPas	8	-2.27	0.053	200	0.27	0.78
Dissolved organic C	Frozen	6	1.30	0.24	32	-0.36	0.72
	PasFro				32	-2.14	0.04*
	FroPas	6	4.06	0.01*	32	-1.97	0.058
Dissolved total N	Frozen	4	1.35	0.14	32	-1.07	0.29
	PasFro				32	-4.47	0.00*
	FroPas				32	-4.69	0.00*

Notes: Treatment *P*-values are omitted where time x treatment interactions are significant. Treatments with significant time x treatment interactions were removed from the model prior to testing for treatment differences. Statistically significant *P*-values (α=0.05) are indicated with *.

Table 3. Mean carbon (C) and nitrogen (N) isotope ratios of fluid released during Chinook salmon carcass decomposition weighted by relative daily weight loss.

		δ^{15}N (‰)		**δ^{13}C (‰)**	
	Observations	Weighted mean	Standard error	Weighted mean	Standard error
Fresh	32	18.1	3.1	-20.0	3.4
Frozen	28	18.4	3.1	-20.8	3.5
PasFro	28	17.5	3.0	-20.2	3.5
FroPas	29	17.2	2.9	-20.8	3.5
Pooled	117	17.8	1.5	-20.2	3.5

Notes: PasFro and FroPas refer to carcasses treated with pasteurization then freezing and freezing then pasteurization respectively. Student's T-tests found no significant difference (α=0.05) in δ^{15}N or δ^{13}C between the weighted fluid means of treated and fresh carcasses. The results were therefore pooled. The pooled δ^{15}N was significantly enriched (d.f.=89, T=2.63, P=0.01) relative to the whole reference carcasses while there was no difference in δ^{13}C. Observations were weighted by the relative daily weight loss of the respective salmon carcass. Total observations derive from n=3 carcasses per treatment over the duration of measurable carcass fluid discharge.

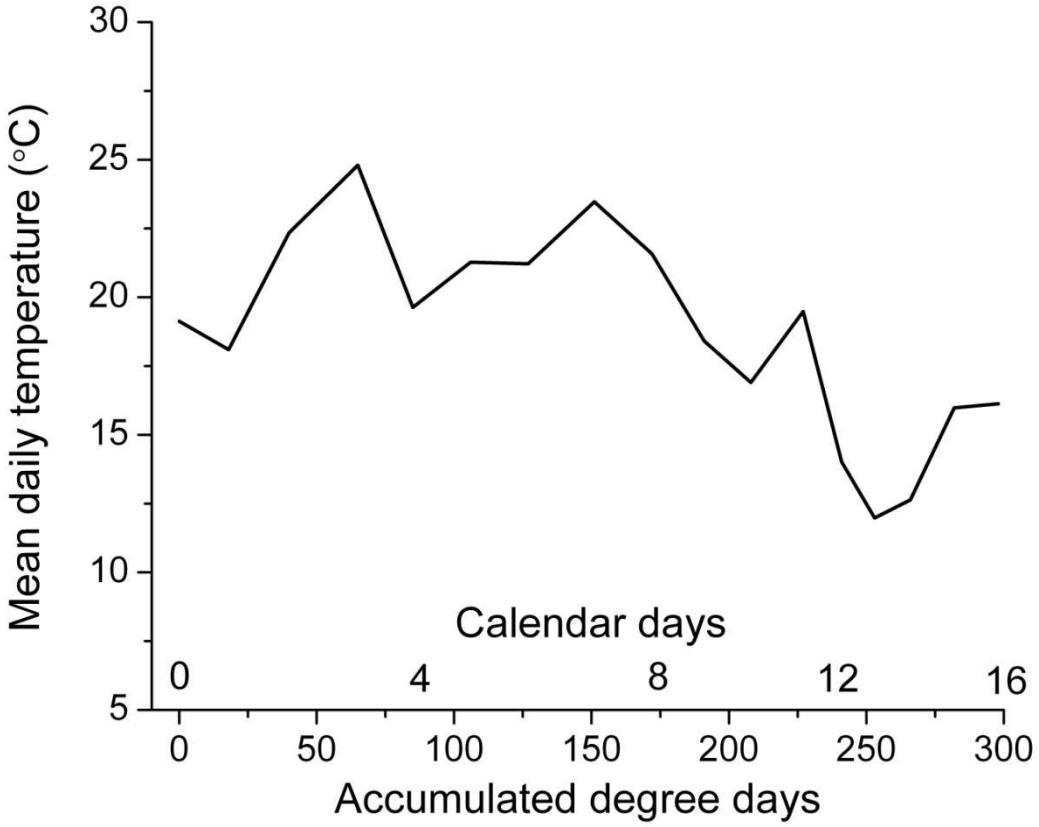
Figure 1. Mean and standard error of daily temperature (˚C) across all three protective enclosures for the 16 day study period (August 25th to September 10th, 2009) referenced against accumulated degree days (ADD). The variance between the daily means of the three temperature sensors was less than 0.02˚ C.

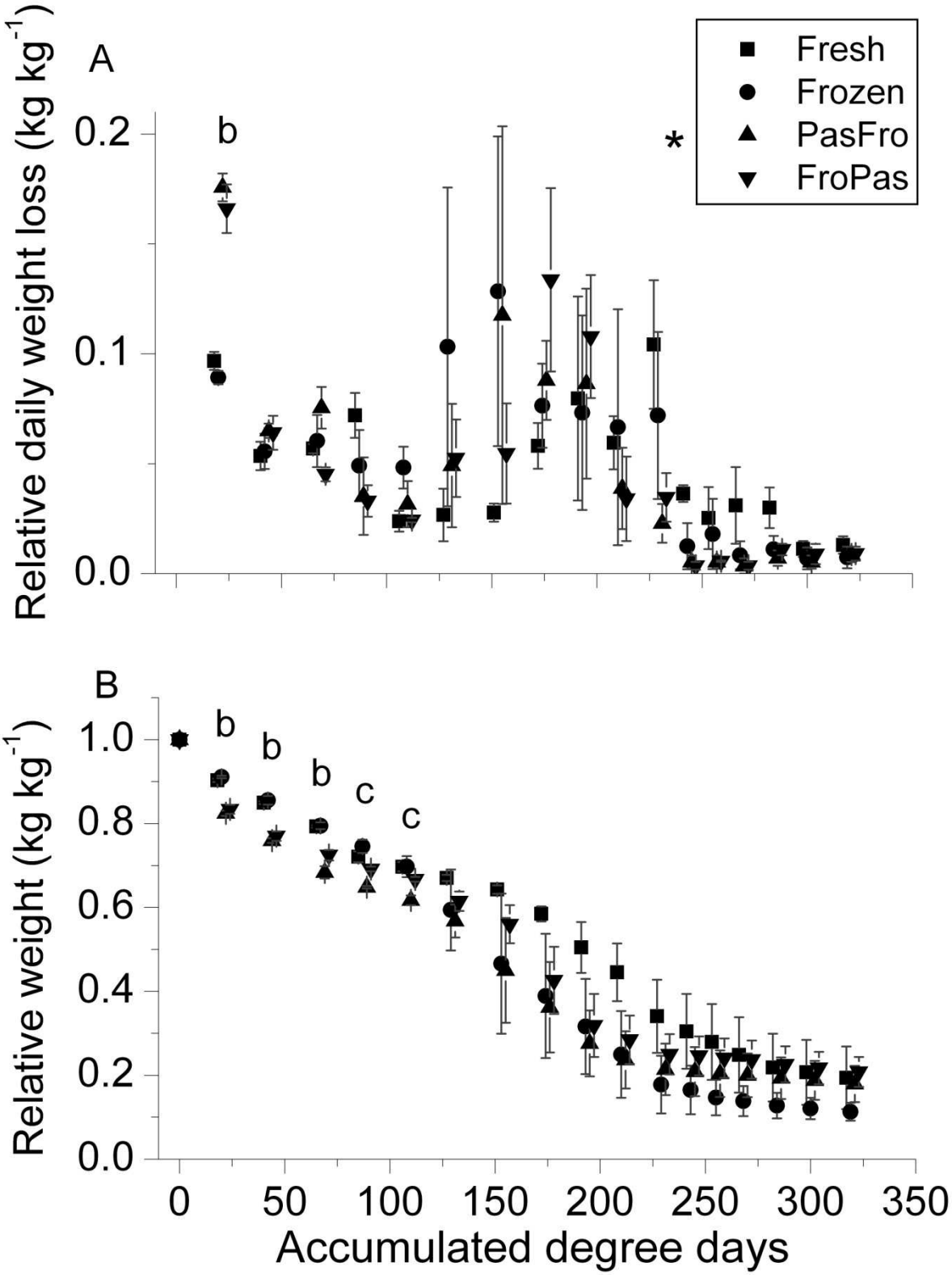
Figure 2. (A) Mean relative daily weight loss and (B) mean relative weight of decomposing Chinook salmon carcasses in relation to accumulated degree days (ADD). Relative weight is the observed weight divided by the weight of the carcass at ADD = 0. Relative daily weight loss is the proportion of weight lost since the previous observation. PasFro and FroPas indicate salmon carcasses treated with pasteurization then freezing and freezing then pasteurization, respectively. * indicates an overall significant difference (P=0.01) in the rate of relative weight loss as compared to fresh carcasses. Significant differences (α=0.05) from fresh carcasses at specific sampling intervals are indicated by (b) when both pasteurized treatments differ and (c) when only frozen then pasteurized carcasses differed. Error bars are standard error of the mean. Treatment values are projected with a 2-6 ADD offset from fresh values to improve resolution.

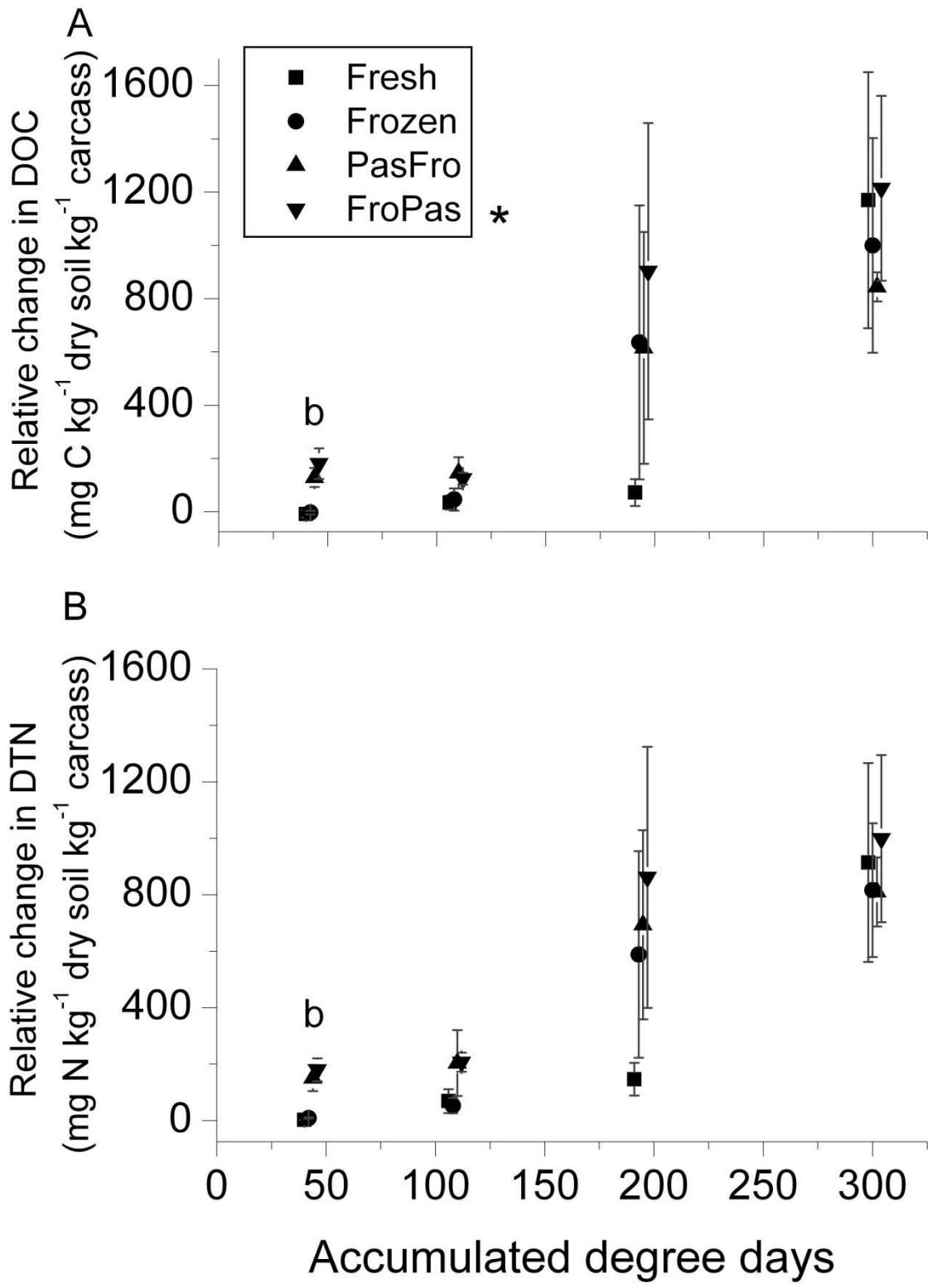
Figure 3. The mean relative change in (A) soil dissolved organic C (DOC) and (B) soil dissolved total N (DTN) pools from pretreatment concentrations beneath Chinook salmon carcasses treated with freezing, pasteurization then freezing (PasFro), and freezing then pasteurization (FroPas). Significant differences (α=0.05) of both pasteurized treatments from fresh carcasses at specific sampling intervals is indicated by (b). Determination of the overall effect of treatment on the rate of soil DOC and DTN accumulation was limited due to time x treatment interactions. * indicates an overall significant difference (P=0.01) in the rate of soil DOC accumulation when compared to fresh carcasses. Error bars represent standard error of the mean. Treatment values are projected with a 2-6 ADD offset from fresh values to improve resolution.

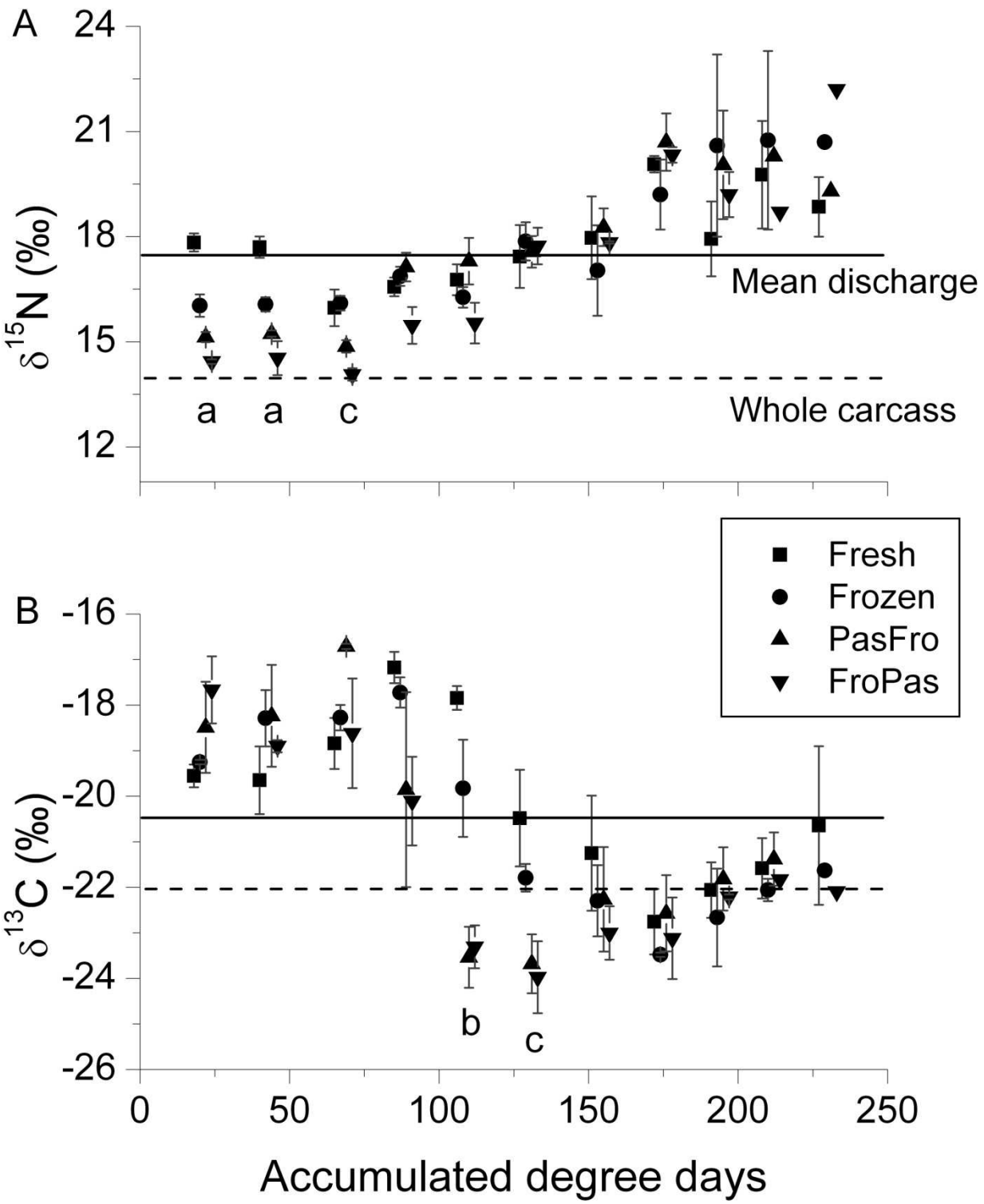
Figure 4. Mean (A) $\delta^{15}N$ and (B) $\delta^{13}C$ of fluids between the salmon carcasses and the soil surface during decomposition for untreated (Fresh) carcasses and carcasses treated with freezing, pasteurization then freezing (PasFro), and freezing then pasteurization (FroPas). Solid and dashed lines denote the mean of the carcass fluids weighted by relative daily weight loss and the mean of the whole reference salmon carcasses, respectively. Significant differences (α=0.05) from fresh carcasses at specific sampling intervals are indicated by (a)

for all three treatments, (b) for both pasteurized treatments and (c) for only the frozen then pasteurized treatment. The weighted mean $\delta^{15}N$ of the fluids was significantly enriched (d.f.=89, T=2.63 , P=0.01) relative to the whole reference carcasses while there was no difference in $\delta^{13}C$. Error bars are standard error of the mean. Treatment values are projected with a 2-6 ADD offset from fresh values to improve resolution.


Mean daily temperature (°C)
30
25
20
15
10
5
Calendar days
0
4
8
12
16
0
50
100
150
200
250
300
Accumulated degree days


A
B
Fresh
Frozen
PasFro
FroPas
Relative daily weight loss (kg kg-1)
Relative weight (kg kg-1)
Accumulated degree days
0.0
0.1
0.2
0.4
0.6
0.8
1.0
0
50
100
150
200
250
300
350
b
b
b
c
c
*


A
Relative change in DOC
(mg C kg-1 dry soil kg-1 carcass)
1600
1200
800
400
0
Fresh
Frozen
PasFro
FroPas
*
b
B
Relative change in DTN
(mg N kg-1 dry soil kg-1 carcass)
1600
1200
800
400
0
b
0
50
100
150
200
250
300
Accumulated degree days


A
24
21
18
15
12
δ15N (‰)
Mean discharge
Whole carcass
a
a
c
B
-16
-18
-20
-22
-24
-26
δ13C (‰)
b
c
Fresh
Frozen
PasFro
FroPas
0
50
100
150
200
250
Accumulated degree days

Chapter 3

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

Abstract

The historic abundance of salmonids returning to their natal streams of the inland Pacific Northwest, USA may have constituted a substantial nutrient influx into these commonly oligotrophic ecosystems. The precipitous decline of salmonid abundance within this inland region over the last century has stimulated recovery goals. However, these efforts are largely informed by investigations conducted in coastal maritime ecosystems. Inland ecosystems tend to be warmer and dryer as well as considerably more nutrient limited than their coastal counterpart. Understanding how these inland conditions influence the soil biogeochemical responses to salmon carcass deposition in inland riparian forests is a vital step in appropriately mitigating for their loss. Therefore, the soil biogeochemical responses of fish carcasses deposition in inland riparian forests of central Idaho were investigated through changes in soil nitrogen (N) and carbon (C) chemistry as well as estimates of total soil N and C loading per fish. This investigation revealed a 480 fold increase in soil inorganic N to 918 (218 SE) mg N kg^{-1} dry soil and a seven fold increase in dissolved organic C to 820 (228 SE) mg C kg^{-1} dry soil at 30 and 60 days following carcass deposition, respectively. The estimates of soil N and C loading only accounted for 30% and 10% of the fish carcass N and C, respectively. It is apparent from these loading estimates that soil microbial mineralization and consumption rates as well as the extent of volatile N loss following salmon carcass deposition in inland semi-arid ecosystems requires further investigation.

Introduction

Setting ecologically defensible recovery goals for endangered species is aided by fully understanding their ecologic role within ecosystems. The Columbia River Basin is believed to have once provided spawning habitat for up to 14.9 million anadramous fish annually (Gresh et al. 2000, NRC 1996). Which would have equated to an annual influx of up to 3 million kg of nitrogen (N) and at least 10.5 million kg of carbon (C) to this basin (Dempson et al. 2009, Gresh et al. 2000). By contrast, current salmon returns to the Columbia River Basin including hatchery fish is less than 5% of historic abundance (FPS 2014). Recovery goals for several salmonid species as low as 2-3% of historical abundance have been set within the 673,400 km^2 of the Columbia River basin (Perry et al. 2004), yet little is known about the ecological influence of salmon in this largely semi-arid region (Koyama et al. 2005, Hilderbrand et al. 1996). This is a large knowledge gap since semi-arid inland ecosystems encompass over 80% of the historic range of anadromous fish in the Pacific Northwest (NRC 1996).

Quantifying the ecological role of salmonids in the riparian forests of the Columbia River Basin is challenging. Knowledge of the effects of salmon carcasses on N and C soil biogeochemistry derives largely from coastal ecosystems in the Pacific Northwestern USA and Alaska (e.g. Holtgrieve et al. 2009, Gende et al. 2007, Drake et al. 2005) and using the primary findings from these studies would hinge on the assumption that environmental conditions in coastal forests differed little from interior forests. However, the environmental conditions are very different. The use of stable isotopes has affirmed the presence and utilization of salmon derived N by vegetation in interior Columbia River Basin forests (Koyama et al. 2005) and extinct grizzly bears (Hildebrand et al. 1996). Yet, relatively little is known about the inland ecosystem responses to this subsidy. For example, salmon carcass decomposition has been documented to take up to 76 days in a coastal maritime climate (Drake et al. 2005) whereas the decomposition in a semi-arid interior environment has been documented at 16 days (Wheeler et al. 2014), potentially due to large temperature differences. This slower relative rate of carcass decomposition in coastal ecosystems and thus nutrient release rate could allow soil nutrient utilization 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 detected soil nutrient responses in these coastal ecosystems, thus

under estimating the magnitude of interior ecosystems soil nutrient responses to the deposition of anadromous fish carcasses.

For these commonly nutrient limited interior ecosystems of the Pacific Northwest (Koyama et al. 2012, 2010, 2005; Stephan et al. 2012; Moore and Mika 1991) the deposition of anadromous fish carcasses may represent a substantial infusion of vital nutrients. These inland forests soils are known to have very low concentrations of inorganic N which potentially limits vegetative (Moore and Mika 1991) and soil microbial productivity (Koyama et al. 2012, 2010, 2005; Stephan et al. 2012) in this region. This lack of inorganic N availability can be partly attributed to soil microbial consumption being nearly equal to mineralization and nitrification rates (Koyama et al. 2010, Henderson et al. 1978). However, resource limitations also keep gross mineralization and nitrification rates quite low in many forests of the Pacific Northwest (Koyama et al. 2012, 2010; Hart et al. 1997; Stark and Hart 1997; Henderson et al. 1978). Forests of this region compensate by exhibiting characteristics of vegetative N retention which can include the reclaiming of N prior to senescence (Tully et al. 2013, Salifu and Timmer 2001, Aerts 1996, Edmonds et al. 1989) and the movement of N from older to younger needles in conifers (Millard and Grelet 2010). This can result in the senescent organic material having a high C:N ratio (>30) as is typical of the conifer forests of this region (Valachovic et al. 2004, Heath et al. 1988). This high C:N ratio leads to a suppression of soil mineralization rates and a further reduction in soil nutrient availability (Booth et al. 2005, Horner et al. 1998). Therefore, the addition of a N rich resource such as salmon carcasses could be expected to elicit a substantial soil microbial response.

There is evidence that soil microbial productivity in inland ecosystems of the Pacific Northwest may also be co-limited by the availability of mineralizable C (Koyama et al. 2010). The deposition of salmon 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 C and N 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 salmon carcasses in inland riparian forests could be expected to result in the potential increased mineralization of these soil C and N reserves.

A decomposing salmon represents a potentially significant source of nutrient enrichment in oligotrophic riparian soils in the interior northwestern USA. Despite the loss of this once abundant resource, the effects of salmon carcass deposition on riparian forest soils have not been investigated outside of more productive coastal riparian forest ecosystems. Thus, the objective of this investigation was to measure the influences of salmon carcass deposition on soil microbial productivity and the abundance of plant available nitrogen in a semi-arid inland riparian forest. Specifically: 1) what do the observable changes in extractable and bulk soil N infer about the underlying soil processes and what potential influence does the carcass carbon contribution have on these processes? 2) Does the addition of organic C from the carcasses result in a priming of soil microbial productivity and the release of sequestered soil N?

Methods

Investigative context

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 (Figure 1). The natal contributions of salmon derived nutrients to this watershed were abruptly severed in the early 1900's by the installation of dams, leaving the weathering of geologic material and atmospheric sources to sustain the productivity of these ecosystems. The soils of this region are characterized by colluvium over bedrock derived from granodiorite (the Idaho Batholith) with alluvial riparian soils ranging from O over C to OBC. The O horizon typically ranges from 0-2 cm in depth. This nutrient poor granitic substrate weathers slowly, contributing little to the sustainable nutrient requirements of these ecosystems (Henderson et al. 1978). The collective fixation and deposition of atmospheric N is also thought to contribute minimally (<3 kg ha^{-1} yr^{-1}) across most of this region (NADP 2014, Jurgensen et al. 1990). These nutrient poor conditions and the complete extirpation of salmonids make this watershed an ideal location to explore the biogeochemical importance of salmon derived nutrients to inland ecosystems throughout the Pacific Northwest.

The climate within the North Fork Boise River watershed is semi-arid with warm, dry summers and cold, moist winters. Natural Resource Conservation Service (NRCS) SNOTEL weather stations within the North Fork Boise River watershed report mean annual

temperatures over the last two decades of 5-8˚ C with mean monthly temperatures of -4˚ C in January and 15.5˚ C in July. The mean annual precipitation is 77-117 cm, predominantly as snow (NRCS 2012). The riparian vegetative communities are 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.

Riparian study design

Regional precipitation and temperature data during this investigation was acquired from Natural Resource Conservation Service (NRCS) SNOTEL climate stations at Graham Guard Station to the north of our 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 air temperature sensors (HOBO Pro, Onset Computer Corporation, USA). These sensors were placed inside vented Polyvinyl chloride (PVC) shelters to protect them from direct solar radiation and suspended 3 meters above the soil surface. The sensors were located in an open area 5-10 m from the stream edge and 5-10 m from the nearest vegetative canopy with a height greater than 1 m.

For this investigation a series of 1 m^2 riparian soil plots were established along four perennial 1-3rd order streams (Table 1). Within the larger project, two of these streams were randomly designated as reference streams while the other two were designated as anadromous fish carcass treatment streams. The riparian area along reference streams contained four plots while treatment streams contained four control (reference) and four treatment plots. The plots were randomly distributed throughout the length of each study reach and plots along treatment streams randomly assigned to treatment or control. This was a multi-year investigation resulting in the selection of new plots each treatment year.

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 (Wheeler et al. 2014, Marcarelli 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 one-half of a 3 kg carcass. The slurry was applied to the plots in an approximately 30 cm x 30 cm area and was 2 cm deep. The carcasses were pureed to prevent scavenger removal. However, time and equipment constraints limited the amount of carcass tissue that could be processed in the field. The blending process required the addition of deionized (DI) water therefore an equivalent amount of DI water (1 litter) was added to the control and reference plots.

In 2010, intact fish carcasses were applied at a rate of 328 g N and 1340 g C per plot. 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 from the periphery of each plot one week prior to the fish carcasses 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 colorimetric 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 $\delta^{15}N$ and $\delta^{13}C$, dissolved organic

carbon (DOC), acid hydrolysable C, and microbial biomass N and C. The release of soil respired CO_2 from the 2010 plots was also quantified. The soil cores were not separated by depth after the 2008 treatment year in order to incorporate the additional N and C parameters.

Extractable N and C

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

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

Combustion analysis

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 $\delta^{15}N$ and $\delta^{13}C$. Additionally, the acid hydrolysable portion of total soil C was assessed following Silbeira et al. (2008) and McLauchlan and Hobbie (2004). Briefly, 1 g of fresh soil sample was refluxed in 25 ml of 6M HCl at 105˚ C for 2 hours then repeatedly rinsed with DI water atop a 0.2 μm vacuum filter membrane. The remaining soil and recalcitrant C was dried at 70˚ C for 48 hrs then ground with a Wig-L-Bug amalgamator and combusted for C content. The relative proportion of the total C pool that was acid hydrolysable was calculated as: Relative hydrolysable C (%) = (1-recalcitrant C/total C) x 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 $\delta^{15}N$ of a marine end member. For the purposes of this investigation the mixing model relationship of: %MDN= ((TEM – SEM)/(MEM – SEM)) x 100 was defined so that the treatment end member (TEM) is the $\delta^{15}N$ observed in the treatment soils, the soil end member (SEM) is the $\delta^{15}N$ observed in the control soils, and the marine end member (MEM) is the mean $\delta^{15}N$ of the fluids that were released from the salmon carcasses to the soil. Traditionally the MEM value has been derived from salmon carcass tissue. However, recent evidence suggests that the $\delta^{15}N$ of carcass fluids entering the soil profile may be significantly enriched relative to whole carcasses (Wheeler et al. 2014). Therefore the $\delta^{15}N$ 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 ± 0.2‰ for $\delta^{15}N$ and ± 0.15‰ for $\delta^{13}C$.

Soil respiration

Soil respired CO_2 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 in both the treatment and reference plots to a depth of 2 cm. These collars were installed two weeks prior to the start of the experiment. Within the treatment plots, one of the salmon carcasses was placed directly over the two 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 salmon 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: $\overline{\delta^{15}N} = \frac{\bar{x}_1 + \bar{x}_2 \ldots \bar{x}_n}{\mathrm{n}}$, where $\bar{x}$ is the mean of daily observations and n is the number of days observed.

Soil N loading estimates

The instantaneous loading of soil N per fish carcass at each sampling interval was estimated utilizing the mean observed soil inorganic N concentration, following Gende et al. 2007, as well as utilizing the observed DTN, and total soil N concentrations. The instantaneous N loading was estimated from the difference between pre and post treatment soil N concentrations observed directly beneath the decomposing carcasses and scaled to the estimated influenced soil mass. This soil mass was comprised of the soil directly beneath the

carcasses and the soil 10 cm horizontally from the edge of the carcasses, both to a depth of 10 cm. The area of the carcasses was determined by importing field images of the carcasses into Image J 1.47 (National Institute of Health, USA). The depth of 10 cm was derived from the observed inorganic N concentrations with depth following the 2008 nutrient amendment. The bulk density of the soil (1.22 g cm^{-3}) was determined by collecting intact 5x10 cm cores from 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 (7700 cm^3 x 1.22 g cm^{-3}) and a soil mass horizontal from the carcasses of 21.7 kg ((25500 cm^{-3} - 7700 cm^3) x 1.22 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.

Results

In the two weeks following the 2008 treatment the average daily temperature within the study reaches was 17.3° C (daily range 2.2 – 33.4) and the SNOTEL sites received 0.8 cm of precipitation. The mean daily temperature within the study sites in the year following the 2008 treatment was 4.8° C (range -28.4 – 38.3) with the mean daily temperature consistently at 0° C or below from December 1st to April 1st. The average precipitation for the year was 94 cm (79 – 109 cm) (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. During this period of decomposition the mean daily temperature within the study reaches was 14.4° C (daily range 1.8 – 34.9) and the SNOTEL sites received approximately 2 cm of precipitation. Within the study reaches, the mean temperature over the one year duration of the investigation was 3.0° C with the mean daily temperature being consistently at 0° C or below from November 1st (100 days post treatment) to May 1st (270 days post treatment). The average precipitation for the year was 120 cm (95 – 146 cm) (NRCS 2012).

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 occurred across all depths at around 30 days post treatment but had declined to near background levels by one year 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 (Figure 2). Placing a linear regression through the maximum observations at each depth suggests that the extent of detectable inorganic N accumulation terminated at around 8.7 cm (Figure 2). Notably, all the maximum values for the three depths were derived from the same combined plots.

The 2010 placement of fish carcasses in these riparian forests yielded significant overall increases in soil extractable and total N (Table 2). 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 3A). Followed by a respective net increase in total extractable N (DTN) and bulk soil N of 1025 (313 SE) and 1556 (649 SE) mg N kg^{-1} dry soil at 60 days post treatment with respective total soil concentrations of 1035 (313 SE) and 3634 (751 SE) mg N kg^{-1} dry soil (Figures 3A 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 2). Soil inorganic N was predominantly ammonium-N. Nitrate-N was not significantly elevated until 300 days post treatment where it reached (24.2 (4.2SE) mg kg^{-1} dry soil) and remained at this level at one year post treatment.

The soil total C pool (38 (3.5SE) 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 contribute to the most labile active pool. The size of the acid hydrolysable pool (active + slow C pools) still prevented detection of the carcass C contribution but it is notable that this portion of the C pool was 25.5% (2.6SE) of the total soil C pool. Where we observed a significant increase in soil C both through time and in amplitude was within the extractable active C (DOC) pool (Figure 4, Table 2). Soil DOC was significantly elevated following carcass decomposition and had increased nearly 7 fold to 820 (228 SE) mg C kg^{-1} dry soil by

60 days post treatment. Treatment soil DOC concentrations had decreased substantial by the onset of winter but remained significantly elevated over control soils through one year post treatment. The total soil C:N ratio within treatment soils significantly decreased from 21 (1.3SE) prior to treatment to 14.6 (0.8SE) at 13 days post treatment and remained at this level through one year post treatment.

The increases in soil N and C resulted in significant soil microbial responses. These results are interpreted cautiously as random fumigation failures resulting in instances of irrefutable 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 5, Table 2). There was a significant overall effect of treatment on microbial biomass N but differences between treatment and control soils were not detected after spring snow melt. There were no differences between treated and control soil microbial biomass C but rather substantial temporal variability (± 100 mg kg^{-1} dry soil) between sampling intervals. These changes in microbial biomass C occurred in both treatment and control soils suggesting they were independent of the treatment. There was a significant overall effect of the treatment on the soil microbial biomass C:N ratio with treatment soil microbial biomass C:N decreasing from 5.4 (0.6SE) pretreatment to 3.6 (0.7SE) by 300 days post treatment. The treatment microbial biomass C:N was not different from the control soils (6.9 (0.6SE)) at one year post treatment (Table 2). Additional evidence of increased soil microbial activity is derived from the evolution of CO_2 from the soil. A 4 fold increase in soil respired CO_2 at 33 days post treatment (Figure 6) suggests that the availability of organic C from the treatment stimulated an increase in microbial productivity. Soil respiration had declined at 96 days post treatment mirroring the substantial decline in extractable DOC but remained significantly elevated relative to the control soils (Table 2). However, soil respiration rates following snowmelt had returned to background rates.

Soil $\delta^{15}N$ increased substantially following the carcass addition from 2.4‰ (0.9SE) pre-treatment to 6.7‰ (2.4SE) at 13 days post treatment and a maximum of 9.2‰ (3.4SE) at

96 days post treatment (Figure 7). The treatment soil $\delta^{15}N$ remained significantly elevated (7.4‰ (1.3SE)) relative to control soils (2.8‰ (0.8SE)) at one year post treatment. Soil $\delta^{13}C$ (-26.3‰ (0.5SE)) was not influenced by the carcass addition.

The $\delta^{15}N$ 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 $\delta^{15}N$ of 10.4‰ (0.2SE) and a $\delta^{13}C$ of -21.5‰ (0.01SE) while the mean $\delta^{15}N$ and of the $\delta^{13}C$ of the carcass fluids entering the soil were 11.9‰ (0.6SE) and -20.3‰ (0.7SE), respectively. The C:N ratio of the whole fish was 4.08.

Loading estimates

The estimates of mean soil N loading beneath the carcasses revealed a consistent N loading of 11.4 – 11.5 g N per fish at 13 days post treatment (Table 3). At 33 days post treatment a divergence in N loading estimates between the utilized N parameters was observed, with the estimates derived from the inorganic N, DTN, and total N reaching 18.6 g N, 20.8 g N, and 25.9 g N per fish, respectively. This suggests that organic and non-extractable N compounds were accumulating within the soil. The soil N loading estimates derived from the inorganic N and DTN concentrations peaked at 33 days post treatment while the estimates derived from total soil N peaked at 31.5 g N per fish at 60 days post treatment.

Discussion

The observed soil biogeochemical responses to the deposition of anadromous fish carcasses not only demonstrates the magnitude and persistence of carcass nutrient contributions in inland nutrient poor ecosystems but also provides insight into the cycling of N and C associated with fish carcass deposition. To explore these responses the instantaneous loading of N then C in the soil directly beneath and surrounding the deposited carcasses was estimated, following Gende et al. (2007). In this discussion, estimates of N mineralization and consumption as well as the observed soil respired CO_2 are incorporated in an effort improve upon these loading estimates. These estimates reveal a number of surprising soil biogeochemical responses as well as highlight some potentially important knowledge gaps in post salmon carcass deposition soil biogeochemical responses.

The estimates of instantaneous loading as well as soil microbial mineralization and consumption were based on the attenuation of soil N concentrations with depth following the

application of pureed carcass material in 2008. There were a couple of concerns with applying this relationship that should be discussed. The first is that the treatment loading rate in 2010 was nearly a four-fold increase over the 2008 treatment loading rate. The second issue is the use of pureed carcass tissue as a surrogate for whole carcasses. However, the consequence of this later concern may have compensated for the first concern. The mechanical destruction of skin and tissue of a decomposing organism (e.g. fish and rats) is reported to increase the rate of decay (Drake et al. 2005, Micozzi 1986). This increase in decomposition rate logically translates into a faster release of nutrient rich fluids to the soil. The sooner these carcass nutrients reach the soil the less likely they are to be diverted to other diffuse pathways, such as invertebrate consumption (Collins and Baxter in press, Hocking and Reimchen 2006, Meehan et al. 2005) or to volatile loss to the atmosphere, as has been confirmed with human cadavers (Hoffman et el. 2009). It appears that the pureeing of the fish carcasses may have amplified the amount of fish N that entered the soil following the 2008 amendment, thus leading to similar soil N concentrations to those observed following the 2010 whole carcass treatments.

It was further assumed that the decrease in soil inorganic N concentration with depth following the 2010 treatment was similar to the linear response observed following the 2008 treatment (Figure 2). Integrating the peak inorganic N concentrations for all three soil depths at 30 days following the 2008 treatment revealed that the soils used to create the linear estimate of soil inorganic N penetration (the upper most values for each depth in figure 2) had an overall inorganic N concentration of approximately 1064 mg kg^{-1} dry soil. This was similar to the mean peak inorganic N concentration from 2010 of 918 (219 SE) mg N kg^{-1} dry soil. It is notable that the three point used for this linear relationship were all derived from the same set of combined plots and thus were assumed to be reflective of soil N attenuation with depth at the observed soil N concentration. This decline in soil N concentration with depth in 2008 suggested that the depth of observable nutrient influence was 8.7 cm. Based on this evidence and to encompass potential variability between plots, the 2010 soil samples were collected to a depth of 10 cm and the estimates of N and C loading calculated to this depth.

The lateral extent of salmon carcass nutrient influence was also based on the vertical attenuation of soil N concentrations. The extent of lateral N movement in coastal soils has been reported at 20-40 cm from the edge of the carcass (Gende et al. 2007, Drake et al. 2005).

However, it was assumed that the gradient of lateral nutrient loading was similar to vertical loading based on the apparent retentiveness of the study soils and conservatively adopted 10 cm as the horizontal extent of N accumulation. Using the linear relationship from the 2008 observations (Figure 2) it was estimate that the average inorganic N concentration across this 0-10 cm expanse was approximately 50% of the peak concentration. There was presumably some additional later movement of nutrients by insects and scavengers that was neglected in these estimates. However, the lateral concentrations of soil nutrients were not quantified and the estimates therefore restricted to a conservative area of lateral nutrient influence.

The rapid decomposition of the fish carcasses and the N limited status of the study soil accentuate some of the underlying processes of N cycling. The visible completion of soft tissue decomposition was reached at 11 days post treatment. However, the peak in inorganic N did not occur until 33 days post treatment and the peak in total extractable and bulk soil N did not occur until 60 days post treatment (Figures 3A and B). This delay in peak N loading would be expected if a large quantity of organic N (DTN minus IN) or even non-extractable N (Bulk soil N minus DTN) was observed in the soil at the end of soft tissue decomposition but no such large increases were detected at 13 days post treatment (Table 3). This lack of difference in non-extractable N would suggest that a portion of the carcass N was initially bound within the >2 mm coarse soil and litter fraction. This would also include a large number of fly larvae that were found in the upper 5 cm of soil with potential digestive tracts full of nutrient rich fluids. These larvae were contained in the coarse fraction that was removed during sieving, potentially leading to an under-estimate of the N loading (Whitney and Zabowski 2004).

The ability to improve upon the instantaneous N loading estimates by incorporating potential soil mineralization and consumption rates is difficult due to a lack of published rates for forest soils following a concentrated organic amendment of this magnitude. However, the estimation of soil N loading provides some insight into the soil biogeochemical processes associated with fish carcass deposition. Beginning with the time period surrounding the salmon carcass decomposition (0-13 days), a reported soil mineralization rate for this region of 1.2 mg N kg^{1} dry soil d^{-1} (Koyama et al. 2010) can be utilized as the pre-treatment mineralization rate. Based on the total soil N content at 13 days post treatment, an approximate mineralization rate of 4.0 mg N kg^{-1} dry soil d^{-1} can be derived from the

relationship between total N and mineralization rates reported by Booth et al. (2005). Assuming that the increase in soil mineralization was somewhat linear, the average daily mineralization rate would have been 2.6 mg N kg^{1} dry soil d^{-1}, resulting in a gross mineralization of N over the 13 days within the estimated carcass influenced soil volume of 1.035 g N. This mineralized N likely contributed little to the observed extractable N pool at 13 days post treatment (11.5 g N) due to potentially equivalent rates of microbial consumption (Koyama et al. 2010, Booth et al. 2005, Stark and Hart 1997), suggesting that a large portion of the observed N at 13 days post treatment may have already been in a mineralized form prior to entering the soil. This is not surprising given the role of fly larvae and microbes in the decomposition of carcasses and the resultant production of ammonia rich metabolic wastes. However, an abundance of inorganic N at the soil surface or within the carcass cavity has implications for atmospheric N loss and the isotopic ratio of the soil N contribution, as will be discussed later.

The exploration of soil microbial mineralization and consumption rates suggests that mineralization rates may exceed published values. In order for the soil inorganic N to reach its peak of 918 mg N kg^{-1} dry soil at 33 days post treatment, 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. This would put the mineralization rate at a substantial departure from the predictive relationships developed by Booth et al. (2005). A second relationship between microbial biomass N and ammonium consumption from Booth et al. (2005) can therefore be utilized to estimate the ammonium consumption rate, where consumption includes microbial assimilation and nitrification. Using the mean observed microbial biomass N value at 33 days post treatment (0.091 g kg^{-1}) the consumption rate can be calculated as: log consumption = 0.48 x log (.091) + 1.102 = 4.0 mg kg^{-1} dry soil d^{-1} (Booth et al. 2005). This estimate seems a bit conservative given the amount of labile N and C added to the soil but is in agreement with reported soil microbial ammonium uptake rates (4 mg kg^{-1} soil d^{-1}) for this region (Koyama et al. 2012). Additionally, soil microbial biomass N only increased at an average rate of 1.8 mg N kg^{-1} dry soil d^{-1} between 13 and 33 days post treatment (Figure 5). Further, the lack of observed soil nitrate would also suggest that the gross nitrification rate was less than 2.5 mg N kg^{-1} soil d^{-1} based on the relationship between gross nitrate consumption rates and gross nitrification rates in forest soils reported by Stark and Hart (1997). Exceeding the estimated

ammonium consumption rate by 18 mg kg^{-1} soil d^{-1} would therefore require a gross mineralization rate of 22.0 mg kg^{-1} soil d^{-1}. Applying this to the estimated influenced soil volume over the 22 day period would suggest that an additional 10.2 g N was mineralized. This brings the estimated N loading at 33 days post treatment based on soil inorganic N to 29.8 g N per fish and the estimate based on total extractable N to 31.7 g N per fish. The soil N loading estimates at 33 days post treatment derived from the extractable N concentrations, when adjusted for soil microbial mineralization and consumption, exceeded the instantaneous N loading estimate derived from total soil N (25.9 g N per fish), suggesting that either the estimated mineralization and consumption rates were in error or that N was being removed from the target soil volume. It is evident from these estimates that further investigation into soil mineralization and consumption rates following fish carcass deposition are needed.

The remaining unknowns for these soil N loading estimates are the removal of N by vegetative consumption and volatile loss. Based on the vegetative uptake of N within forests of the north-central Oregon Coast Range with similar total soil N content, the uptake of N by vegetation may have accounted for the removal of roughly 0.3 g N (Perakis and Sinkhorn 2011) in the 33 days following carcass deposition. Looking at volatile loss in interior ecosystems, salmon spawn in this region during the late summer to fall when climate conditions are the driest and warmest of the year (Fulton 1968). These conditions would 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 $\delta^{15}N$ enrichment of the carcass fluids entering the soil, as reported by Wheeler et al. (2014) and again in this investigation, suggests that discriminatory loss of N is occurring. However, to our knowledge the volatile loss of ammonia following anadromous fish carcass deposition has not been directly investigated.

Exploring these loading estimates further using the linear two-member mixing model to calculate the percentage of the bulk soil N pool comprised of marine derived N (Gende et al. 2007) suggests the portion of total soil N comprised of salmon N at 33 days post treatment was 65%. This attests not only to the N deficient status of this region but also the potential importance of salmon as a nutrient subsidy in this region. Based on a total soil N at 33 days post treatment of 3.4 (0.5 SE) g N kg^{-1} dry soil and utilizing the estimates of influenced soil

volume and N concentration, the mixing model suggests a mean loading of 44.3 g N per fish. By comparison, the estimate using the net change in total soil N from the pretreatment concentrations at 33 days post treatment suggests the contribution was 25.9 g N per fish. At 60 days post treatment the estimates of peaking soil N loading based on the change in total soil N and the isotopic mixing model were 31.5 g N and 50.9 g N per fish, respectively.

Looking specifically at the soil under the carcasses at 33 days post treatment to rule out any error propagated by the assumptions of horizontal nutrient movement, the N loading based on the change in total soil N was 12.0 g N per fish while the N loading based on the mixing model was 20.5 g N per fish. The change in total soil N was utilized for this comparison, rather than the estimated soil N contribution using the mineralization and consumption rates, as the soil $\delta^{15}N$ utilized for the mixing model estimate was derived from the total soil N pool. The difference in loading estimates between the total soil N pool and the mixing model estimate may arise from several influencing factors. The first may be that the observed net increases in total soil N do not adequately account for N removal by vegetation. Forests of the Pacific Northwest with severe nutrient limitations are known to have a higher fine root biomass relative to more fertile sites (Edmonds et al. 1989) and thus a greater N uptake and storage potential.

A second potential source of error in the N loading estimates is an inability to account for volatile N loss, which is related to a third factor of potential fractionation during volatile N loss. Ammonium (NH_4^+) and ammonia (NH_3) coexist in a state of equilibrium which favors ammonia with increasing temperature (Zhou et al. 2009, Marschner and Rengel 2007). The daily temperatures within the study areas frequently reached highs of 30-34˚ C in the month following treatment, suggesting the presence of ammonia was favored. The energetic properties and preferential volatilization of $^{14}NH_3$ would result in an enrichment of the remaining N pool (Fry 2006). The fourth factor is potential fractionation during soil microbial mineralization and nitrification. The metabolism of N results in the biotic accumulation of ^{15}N and a depletion of $\delta^{15}N$ in waste products (Fry 2006). The removal of these waste products (i.e. ammonium and nitrate) from the soil pool by vegetative consumption or atmospheric loss, results in enrichment of the remaining N pool. Leaching would be a fifth N loss pathway. Though, the apparent N retentiveness of the study soils based on the 2008 depth observations and the small amount of precipitation received during this time period (~2 cm) would suggest

a minimal loss of N through leaching. Any or all of these mechanisms working in concert could greatly reduce the abundance of soil N as well as inflate the N loading estimates based on the soil isotopic N ratios.

It is therefore concluded based on these potential pathways of N loss and isotopic enrichment that the actual soil N loading from each fish carcass likely fell within the range of the estimates derived from the total soil N and isotopic mixing model methods. The N loading estimates previously derived utilizing the extractable soil N in conjunction with mineralization and consumption rates also supports this conclusion. These estimates of soil N loading in close proximity to the carcass were considerably lower than the total N contained within each fish carcass. However, it is emphasized that a large proportion of the total soil N pool was potentially comprised of fish carcass N. It is further emphasized that diffuse pathways of nutrient redistribution within the ecosystem would not be accounted for in these estimates. This would suggest that salmon nutrient contributions may have historically been a substantial source of N within these oligotrophic inland ecosystems.

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 to soil nutrient cycling. This may be largely due to the 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). The pre-treatment total soil C was 38 g 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 is about 50% of the C in the bulk soil. However, a large portion of this contribution was potentially consumed by an abundance of fly larvae and microbes during carcass decomposition (Collins and Baxter in press, Hocking and Reimchen 2006, Meehan et al. 2005). The primary evidence of a significant soil C contribution was from the extractable DOC pool (Figure 4). Calculating an instantaneous net extractable C loading as done with N suggested a mean net loading of 5.8, 11.6, and 14.2 g C at 13, 33, and 60 days post treatment, respectively. Using the C:N of the whole fish carcasses (4.08) and the total soil N loading estimates a C contribution of roughly 46.9, 105.6, and 128.5 g C per fish would have been expected in the absence of C consumption.

This is a large gap in the C budget and only a small portion of it was accounted for as soil respired CO_2. Extrapolating the observed peak soil respiration rate (10.59 μmol m^{-2} s^{-1}) to the surface area of the carcasses (770 cm^2) and the 10 cm surrounding the carcasses (1780 cm^{-2}) with the expectation that the respiration in the soil surrounding the carcasses was 50% of the max, the respiratory loss of C reached approximately 1.8 g C d^{-1}. The steep upward trend in soil microbial biomass N suggests that the microbial response to the nutrient addition was rapid. If it is assumed that the average respiratory C loss reached 1.8 g C d^{-1} by 13 days post treatment (average of 0.9 g C d^{-1} between 0-13 days) then soil respired CO_2 would account for approximately 28%, 38%, and 42% of the consumed C from 0-13, 13-33, and 33-60 days post treatment, respectively. A sizable portion of the missing C at 13 days post treatment could be attributed to above ground metabolic consumption by fly larvae and microbes during carcass decomposition. This would support the assertion that most of the observed N at 13 days post treatment was already mineralized prior to entering the soil. Our inability to detect significant contributions of carcass C to the larger soil C pools or microbial biomass C leaves the fate of the remaining C largely unknown. However, DOC remained consistently elevated at one year post treatment suggesting that this C was slowly being liberated to the active C pool.

We were unable to confirm the liberation of sequestered N 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 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 assess 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. These responses as well as the possibility of long term priming of microbial productivity require further examination.

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 carcass deposition event has on the availability of soil N. This availability of N would not only benefit those vegetative species capable of capitalizing on and storing this pulse of N (Drake et al. 2006) but the persistence of available soil N would ensure species with annual or

perennial life cycles would also benefit 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). This has been shown in relation to the decomposition and feeding behaviors of ungulates in numerous ecosystems (Bump et al. 2009,Parmenter and MacMahon 2009, Danell et al. 2002, Towne 2000). For example, the spatial patterns of wolf predation on moose in Isle Royale National Park have been attributed to changes in soil nutrient availability, soil microbial biomass, and vegetative quality patterns within this ecosystem (Bump et al. 2009). This increase in vegetative quality leads to soil nutrient feedbacks through increased senescent litter quality and increased herbivory by moose and thus soil nutrient feed backs through metabolic wastes. Not to mention the increased likelihood of additional wolf predation in these areas. The persistence and availability of soil N to herbaceous plants in the growing season following carcass deposition may accelerate or amplify these effects through increased biomass production and increased litter feedbacks.

A salmon carcass lacks the large biomass of an ungulate like a moose but this is offset by the high number of salmon carcasses potentially deposited within the forest both spatially and temporally. A salmon carcass has been demonstrated to have a localized influence on soil nutrients (Gende et al. 2007, Drake et al. 2005). However, the spatial distribution of these numerous carcasses may be more advantageous to the forested ecosystem than the death of a few ungulates by creating nutrient access to a larger portion of the soil and plant community. The historic abundance of salmonids returning to spawn within the Columbia River Basin has been estimated at between 8 – 14.9 million fish (Gresh et al. 2000, NRC 1996). These salmonids spawned within approximately 21,325 km of historically accessible stream habitat (NRC 1996). Bears have been observed carrying carcasses 200 meters into the forest (Gende and Quinn 2004) but isotopic and carcass deposition evidence suggests most are deposited much closer to the stream edge (Gende et al. 2004, Bilby et al. 2003, Hilderbrand et al. 1999). If we assume the mean riparian area of greatest deposition is within 50 m of the stream and a carcass deposition rate of 25% of the run (Quinn et al. 2009, 2003), there would have been an average deposition across the Columbia River Basin of 93-175 carcasses ha^{-1} yr^{-1} within these riparian corridors. Using the calculated 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, Hilderbrand et al. 1999). By comparison, the vast majority of the Pacific Northwest is reported to receive less than 1 kg N ha^{-2} yr^{-1} from atmospheric deposition (NADP 2012, 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^{-2} yr^{-1} (Jurgensen et al. 1990). This places the deposition of anadromous 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 increases in soil available N directly below salmon carcasses that not only exceeded coastal observations but also remained elevated at one year post treatment. This persistence of available soil N through the subsequent growing season results in the accessibility of N by a larger number of vegetative species with differing life cycles. The deposition of a salmon carcass has been deemed a localized event but through the N attenuation of these inland ecosystems and the eventual probability of vegetation near salmon bearing streams encountering a salmon carcass or the metabolic remains of one, the productivity of these forests may have been historically maintained. 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 by the emigration of fly larvae as well as through consumption by other insects and scavengers. It is apparent from these observations that mitigation efforts in inland ecosystems would benefit from further investigation into the influence of salmon derived nutrients in semi-arid inland riparian forests, particularly in regard to soil microbial responses following salmon carcass deposition as well as the potential for volatile N loss.

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

Stream	Latitude (N)	Longitude (W)	Elevation (m)	Sand/Silt/Clay (%)
Banner Creek	43˚59'14"	115˚32'46"	1625	73/6/21
Beaver Creek	43˚49'30"	115˚31'08"	1311	94/4/2
Little Beaver Cr.	43˚58'07"	115˚35'48"	1572	75/25/1
Trail Creek	43˚54'00"	115˚23'12"	1571	68/28/5

Table 2. 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.

		One-tailed t-tests					
		Days since treatment					
Dependent Soil Variable	Repeated Measures ANOVA	13	33	60	96	300	361
Inorganic N	DF = 14	DF = 13	DF = 13	DF = 14	DF = 14	DF = 14	DF = 14
	T = -14.1	T = 21.8	T = 21.8	T = 11.9	T = 9.4	T = 9.4	T = 2.77
	***P* < 0.01**	***P* < 0.01**	***P* < 0.01**	***P* < 0.01**	***P* < 0.01**	***P* < 0.01**	***P* < 0.01**
Organic N	DF = 14	DF = 13	DF = 14	DF = 14	DF = 14	DF = 14	DF = 13
	T = 3.5	T = 1.6	T = 2.5	T = 2.3	T = 3.0	T = 2.9	T = 2.3
	***P* < 0.01**	*P* = 0.07	***P* = 0.01**	***P* = 0.02**	***P* < 0.01**	***P* < 0.01**	***P* = 0.02**
Total N	DF = 14	DF = 14	DF = 14	DF = 14	DF = 14	DF = 14	DF = 14
	T = -2.3	T = 1.8	T = 2.6	T = 2.6	T = 2.0	T =n 2.4	T = 2.2
	***P* = 0.04**	***P* < 0.05**	***P* = 0.01**	***P* = 0.01**	***P* = 0.03**	***P* = 0.01**	***P* = 0.02**
Total C	DF = 14	NA	NA	NA	NA	NA	NA
	T = -0.7						
	P = 0.5						
Total C:N	DF = 14	DF = 14	DF = 14	DF = 14	DF = 14	DF = 14	DF = 14
	T = -4.5	T = 5.8	T = 3.8	T = 6.5	T = 6.5	T = 3.4	T = 3.1
	***P* < 0.01**	***P* < 0.01**	***P* < 0.01**	***P* < 0.01**	***P* < 0.01**	***P* < 0.01**	***P* < 0.01**
Relative Hydrolysable C	DF = 14	NA	NA	NA	NA	NA	NA
	T = -0.5						
	P = 0.6						
Dissolved Organic C	DF = 14	DF = 14	DF = 14	DF = 14	DF = 14	DF = 14	DF = 14
	T = -4.1	T = 5.0	T = 3.4	T = 4.4	T = 3.1	T = 2.5	T = 3.3
	***P* < 0.01**	***P* < 0.01**	***P* < 0.01**	***P* < 0.01**	***P* < 0.01**	***P* = 0.01**	***P* < 0.01**
Soil Respired CO_2		Not measured	DF = 14	Not measured	DF = 14	DF = 14	DF = 14
			T = 3.9		T = 1.8	T = 0.2	T = 0.6
			***P* < 0.01**		***P* < 0.05**	*P* = 0.8	*P* = 0.3
Microbial	DF = 14	DF = 11	DF = 13	DF = 13	DF = 10	DF = 11	DF = 11

Biomass N	T = 2.9	T = 1.0	T = 2.7	T = 3.6	T = 3.4	T = 0.1	T = 0.4
	***P* = 0.01**	*P* = 0.2	***P* = 0.01**	***P* < 0.01**	***P* < 0.01**	*P* = 0.4	*P* = 0.4
Microbial	DF = 14	NA	NA	NA	NA	NA	NA
Biomass C	T = 0.3						
	P = 0.7						
Microbial	DF = 14	DF = 10	DF = 12	DF = 10	DF = 10	DF = 10	DF = 8
Biomass C:N	T = -6.4	T = 4.6	T = 4.3	T = 4.5	T = 14.3	T = 15.2	T = 0.3
	***P* < 0.01**	***P* < 0.01**	***P* < 0.01**	***P* < 0.01**	***P* < 0.01**	***P* < 0.01**	*P* = 0.7

Notes: The repeated measures ANOVA for soil respired CO_2 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 3. Estimates of instantaneous soil N loading in soils directly under and 10 cm surrounding anadromous fish carcasses based on net increases in soil inorganic N, dissolved total N (DTN), and total soil N concentrations.

	Instantaneous N loading (g) using:		
Days Since Treatment	IN	DTN	Total Soil N
13	11.4	11.5	11.5
33	18.6	20.8	25.9
60	16.3	20.8	31.5

Figure 1. A map of the study area within the North Fork Boise River watershed with an inset showing the study location within the western United States. The study streams of Little Beaver Creek and Trail Creek received anadromous fish carcass amendments while Banner Creek and Beaver Creek were utilized as reference streams. Image created by Jamey Anderson.

Figure 2. 2008 soil inorganic N (NH_4^+-N + NO_3^--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 maximum values across all three depths derive from the same set of homogenized soil samples.

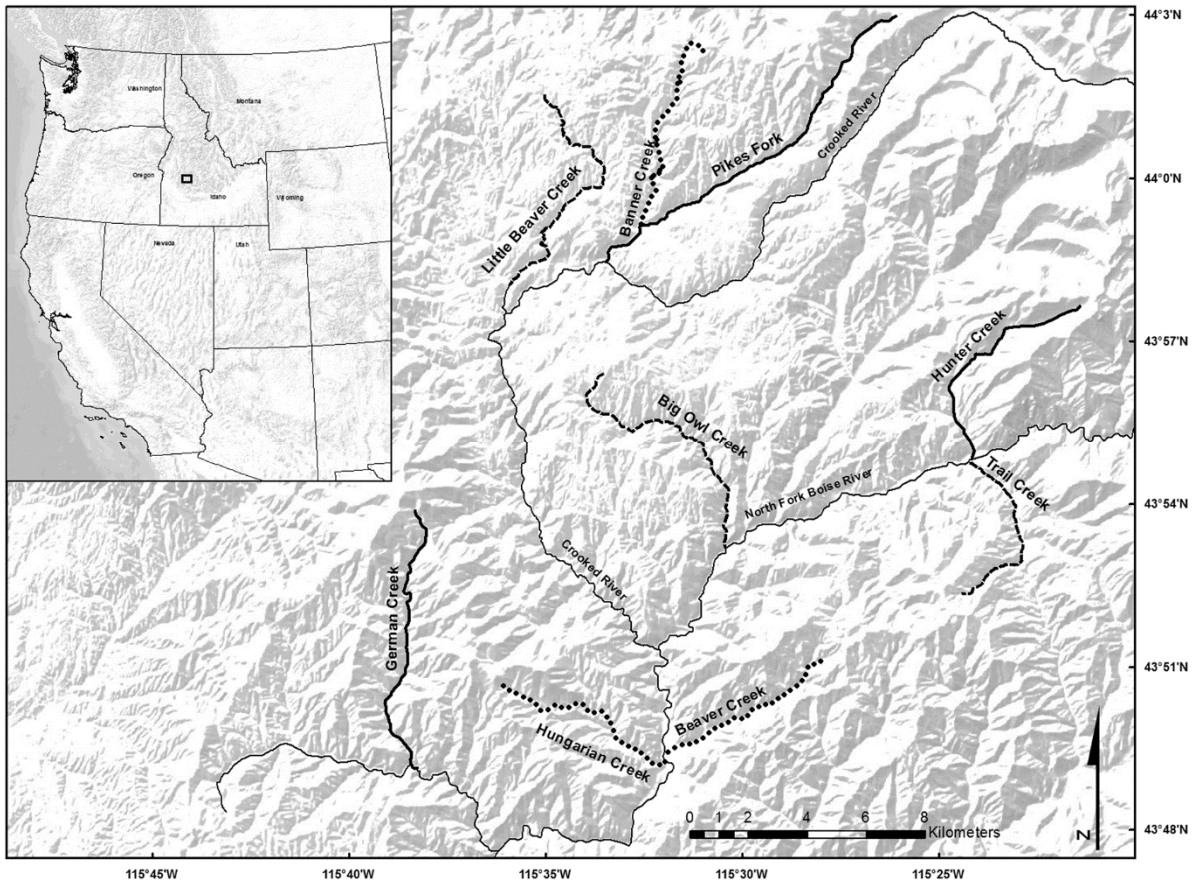
Figure 3. The mean A) Soil extractable inorganic and organic N and B) Bulk soil total N for n=8 soil plots in relation to days since treatment in 2010. The values to the left of zero are pretreatment values. There was an overall significant effect (P<0.05) of treatment on all three N concentrations (Table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

Figure 4. 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 2). The asterisks indicate significant (P<0.05) point in time differences between treated and control soils.

Figure 5. 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 2). The asterisks indicate significant (P<0.05) point in time differences between treated and control soils.

Figure 6. 2010 soil respired CO_2 (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 CO_2 (Table 2). The asterisks indicate significant (P<0.05) point in time differences between treated and control soils.

Figure 7. The soil $\delta^{15}N$ 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 $\delta^{15}N$ 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.


Washington
Montana
Oregon
Idaho
Wyoming
Nevada
Utah
Little Beaver Creek
Banner Creek
Pikes Fork
Crooked River
Hunter Creek
Big Owl Creek
North Fork Boise River
Trail Creek
Crooked River
German Creek
Beaver Creek
Hungarian Creek
0 1 2 4 6 8
Kilometers
N
44°3'N
44°0'N
43°57'N
43°54'N
43°51'N
43°48'N
115°45'W
115°40'W
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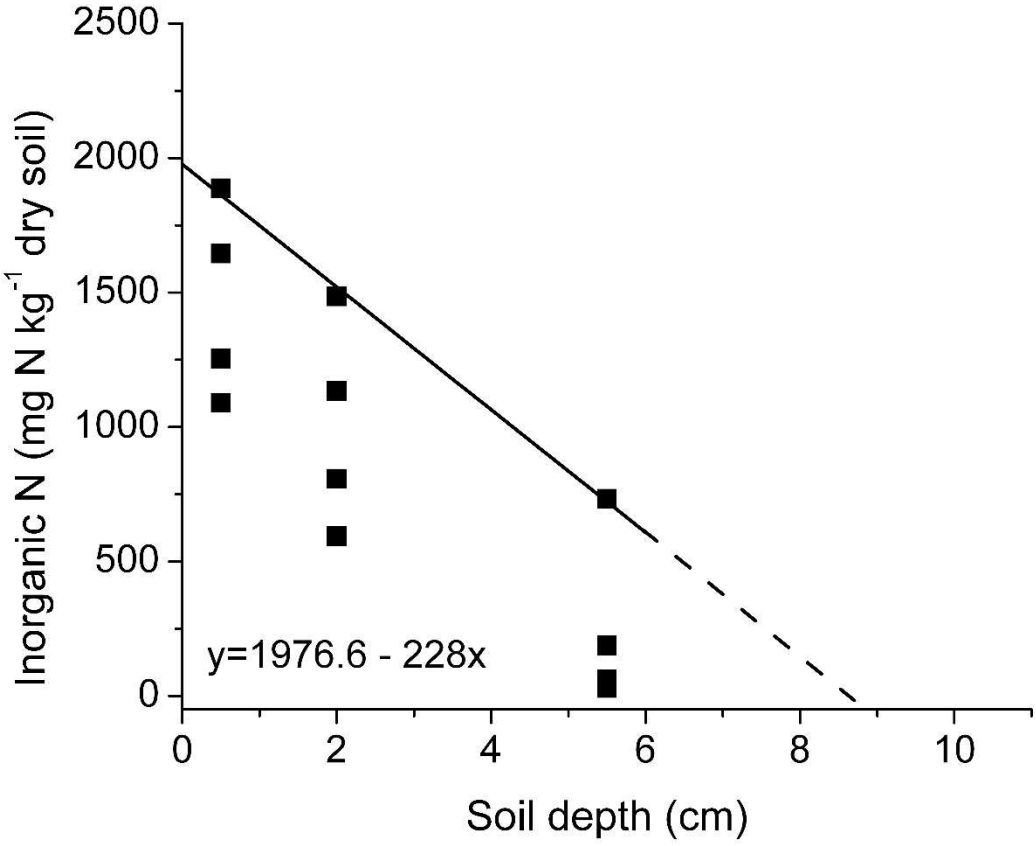
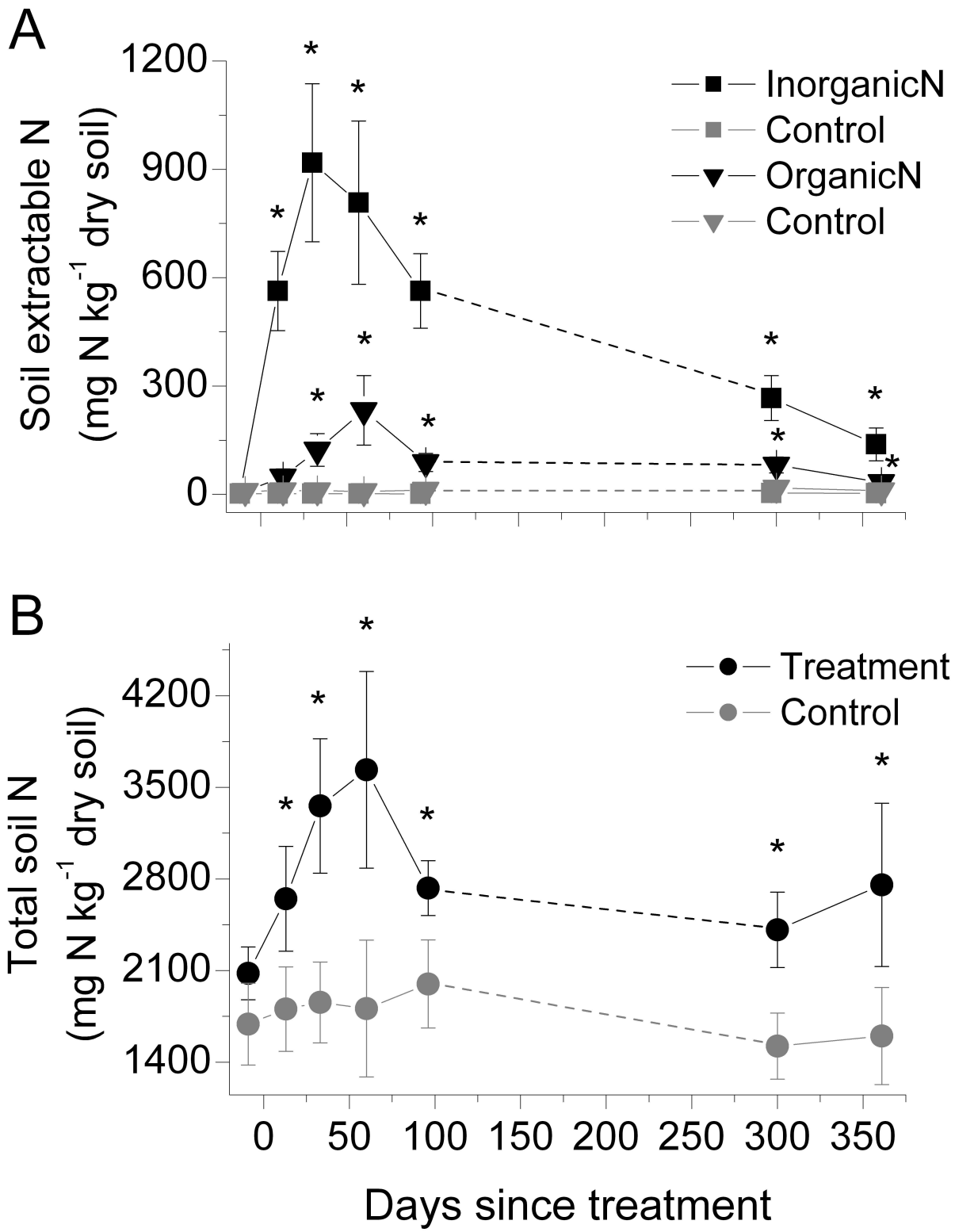
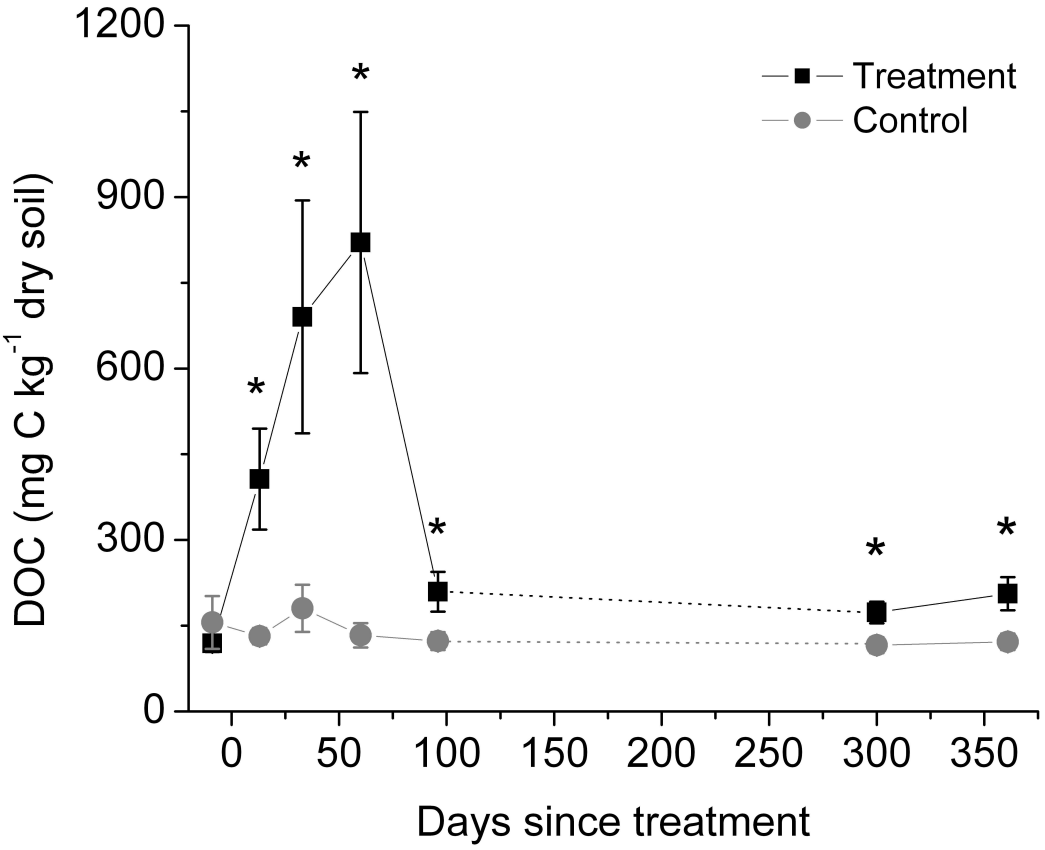
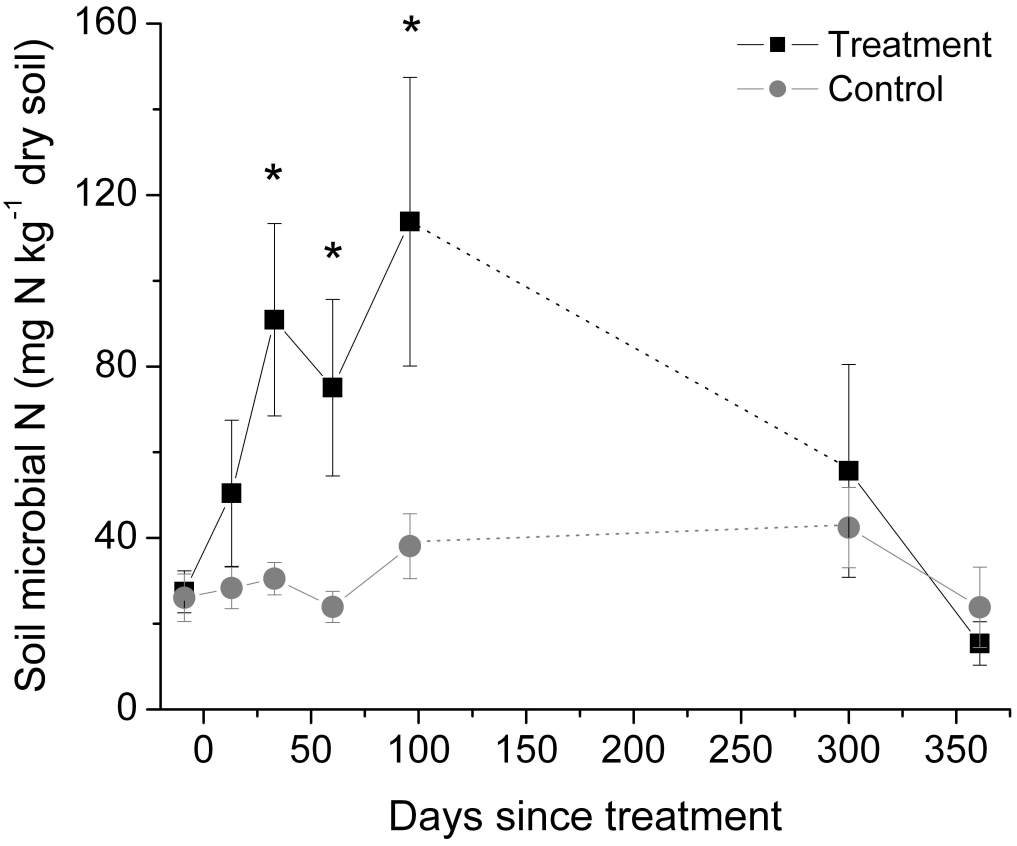
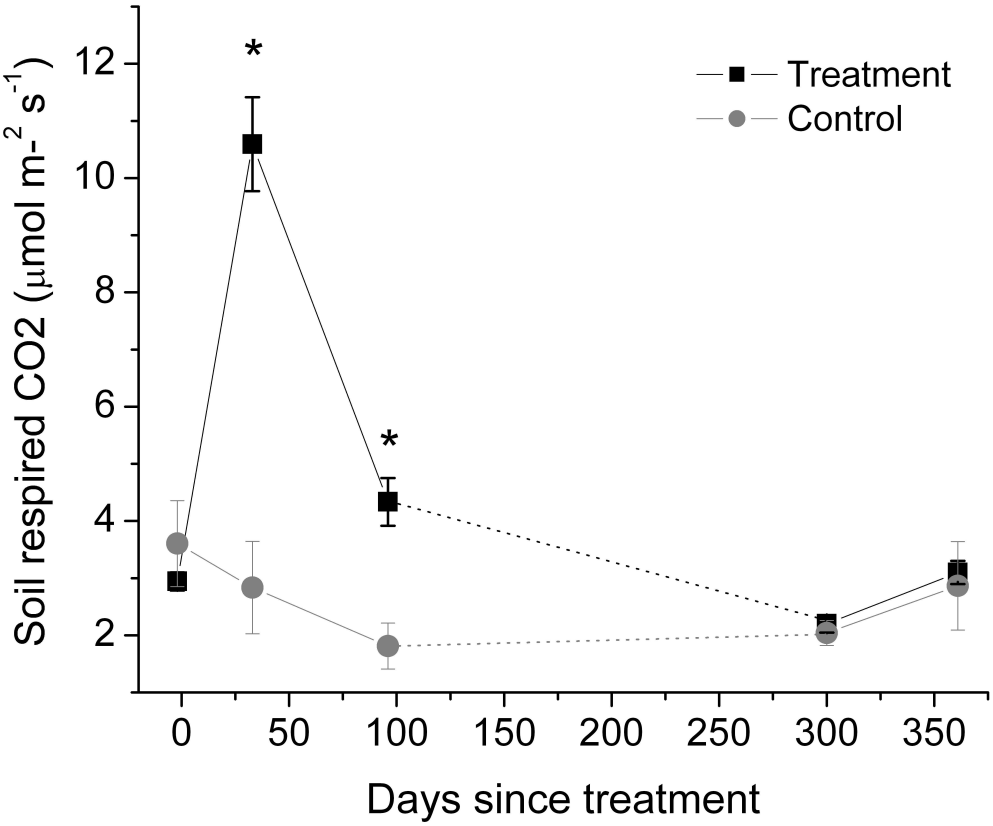
115°30'W
115°25'W

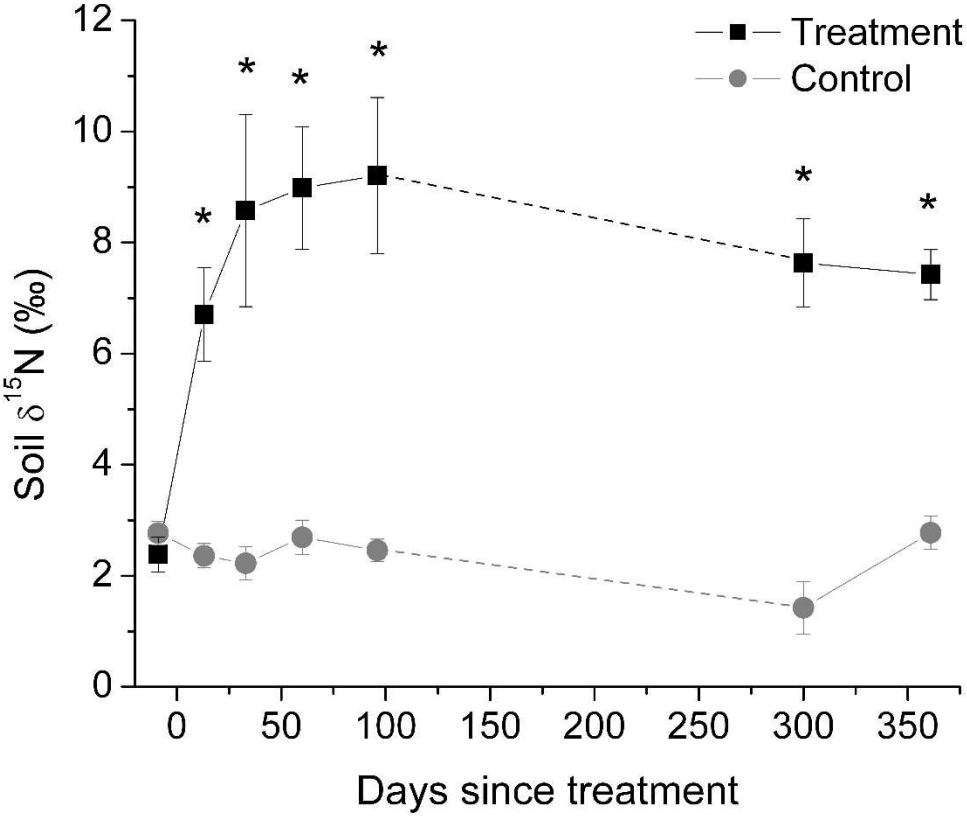

2500
2000
1500
1000
500
0
Inorganic N (mg N kg-1 dry soil)
y=1976.6 - 228x
0
2
4
6
8
10
Soil depth (cm)


A
Soil extractable N
(mg N kg-1 dry soil)
1200
900
600
300
0
InorganicN
Control
OrganicN
Control
B
Total soil N
(mg N kg-1 dry soil)
4200
3500
2800
2100
1400
Treatment
Control
0
50
100
150
200
250
300
350
Days since treatment


1200
900
600
300
0
DOC (mg C kg-1 dry soil)
0
50
100
150
200
250
300
350
Days since treatment
Treatment
Control
*
*
*
*
*
*


Soil microbial N (mg N kg-1 dry soil)
Days since treatment
Treatment
Control
*
*
*
0
40
80
120
160
0
50
100
150
200
250
300
350


Soil respired CO2 (µmol m-2 s-1)
12
10
8
6
4
2
0
50
100
150
200
250
300
350
Days since treatment
*
*
Treatment
Control


Treatment
Control
Soil δ15N (‰)
Days since treatment
0
2
4
6
8
10
12
0
50
100
150
200
250
300
350
*
*
*
*
*

Chapter 4

Marine derived nutrients and understory vegetation in oligotrophic inland riparian forests of the Pacific Northwest, USA

Tadd A. Wheeler, Kathleen L. Kavanagh

Abstract

The flow of nutrients within and between ecosystems as well as the productivity of these ecosystems is often attributed to complex biogeochemical interactions between multiple trophic levels. There is mounting evidence that the annual return of salmonids to their natal streams and the productivity of forested riparian ecosystems is one of these complex relationships. The transfer of salmon-born nutrients into riparian ecosystems and the utilization of these nutrients have been reported. However, nearly all of these investigations have taken place in maritime coastal ecosystems where returning salmonids remain abundant and the forests highly productive. By contrast, a vast majority of historic Pacific Northwest, USA spawning habitat was within semi-arid, oligotrophic inland ecosystems. The objective of this investigation was to quantify the annual and inter-annual responses of riparian vegetation to the deposition of anadromous fish carcasses in oligotrophic inland riparian forests. Perennial vegetation growing near carcass treated streams but not receiving direct deposition of carcass tissue revealed the transfer of aquatic nutrients into the riparian forest within 30 days of the stream treatment. The observations of understory perennial vegetation following direct carcass deposition revealed that herbaceous species had assimilated this nutrient subsidy by one year post treatment but that the resource contribution was depleted within two to three years. Conifer seedlings appear to have a much higher nutrient acquisition and storage capacity as foliage produced three years after carcass nutrient additions contained large quantities of marine derived nitrogen. It is evident that additional consideration of the annual and inter-annual flow of salmon derived nutrients within these inland ecosystems in needed before mitigation for the decline of this subsidy can be appropriately prescribed.

Introduction

Understanding how the flow of nutrients within and between ecosystems influences productivity is vitally important for the comprehensive management of ecosystems. Understanding these nutrient fluxes can be a difficult task when the flow of nutrients is influenced by the productivity and migratory patterns of fish and wildlife (Bump et al. 2009, Danell et al. 2002, Hocking and Reynolds 2011) or dependent on interactions between species (Bump et al. 2009, Gende et al. 2004, Helfield and Naiman 2006, Hilderbrand et al. 1999, Holtgrieve et al. 2009). The return of anadromous fish to their natal streams is an example of this complex interaction.

Pacific salmonids returning to their natal streams are preyed upon in great numbers by bears (Quinn et al. 2009, Quinn et al. 2003), wolves (Adams et al. 2010), and other scavengers (Cederholm et al. 1989) who deposit partially consumed carcasses and metabolic wastes (Hilderbrand et al. 1999) throughout the riparian ecosystem (Holtgrieve et al. 2009), creating spatially heterogeneous gradients of soil nutrient availability (Bartz and Naiman 2005, Drake et al. 2005, Gende et al. 2007, Gende et al. 2004, Hilderbrand et al. 1999). Salmon derived nutrients also move into the riparian forest through hyporheic transfer of dissolved nutrients (O'Keefe and Edwards 2003). The use of these nutrients, particularly nitrogen (N), by riparian vegetation has been demonstrated using natural gradients in ^{15}N abundance between marine and natal ecosystems (Ben-David et al. 1998, Bilby et al. 2003, Hilderbrand et al. 1999, Hocking and Reynolds 2011, Koyama et al. 2005). The presence of spawning salmon has also been attributed to increases in vegetative growth (Helfield and Naiman 2001) as well as abundance driven gradients in biodiversity and productivity across a landscape (Hocking and Reynolds 2011). However, with the exception of Koyama et al. (2005), all of these investigations have focused on coastal maritime ecosystems where salmon returns remain relatively robust (NRC 1996) and the forests characterized by their high productivity (Grier 1979).

By contrast, more than 80% of historic natal salmon habitat in the Pacific Northwestern USA is contained within the Columbia River Basin (NRC 1996). This inland region is dryer and warmer than coastal ecosystems as well as substantially more N limited (Koyama et al. 2010, Koyama et al. 2012, Moore and Mika 1991, Stephan et al. 2012, Wheeler and Kavanagh in review). This N limitation can be partially attributed to low rates of

atmospheric deposition (< 1 kg N ha^{-1} yr^{-1}, NADP 2014) and N-fixation (< 2kg N ha^{-1} yr^{-1}, Jurgensen et al. 1990) as well as the weathering of granitic parent geology in some sub-basins that contributes little to the N needs of the ecosystem (Henderson et al. 1978). The lack of N availability in forested ecosystems has been associated with N retentive responses in woody vegetation including the reclaiming of N prior to senescence and the translocation of N from older to newer needles (Aerts 1996, Edmonds et al. 1989, Millard and Grelet 2010, Salifu and Timmer 2001, Tully et al. 2013). The decades long retention and internal recycling of N within conifers of these inland riparian forests has been reported (Koyama et al. 2005). However, these adaptations have the resultant nutrient cycling consequence of increasing the litter C:N ratio (Heath et al. 1988, Valachovic et al. 2004) which can lead to the suppression of soil microbial mineralization rates and the low extractable N availability that is common in many forests of the inland Pacific Northwest (Hart et al. 1997, Henderson et al. 1978, Koyama et al. 2012, Koyama et al. 2010, Stark and Hart 1997). This suggests that the spatial distribution and frequency of salmon nutrient deposition may be even more important to the productivity and diversity of inland riparian forests both on an annual and inter-annual time scale.

The soil biogeochemical responses to the deposition of anadromous fish carcasses in semi-arid inland riparian forests differ considerably from those of the coastal Pacific Northwest (Wheeler et al. 2014, Wheeler and Kavanagh in review). Anadromous fish carcasses decompose in as little as two weeks in these inland ecosystems (Wheeler et al. 2014, Wheeler and Kavanagh in review) whereas decomposition in a coastal forest has been reported to take up to 10 weeks (Drake et al. 2005). This rapid decomposition rate in inland ecosystems can lead to substantially higher concentrations of soil N availability (Wheeler and Kavanagh in review) relative to coastal ecosystems (Drake et al. 2005, Gende et al. 2007, Holtgrieve et al. 2009). The N retentiveness of these soils also appears to lead to the persistence of elevated soil N for at least one year following the deposition of fish carcasses (Wheeler and Kavanagh in review). By contrast, one coastal investigation reported soil N returning to background concentrations in as little as three months (Gende et al. 2007). A second study reported elevated but declining concentrations of soil N at six months post carcass deposition but did not report values beyond six months (Drake et al. 2005). A third study reported soil N concentrations returning to reference conditions by one year following

carcass deposition (Holtgrieve et al. 2009). However, the Holtgrieve et al. (2009) investigation only reported soil N concentrations at the time of carcass deposition and at one year following deposition. The persistence of elevated soil N levels in inland ecosystems through the growing season following carcass deposition is a drastic contrast to the N limited characteristics of this inland region (Koyama et al. 2012, Koyama et al. 2010, Wheeler and Kavanagh in review) and could lead to dramatic responses in vegetative productivity and biodiversity within these ecosystems (Hocking and Reynolds 2011).

Following the deposition of anadromous fish carcasses we would expect both short term (weeks to months) and longer term (years) vegetative responses. The first is an increase in foliar N concentrations. This increased foliar N could in turn be expected to elicit short term increases in leaf level photosynthetic capacity (Gough et al. 2004, Mitchell and Hinckley 1993). Ultimately, this extra N should lead to long term increases in above ground biomass production (Balster and Marshall 2000, Gough et al. 2004, Moore and Mika 1991) and a decline in foliar N concentrations and leaf level photosynthetic capacity towards pre-treatment levels (Gough et al. 2004) in exchange for increased whole plant photosynthetic capacity via increased photosynthetic biomass. One method of assessing this short term increase in photosynthesis is through the use of the ratio of carbon isotopes $^{13}C/^{12}C$ or $\delta^{13}C$ in the foliage (Farquhar et al. 1982). Photosynthesis requires the movement of CO_2 from the atmosphere through several restrictive layers of the leaf, the stomata and the mesophyll, which results in discrimination against the heavier ^{13}C and thus a foliar $\delta^{13}C$ that is lower than the atmosphere (Farquhar et al. 1982). However, if the rate of photosynthesis were to increase without increasing gas exchange capacity (i.e. more stomata) the foliar $\delta^{13}C$ would be expected to increase (Farquhar et al. 1982). This is due to an increased demand for CO_2 that begins to override ^{13}C discrimination. Though this method can be confounded by the influences of water use efficiency on the C isotope ratio (Farquhar et al. 1982, Mitchel and Hinckley 1993), it assesses potential increases in leaf level photosynthesis within the natural constraints of the environment rather than maximum photosynthetic potential (A_{max}) of the leaf. This may be more biologically representative of actual vegetative responses to salmon carcass deposition.

The deposition of millions of salmon carcasses along the 21,325 km of accessible streams and rivers of the Columbia River Basin (NRC 1996) was historically an annual event. The associated influx of N into riparian ecosystems through aquatic nutrient transfer (O'Keefe

and Edwards 2003) and salmon carcass deposition (Hilderbrand et al. 1999, Holtgrieve et al. 2009, Quinn et al. 2009, Quinn et al. 2003) may have constituted a vital N source for vegetation of these oligotrophic riparian ecosystems. However, the large amounts of biomass and thus large reservoirs of internally cycled N in forests make it difficult to quantify the short term vegetative responses to renewed salmon carcass deposition. Thus, the objective of this investigation was to measure the influences of salmon carcass deposition on understory herbaceous and tree seedling N utilization and productivity in semi-arid inland riparian forests. Specifically: 1) what changes in foliar C and N chemistry are elicited in herbaceous vegetation growing near the stream edges following the deposition of anadromous fish carcasses into the streams? 2) What changes in foliar C and N biochemistry are elicited in herbaceous species growing within the riparian forests following the terrestrial deposition of anadromous fish carcasses? 3) What changes in foliar C and N biochemistry and above ground biomass production are elicited in conifer seedlings following the terrestrial deposition of anadromous fish carcasses?

Methods

Study location

This investigation is nested within collaborative research efforts in central Idaho, USA to quantify ecosystem wide biogeochemical responses to salmon nutrient deposition within the oligotrophic North Fork Boise River watershed (Collins and Baxter in press, Marcarelli et al. 2014, Wheeler et al. 2014, Wheeler and Kavanagh in review). The natal contributions of salmon derived nutrients to this watershed were abruptly severed in the early 1900's by the installation of dams, leaving the weathering of N poor geologic material (the granitic Idaho Batholith) as well as low levels of atmospheric deposition and N fixation (<3 kg N $ha^{-1}yr^{-1}$ Jurgensen et al. 1990, Henderson et al. 1978, NADP 2014) to sustain the productivity of these riparian ecosystems.

The climate within the North Fork Boise River watershed is semi-arid with warm, dry summers and cold, moist winters. Snotel weather stations within the North Fork Boise watershed report mean annual temperatures over the last two decades of 5-8˚ C with mean monthly temperatures of -4˚ C in January and 15.5˚ C in July. The mean annual precipitation is 77-117 cm, predominantly as snow (NRCS 2012). The riparian vegetative communities are open mixed conifer forests comprised of willows (*Salix.* spp), dogwood (*Cornus* spp.), and

tall grasses (*Festuca* spp.) (Marcarelli et al. in press) residing under predominantly Douglas-fir (*Pseudotsuga* menziesii) and lodgepole pine (*Pinus contorta*) canopies.

Experimental design

Within the Boise River watershed, six 1^{st} – 3^{rd} order streams were randomly assigned as salmon carcass treatment streams or reference streams (Table1). In early August, for three consecutive years (2008-2010), anadromous fish carcasses (*Oncorhynchus* spp.) of hatchery origin were placed in the treatment streams at a historically estimated rate of 0.5 carcass m^2 (IDFG 1985). These carcasses were treated with freezing and pasteurization (heated until the cranial cavity exceeded 60˚ C for 20 minutes) due to concerns of spreading pathogens between watersheds (Compton et al. 2006, Noga 2000) but these treatments were not expected to dramatically alter the ecologic outcomes of the investigation (Wheeler et al. 2014).

It is important to note that within this investigation the foliar C and N of common horsetail (*Equisetum arvense*) is investigated within two different geographic settings, each of which has a different nutrient import vector. One location is near the stream edge where horsetails would access diffuse nutrients from the in-stream deposition of anadromous fish carcasses whereas the other location is 10-30 meters away from the stream and subjected to the direct terrestrial deposition of fish carcasses. In an effort to separate these two components of the investigation they will be referred to as "streamside" horsetail and "forest" horsetail.

To investigate the annual and inter-annual influences of aquatic nutrient transfer from in-stream carcass deposition on the foliar C and N chemistry of herbaceous species growing near the stream edge, the foliage of non-fertile common horsetail was collected prior to treatment, at 30 days post treatment, and at one year post treatment for each of the three treatment years. This "streamside" horsetail foliage was collected from 6-8 locations along each stream and was equally collected from the bank full water line as well as directly adjacent to the active water line. This horsetail foliage was dried at 70 ˚C for 72 hours then ground in a ball mill and combusted for $\delta^{15}N$, $\delta^{13}C$, %N, and %C 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 ± 0.2‰ for $\delta^{15}N$ and ± 0.15‰ for $\delta^{13}C$.

At the same time as the stream treatments, anadromous fish carcasses were also added to 1 m^2 plots within the riparian forests. These plots were located a mean distance of 15 m (range 2 – 30 m) from the stream edge. These plots were comprised of four randomly assigned treatment and four control plots. Four control plots were also placed along the reference streams as a validation of control plots along treatment streams. In 2008 and 2009, the riparian forest plots were equally associated with common horsetail and conifer seedlings (*Pseudotsuga menziesii* and *Pinus contorta*). In 2010, the treated and control plots were only associated with conifer seedlings and no "forest" horsetail plots were established. This change was based on a need to devote resources to conifer seedlings due to mortality and grazing of the conifer seedlings reducing sample size in the first two years of the experiment. One pair of control and treatment streams was excluded from riparian vegetation plot sampling due to the dominance of dense willow and thus a total lack of target species. Plots were treated with salmon carcasses in 2008 at a rate of 75 g N while the 2009 and 2010 plots were treated at a rate of 249 g N (~5 kg of carcasses) and 328 g N (~6.4 kg of carcasses), respectively. Deionized water (DI) equivalent to the moisture content of the carcass amendments was added to the control plots to compensate for any biogeochemical responses of the moisture in the carcasses. New plots were established for each treatment year. The 2008 treatment rate was substantially lower than subsequent treatments but the observed increase and timing of soil inorganic N was similar to responses observed during subsequent treatment years (Wheeler and Kavanagh in review). This similarity between the 2008 soil N responses and the responses in subsequent treatment years was likely the result of our manipulation of the carcass tissue. In 2008 we pureed the carcasses to prevent scavengers from removing them from the plots. It is believed that the destruction of tissue integrity resulted in faster nutrient release from the carcasses to the soil (Drake et al. 2005, Miccozi 1986). A faster rate of nutrient release to the soil could result in less nutrient removal from the plot by insects and other scavengers (Collins and Baxter in press, Hocking and Reimchen 2006, Meehan et al. 2005) and thus the similarity of the 2008 soil N concentrations to our later treatments. Subsequent treatment years utilized whole carcasses held in place with wooden stakes. Only one carcass was removed over these two years.

Within the riparian forest plots, horsetail foliage and current year conifer seedling foliage were collected prior to treatment and again at one year post treatment. Riparian forest

horsetail was not collected in the weeks following the treatments as the application of DI water to the control plots resulted in the horsetail turning white and senescing for the year. The horsetail appeared to grow back normally the following year. The carcass treated plots did not senesce early. In addition to the pre and one year post treatment foliage collection following the 2008, 2009, and 2010 treatments, an additional collection of riparian forest horsetail and conifer needles for all 2008 and 2009 plots was conducted in 2011. The conifer needle collection from the 2008 and 2009 trees included a sampling of each needle age class from one year post treatment to the current 2011 age class. Soil samples were also collected from the top 10 cm of the 2009 and 2010 riparian plots. The methodology and results of the 2010 soils are detailed in Wheeler and Kavanagh (in review). These foliar and soil samples were dried at 70 ˚C for 72 hours then ground in a ball mill or Wig-L-Bug amalgamator and combusted for $\delta^{15}N$, $\delta^{13}C$, %N, and %C. All isotopic 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 ± 0.2‰ for $\delta^{15}N$ and ± 0.15‰ for $\delta^{13}C$.

To determine above ground biomass of the conifer seedlings, the basal diameter of each tree was measured prior to treatment and again at one year post treatment. Seedlings from 2008 and 2009 were also measured in subsequent years. It was desirable to preserve our trees for possible future observation so allometric relationships of above and below ground biomass based on basal area were determined from the destruction of non-target seedlings from throughout the watershed. The basal diameter of these trees were measured and the above biomass relationships determined by drying the seedling components at 70˚ C for 72 hours. Additionally, conifer seedling foliage was collected from the 2010 treatment and control seedlings in August of 2011 to test for differences in specific leaf area (SLA), the projected area of leaf per unit mass. Needle samples were typically between 15-45 needles but ranged from 5-60 needles depending on seedling size and needle availability. The needles were weighed within one week of collection and the projected leaf area determined using ImageJ software version 1.47 (National Institute of Health, USA).

Climate data

Regional precipitation and temperature data was retrieved from Natural Resource Conservation Service (NRCS) SNOTEL climate stations at Graham Guard Station to the north of our 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 air temperature sensors (HOBO Pro, Onset Computer Corporation, USA). These sensors were placed inside vented PVC shelters to protect them from direct solar radiation and suspended 2.5 meters above the soil surface in an open area 5-10 meters from the stream edge. Soil moisture data was collected at depths of 1, 3, 8, and 10-30 cm using Decagon Em5b data loggers with EC10 and EC20 probes (Decagon Devices, Pullman, Washington, USA).

Statistics and foliar nitrogen calculations

Where the responses of riparian vegetation were consistent in timing and amplitude between years and following statistical verification of no difference ($P>0.05$) between years the results were pooled for inferential strength and simplicity. To avoid inflating the risk of type 1 errors, treatment effects were assessed within years prior to summarizing across years. This was applicable to all data except the streamside horsetail and soil $\delta^{15}N$. A mixed effects repeated measures analysis of variance (ANOVA) was utilized to test for an overall effect of salmon carcass nutrients on streamside horsetail and riparian plot soils. The fixed variables were treatment and time while the random variable was the individual plots through time. The possibility of a time x treatment interaction was also assessed. All remaining comparisons between treatments and controls as well as tests for differences between years and between pre and post treatment were conducted using two-way ANOVA's. 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). The inferences derived from these analyses are assumed to be representative of community level responses and are therefore reported as means and standard errors of the mean (SE) unless otherwise noted.

We also estimated the marine derived N content of conifer seedling foliage using an isotopic mixing model and the allometric relationships between basal area and current year

foliage biomass. The isotopic mixing model was used to estimate the proportion of foliar N comprised of marine derived N (MDN) where: % MDN = (δ^{15}N treatment foliage - δ^{15}N control foliage) ÷ (δ^{15}N carcass fluids - δ^{15}N control foliage) x 100. The treatment and control foliage δ^{15}N derive from the mean of the target year foliage from the treated and control seedlings and the carcass fluid δ^{15}N is the mean δ^{15}N value of the carcass fluids observed exiting the carcasses to the soil in 2010 (Kavanagh and Wheeler in review). This value was then multiplied with the foliar %N and again with the estimated foliar biomass to determine approximate MDN content. For simplicity all estimates were conducted using mean values. This estimate was made for the one year post treatment foliage of the 2010 treatment seedlings as well as for each of the post treatment age classes of the 2008 and 2009 seedlings. The sample size of the 2008 and 2009 conifer seedlings had decreased (n=2-3) by 2011 due to herbivory and mortality so the foliar data from these years were pooled by years since treatment. We also included three seedlings treated with whole carcasses in 2008. These are not included elsewhere as their treatment rate was higher than the other 2008 treatment plots. However, the foliar N and growth responses of these seedlings were not different from the other seedlings.

Results

The climate over the three year duration of the investigation was comparable to the long term climate average. The average precipitation over the three treatment years (August 2008-August 2011) was 84 cm (79-94 cm) at Graham Guard Station and 123 cm (110-146 cm) at Mores Creek Summit (NRCS 2012). The majority of this precipitation fell during the cold winter months of November through April with only 4 – 8 cm of precipitation on average received per month from July through September (Figure 1). The availability of soil moisture within the riparian study areas closely tracked precipitation (Figure 1). The soil water content (SWC) of the soils 1-30 cm deep declined rapidly in early June of each year and remained low until early October. The reported SWC at Beaver Creek (Figure 1) is indicative of the seasonal SWC trends expected in this study region. The Banner Creek SWC (Figure 1) was influenced by the progressive construction of beaver dams over the duration of the investigation. The SWC of the riparian soils associated with the treatment streams are not reported as the accuracy of the soil moisture sensors was altered by the placement of salmon carcasses in close proximity. Salmon carcasses were placed over the soil moisture sensors to

measure the influence of the carcass fluids on soil moisture. However, following the deposition of the carcasses the sensors reported SWC values that exceeded maximum SWC by three fold. The soil moisture sensors continued to report these values for up to nine months following the treatment irrespective of seasonal declines in soil moisture content. It is suspected that the functionality of the soil moisture sensors was influenced by potential alterations to the electrical conductivity of the soils by the carcass fluids (Decagon Devices Inc, personal communication, April 25, 2014). The average annual air temperature within the riparian areas was a 2.1 – 4.5˚ C with an average monthly temperature for January of -5.0˚ C and for July of 14.1 – 15.9˚ C. Though this average temperature of July appears rather mild, the diurnal temperatures in these sites regularly approached 39˚ C during the day then decreased to near 0˚ C at night (Figure 2).

The placement of fish carcasses within the streams elicited a rapid (30 days) and sustained response in streamside horsetail isotope composition indicating a terrestrial transfer and rapid utilization of N from the salmon carcasses placed in the stream (Figure 3). There was a significant overall influence of treatment on streamside horsetail folar $\delta^{15}N$ (DF=24, T=-2.80, P=0.01) using the repeated measures analysis. This was driven by an increase in foliar $\delta^{15}N$ of roughly 2.5 ‰ within 30 days of the 2008 carcass addition with streamside horsetail foliar $\delta^{15}N$ remaining elevated ($F_{1, 20\text{-}32} > 7.97$, P<0.01) at each sampling interval for the duration of the three year investigation (Figure 3). There were no control stream samples collected 30 days after the 2008 carcass addition. Therefore, the 2008 post treatment values at 30 days were tested against the pretreatment values. The overall trend in foliar $\delta^{15}N$, with the exception of streamside horsetail observations in August 2011, revealed a slight decline in $\delta^{15}N$ at one year post treatment but the addition of anadromous fish carcasses to these streams in August of 2009 and 2010 maintained an overall elevated level of foliar $\delta^{15}N$ in the streamside horsetail. There were no differences in foliar %N, $\delta^{13}C$, or %C observed between the control and treatment streamside horsetail with an average %N of 2.1 (0.1 SE), a $\delta^{13}C$ of -29.2 (0.2 SE), a %C of 35.6 (0.3 SE), and a C:N of 17.6 (0.6 SE).

Changing focus now from the horsetail growing along the stream edge to the horsetail within the riparian forest, the deposition of anadromous fish carcasses in the riparian forest plots resulted in a 4.6‰ (1 SE) increase in foliar $\delta^{15}N$ of riparian forest horsetail to 9.0 ‰ (0.9SE) at one year post treatment (Figure 4A). Surprisingly, this significant ($F_{1,13}$=31.04,

P<0.001) increase in $\delta^{15}N$ was not associated with an increase in %N, $\delta^{13}C$, or %C with an average %N of 1.8 (0.1 SE), a $\delta^{13}C$ of -28.5 (0.4 SE), a %C of 36.3 (0.5 SE), and a C:N of 22.0 (1.3 SE) in the treated and control foliar samples. The August 2011 collection of riparian forest horsetail from the 2008 and 2009 plots suggests that very little of the enriched N remained in the plots at two years post treatment and that none was available by three years post treatment (Figure 4B). The two year data was derived from 2009 plots and the three year from 2008 plots. A significant differences between treated and control riparian horsetail $\delta^{15}N$ was not observed for the two and three year post treatment foliage. However, the samples size for the two year post treatment was considerably smaller (n=2 for treatments, n=4 for controls), adding uncertainty to this conclusion.

The conifer seedlings rapidly assimilated the N released from the carcasses as is evident by an increase in both foliar $\delta^{15}N$ ($F_{1, 28}$=45.2 *P*<0.001) and %N ($F_{1,28}$=10.0 *P*<0.01) at one year post treatment with increases of 4.8‰ (0.6 SE) and 0.6% (0.2 SE), respectively (Figure 5A). The two and three years of post-treatment foliar age classes collected from the 2008 and 2009 treatment seedlings found that foliar $\delta^{15}N$ remained elevated ($F_{1, 7\text{-}11}$>13.1, *P*<0.01) for several years but that foliar %N did not remain elevated (Figure 5B). The $\delta^{13}C$ and %C of the foliage at one year post treatment remained unchanged at -28.1‰ (0.2SE) and 50.3% (0.4SE), respectively but the C:N had significantly decreased ($F_{1,28}$=19.2 *P*<0.001) from 35.3 (1 SE) to 26.2 (1.8SE) at one year post treatment (Figure 6). There was a significant decline ($F_{1, 29}$=5.3 *P*=0.03) in the C:N of the control plots as well but the amplitude of decrease in the treated conifers was twofold.

There was an overall influence of the salmon carcasses on the soil $\delta^{15}N$ (DF=23, T=-8.6, *P*<0.01). The $\delta^{15}N$ of the 2009 plot soils increased significantly ($F_{1,6}$=39.6, *P*<0.01) within 14 days of the carcass treatment from 3.0‰ (0.2SE) pre-treatment to 6.1‰ (0.8SE) and continued to increase to a maximum of 13.7‰ (3.2SE) at 97 days post treatment (Figure 7). Treatment soil δ15N remained significantly elevated ($F_{1,6}$>22.2, *P*<0.01) relative to control soils through one year post treatment. No differences in soil $\delta^{15}N$ were found between the horsetail plot and conifer seedling plot soils. The soil $\delta^{15}N$ responses to the 2009 carcasses treatments were also similar to the responses of the 2010 soil $\delta^{15}N$ reported in Wheeler and Kavanagh (in review).

The application of allometric equations for above ground biomass of Douglas-fir and lodgepole pine seedlings based on basal area revealed no differences in relative above ground biomass production at one year post treatment. The relative growth rate ranged from 0 – 80 g of above ground biomass per cm^2 of basal area growth independent of treatment. The sample size in 2008 and 2009 were relatively small and one treatment seedling was lost to grazing but monitoring of these seedlings during subsequent growing seasons did not reveal any difference in relative above ground biomass production between the treated and control seedlings. The comparison of specific leaf area (SLA) of 2011 conifer needles at one year post treatment also did not reveal any differences between treated and control seedlings. The average SLA was 69.2 (4.9 SE) $cm^2\ g^{-1}$. The development of allometric relationships from 21 lodgepole pine seedlings ranging in basal diameter of 2.2-26 mm and Douglas-fir seedlings ranging in basal diameter from 3.7-25.1 mm were highly correlative ($R^2 > 0.92$) when both variables were natural log transformed. Reports of these relationships in natural ecosystems are largely lacking for this size range, particularly for a semi-arid N limited ecosystem, and thus are reported here (Table 2). The estimates of foliar marine derived N content in conifer seedlings revealed an estimated foliar MDN content of 46.4 mg N per seedling in the current year foliage (2011 foliage) one year following the 2010 fish carcass amendments (Table 3). The estimates across the post treatment age classes of the 2008 and 2009 seedlings suggest an MDN content of 35.5 mg, 49.5 mg, and 60.9 mg N per seedling for the 1, 2, and 3 year post treatment age classes, respectively (Table 3). This would yield an approximate foliar accumulation of 145.9 mg N per seedling in the three years following a carcass deposition event.

Discussion

The dramatic responses of foliar N to the deposition of anadromous fish carcasses demonstrate the potential influence of marine derived nutrient contributions in oligotrophic inland ecosystems. The response of vegetation to the deposition of carcasses further suggests that there are both transient and cumulative responses to this nutrient subsidy. The decline in foliar $\delta^{15}N$ of the riparian forest horsetail within two years of treatment further emphasized that herbaceous species are more reliant on soil N turnover for maintaining productivity than are conifer species with nutrient storage and internal recycling strategies (Aerts 1996, Edmonds et al. 1989, Millard and Grelet 2010, Salifu and Timmer 2001, Tully et al. 2013).

This is consistent with the annual deposition of carcass nutrients being of high importance for maintaining understory productivity. We did not quantify changes in horsetail biomass but the lack of observed increase in %N in conjunction with the increase in $\delta^{15}N$ would indicate a potential increase in biomass production that diluted the assimilated N (Gough et al. 2004). We did not observe changes in leaf level photosynthesis or above ground biomass in conifer seedlings despite increases in foliar N concentrations but this may be due to other physiologic limitations such as soil moisture and light in this semi-arid shaded understory environment (Coomes and Grubb 2000).

The amplitude of foliar $\delta^{15}N$ response in the streamside horsetail, which only received N from the carcasses deposited in the stream, demonstrates the ability of vegetation within the stream margins to capitalize on the nutrients from anadromous fish carcasses. This response was particularly impressive given that roughly half of the horsetail was collected from the upper edges of the active stream channel (~0.3-0.8 m above stream level) and up to one meter horizontally from the water's edge. Horsetail is known for its extensive root system and its ability to bioaccumulate nutrients in above ground biomass (Marsh et al. 2000) but the amplitude of this response could also suggest a substantial hyporheic transfer of nutrients into the adjacent riparian forest (O'Keefe and Edwards 2003). The movement of some carcasses from the stream to the stream margin by wildlife was observed (Collins and Baxter in press). However, there were no visible indications of scavenger carcass deposition near the streamside horsetail collection sites.

The elevated foliar $\delta^{15}N$ of the streamside horsetail at one year post treatment also demonstrates that reservoirs of streamside marine derived N can persist into the following growing season. This enrichment may derive from several pathways. Senescent horsetail tissue decomposes rapidly (Marsh et al. 2000) and likely results in some recycling of marine derived N but the extent of foliar $\delta^{15}N$ in the following year's biomass would also suggest a much larger reservoir of N was accessed. The most likely source would be the accumulation of marine derived N in the extensive root system of the horsetail during the previous year (Bastiene et al. 2006). Despite this reservoir of marine derived N, it is apparent from the declining trend in foliar $\delta^{15}N$ of the streamside horsetail at one year post treatment that this resource is finite within the herbaceous community. Herbaceous species are major nutrient contributors to the forest litter pool with bioaccumulating species like horsetail contributing

nutrients at higher rates than other species (Marsh et al. 2000). The decomposition of senescent herbaceous tissue and the subsequent competition for recycled nutrients between soil microbes and dominant forest vegetation can quickly deplete soil nutrient availability (Hodge et al. 2000, Kaye and Hart 1997). This would make the annual influx of anadromous fish important for maintaining the established productivity of herbaceous species.

The increases in foliar $\delta^{15}N$ of the streamside horsetail following the 2009 and 2010 carcass deposition events were much smaller than the response in 2008 (Figure 3). This would suggest that while N remained a limiting resource in the growing season following carcass deposition that either a secondary resource became limiting (Anderson and Lundergardh 1999) or that the foliar $\delta^{15}N$ of the streamside horsetail had reached isotopic equilibrium with the marine derived nutrient pulse. Though the possibility of co-limiting resources cannot be ruled out, the nutrient bioaccumulating characteristic of horsetail make it unlikely (Marsh et al. 2000). Rather, there is evidence for the later possibility of isotopic equilibrium. The $\delta^{15}N$ of the anadromous carcasses placed in the streams was reported by Marcarelli et al. (2014) as 12.0 ‰ (0.2SE). However, the mobility and mixing of this carcass N with native N within the stream and streambed would be expected to dilute the $\delta^{15}N$ of this resource pulse. This is supported by the $\delta^{15}N$ of stream biofilm which only increased from 3.3 to between 6.0 and 7.9 ‰ at one month post treatment despite a nearly 8 fold increase in biofilm standing crop (Marcarelli et al. 2014). Additionally, at least 25% of the daily dissolved N within the streams during this time could be attributed to local and less enriched N sources (Marcarelli et al. 2014).

The dramatic responses of riparian "forest" horsetail to the direct deposition of anadromous fish carcasses on the forest soil further supports the assertion of isotopic equilibrium between the horsetail and the exchangeable N pool. The deposition of anadromous fish carcasses within the riparian forest plots resulted in an increase in the foliar $\delta^{15}N$ of the riparian forest horsetail to 9.0 ‰ (0.9SE) at one year post treatment. This foliar isotopic enrichment was reflective of soil $\delta^{15}N$ within the riparian forest plots that averaged 9.2 ‰ (0.9SE) across the 2009 post treatment observations (Figure 5). Though the soil $\delta^{15}N$ following the 2009 treatment reached as high as 13.7 ‰ (3.2SE) the average of post treatment observations may be more representative of the cumulative utilization of this resource by the riparian forest horsetail. This similarity between the foliar $\delta^{15}N$ of the riparian horsetail and

the mean soil $\delta^{15}N$ supports the assertion of isotopic equilibrium between the horsetail and the resource. The subsequent decline of foliar $\delta^{15}N$ within the riparian forest horsetail to near reference values by two years' post treatment also supports the previous assertion that the herbaceous community in these forests is a short term MDN reservoir. However, this also raises the question of how much MDN is annually sequestered by riparian vegetation and later recycled through detrital pools. This sequestering of N by vegetation will be explored a bit further using the conifer seedlings.

Conifer productivity in the Pacific Northwest is often N limited (Chapin et al. 1986, Henderson et al. 1978, Moore and Mika 1991) yet despite large increases in foliar $\delta^{15}N$ and an increase in total foliar N of the conifer seedlings at one year post treatment, an increase in productivity was not observed. This lack of response in conifer productivity is most likely due to the harsh environmental constraints of soil moisture and light availability (Balster and Marshall 2000) in this semi-arid understory environment. There can be other biogeochemical inhibiters of increased productivity in fertilized conifer seedlings such as: high substrate ammonium: nitrate concentrations (Bown et al. 2010, Everett et al. 2010) and a lack of N conversion into the enzyme Rubisco used in photosynthesis (Bown et al. 2010, Manter et al. 2005). However, the high expected rates of forest nitrate consumption in the Pacific Northwest (Stark and Hart 1997, Perakis and Sinkhorn 2011) in conjunction with the observed increase in foliar N at one year post treatment followed by the high foliar content of MDN in subsequent foliar years (Table 3) suggests that the environmental constraints were the limiting influence on quantifiable productivity increases in these conifer seedlings.

The granitic soils of these forests (Table 1) tended to dry down quickly following snow melt and with smaller less frequent precipitation events during the months of June through September plant available water likely became quite limiting for these shallow rooted conifer seedlings (Figure 1). The conifer seedlings collected for the development of allometric relationships were generally rooted at less than 30 cm deep (data not shown). The other possible environmental influence on productivity was the availability of direct solar radiation within the understory (Balster and Marshall 2000). The natural distribution of conifer seedlings in these riparian forests resulted in the use of seedlings across a wide range of light environments. The understory light levels were not directly quantified but the overstory leaf area index (LAI) of these forests ranged within and between the riparian study areas from near

zero to 4 m^2 of leaf area per m^2 of ground area (A Noble-Stuen unpublished data) which has been shown to influence light penetration within and through the forest canopy (Balster and Marshall 2000). Within the heterogeneity of this light environment, several of the observationally more heavily shaded carcass treatment seedlings exhibited very low foliar $\delta^{15}N$ responses (<1‰) and relative growth rates of near zero, while some untreated control seedlings growing in relatively open canopy gaps expressed growth rates as high as 80 g biomass cm^{-2} of basal area growth. It is quite possible that this heterogeneity in understory light conditions as well as the seasonal soil moisture limitations in this region prevented the detection of treatment effects on conifer seedling productivity. However, it is important to recognize that these limitations on productivity are not uncommon in understory conifer seedlings (Palik et al. 2003) and may not be reflective of overstory tree productivity potential from annual applications of MDN. For example, the overstory conifer trees within the riparian study area did not exhibit reductions in pre-dawn water potentials until the last few weeks of September (A Noble-Stuen unpublished data). Suggesting that these trees were accessing deeper water resources and thus would have higher productivity potentials.

Despite our inability to detect changes in vegetative productivity, it is judicious to explore further the time scale and potential amount of N sequestered within riparian vegetation following the deposition of anadromous fish carcasses. Using the linear mixing model and the allometric relationship for current year foliage, we estimated the amount of marine derived N sequestered in the 2011 needles of the 2010 treatment seedlings to be roughly 40.2 mg MDN per seedling or 33% of foliar N (Table 3). Since it was desirable to preserve the treated seedlings for potential future observations, we did not measure the $\delta^{15}N$ of roots or stems and are thus unable to directly quantify the MDN in these structures. However, we can make some estimates based on the N content relationships between roots, stem, and foliage reported by Everett et al. (2010). This investigation of hydroponically grown Douglas-fir seedlings reported root and stem N contents that were roughly 80% and 20% of total foliar N content, respectively. Applying an average post treatment basal area of 91.1 mm^2 to the allometric relationship for total foliage and assuming that the foliar N content of the foliage is 2%, yields a total foliar N content of 302 mg N. Further, assuming that the MDN content of the root and stem is 33% of the N pool based on the mixing model estimate and applying the respective 80% and 20% relationships yields an approximate root and stem

MDN content of 79.7 and 12.0 mg, respectively. This would suggest a total MDN uptake of approximately 131.9 mg MDN per average seedling.

There is further evidence that MDN sequestration by conifer seedlings was even higher. The 2011 analysis of the needles from one, two, and three year post treatment age classes of the 2008 and 2009 conifers seedlings revealed all of these foliage age classes to be enriched in $\delta^{15}N$ by 5-6 ‰ on average, relative to control seedlings. Applying the basal area measured in each of these years to the allometric relationship for current year foliage suggests that the one, two, and three year post treatment foliage contained a respective 30.2, 43.4, and 54.4 mg MDN per seedling. This puts the average MDN sequestered in foliar biomass at 128.0 mg MDN per seedling. The level of foliar $\delta^{15}N$ enrichment in the third year foliage would further suggest that additional MDN reserves remained in the conifer roots. Given the amount of time (2-3 years) since the deposition of fish carcasses near these conifer seedlings, the uncertainty of root $\delta^{15}N$ and N content prevents an estimate of root MDN content using the previous root N to foliar N relationships of Everett et al. (2010). However, it is evident from these observations that conifers are capable of acquiring and storing large quantities of MDN for later utilization.

Implications

The dramatic foliar N responses of riparian vegetation to the deposition of anadromous fish carcasses demonstrate the potential influence of this resource subsidy on the immediate and sustained vegetative N chemistry of these N limited inland ecosystems. These responses further suggest that conifer forests of this region may be substantial short and long term N sinks. The soil nutrient hotspots associated with the deposition of anadromous fish carcasses appear to dissipate within a few months (Drake et al. 2005, Gende et al. 2007, Holtgrieve et al. 2009) to over a year (Wheeler and Kavanagh in review). However, reservoirs of MDN remain within the riparian vegetation. Conifers are clearly adept at sequestering N and there is evidence from conifers within this region that they can retain this N for decades (Koyama et al. 2005). This preponderance to store and internally recycle N (Aerts 1996, Edmonds et al. 1989, Millard and Grelet 2010, Koyama et al. 2005, Salifu and Timmer 2001, Tully et al. 2013) may play a major role in the sustained conifer productivity of these N limited forests. However, this N retentiveness also lends to negative feedbacks on soil litter quality and soil microbial productivity (Heath et al. 1988, Johnson 1992, Valachovic et al. 2004).

By contrast, the herbaceous community may serve as a short term MDN reservoir in these forests. The accumulation of MDN in herbaceous biomass and the subsequent contribution of this biomass to the soil litter pool could stimulate microbial productivity and increase soil nutrient availability, thus possibly extending the influence of MDN on the productivity of these forests beyond the resident time of the carcass nutrient hotspots. However, this potential resource reservoir remains un-quantified. The utility of stable isotopes and isotopic mixing models to confirm the presence of marine derived N by riparian vegetation has advanced the understanding of salmon influence on riparian forests (Bilby et al. 2003, Hilderbrand et al. 1999, Hocking and Reynolds 2011, Koyama et al. 2005) but the potential for advancing this knowledge by applying this isotopic mixing model to vegetative biomass estimates is apparent. Understanding the temporal and spatial dynamics of these MDN reservoirs could advance the ability to appropriately manage these anadromous fish driven ecosystems.

Conclusion

The renewed deposition of anadromous fish carcasses within streams and riparian forests of the inland Pacific Northwest revealed the potential magnitude of influence this nutrient subsidy has on vegetation of this oligotrophic semi-arid region. Common horsetail growing near the stream edge readily assimilated carcass nutrients from stream water as was evidenced by increased foliar $\delta^{15}N$ but annual carcass deposition events were required in order to maintain this foliar N enrichment. In the absence of subsequent carcass deposition events, horsetail growing within the riparian forest indicated that MDN contributions become exhausted within two to three years. Conifer seedlings demonstrated the advantage of nutrient uptake and storage capacity with dramatic increases in foliar $\delta^{15}N$ that continued beyond the depletion of soil MDN. The use of an isotopic mixing model in conjunction with allometric relationships for conifer seedling biomass revealed that a considerable amount of MDN can be sequestered within riparian vegetation. The subsequent flow of these nutrients through the understory producer and consumer community as well as the complex interactions within this community may play a major role in the productivity and diversity of these ecosystems. However, it is evident from this investigation that additional consideration and exploration of these potential underlying drivers of ecosystem productivity are needed in order to appropriately mitigate for the decline of salmonids.

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

Stream	Latitude (N)	Longitude (W)	Elevation (m)	Sand/Silt/Clay (%)
Banner Creek	43˚59'14"	115˚32'46"	1625	73/6/21
Beaver Creek	43˚49'30"	115˚31'08"	1311	94/4/2
Big Owl Creek	43˚53'25"	115˚30'33"	1417	NA
Little Beaver Cr.	43˚58'07"	115˚35'48"	1572	75/25/1
Hungarian Cr.	43˚49'04"	115˚32'20"	1307	NA
Trail Creek	43˚54'00"	115˚23'12"	1571	68/28/5

Note: NA indicates that sand/silt/clay content of these soils was not quantified.

Table 2. The allometric relationships between stem basal area (mm^2) and biomass of Douglas-fir (DF) and lodgepole pine (LPP) seedlings.

Biomass ~ Basal area (mm^2)	**Equation**	**R^2**	**Correction**
Current year branch (g)	(DF) ln Y = ln BA x 1.12 – 5.02	0.92	1.06
	(LPP) ln Y = ln BA x 1.26 – 5.80	0.94	1.11
Total branch (g)	(DF) ln Y = ln BA x 1.34 – 4.00	0.94	1.06
	(LPP) ln Y = ln BA x 1.56 – 5.74	0.92	1.21
Current year foliage (g)	(DF) ln Y = ln BA x 1.04 – 3.03	0.92	1.05
	(LPP) ln Y = ln BA x 1.14 – 3.60	0.92	1.15
Total foliage (g)	(DF) ln Y = ln BA x 1.22 – 2.79	0.96	1.04
	(LPP) ln Y = ln BA x 1.24 – 2.94	0.98	1.03
Stem (g)	(DF) ln Y = ln BA x 1.35 – 3.55	0.99	1.01
	(LPP) ln Y = ln BA x 1.37 – 3.77	0.99	1.02
Total Above ground (g)	(DF) ln Y = ln BA x 1.28 – 1.95	0.99	1.01
	(LPP) ln Y = ln BA x 1.33 – 2.63	0.99	1.02
Below ground – course root (g)	(DF) ln Y = ln BA x 1.22 – 3.19	0.97	1.03
	(LPP) ln Y = ln BA x 1.22 – 3.48	0.99	1.01

Notes: BA is the basal area in mm^2 of the conifer seedlings at one inch above the root collar. Conversion of the y-variable into allometric biomass requires multiplying the untransformed Y parameter with the correction factor.

Table 3. Isotopic mixing model estimates of foliar marine derived nitrogen (MDN) content (%) and total MDN content (grams) for allometrically derived foliar biomass of conifer seedlings at one year following anadromous fish carcasses deposition and across a foliar age sequence of seedlings treated in previous years.

Treatment year	Foliar years since treatment	Foliar $\delta^{15}N$ (‰)	% foliar N from MDN	Foliar N (%)	Foliar biomass (g)	Total MDN in foliar biomass (mg)
2010	1 year	(T) 3.8 (2.3) (C) -0.2 (0.8)	33.0	2.1 (0.1)	5.8 (5.5)	40.2
2009 2008	1 year	(T) 5.1 (2.3) (C) -0.9 (0.9)	46.9	1.4 (0.2)	4.6	30.2
2009 2008	2 years	(T) 5.4 (3.1) (C) -0.7 (1.1)	48.4	1.4 (0.1)	6.4	43.4
2008	3 years	(T) 5.2 (2.3) (C) 0.1 (1.0)	43.2	1.5 (0.1)	8.4	54.4

Notes: (T) and (C) represent foliage from treated and control trees, respectively. Values in parentheses are standard deviations of the mean. The % foliar MDN and total foliar MDN were calculated using mean values only. The values for one and two year foliage from the 2008 and 2009 trees were pooled to add inferential simplicity.

Figure 1. The daily soil water content (SWC) and monthly precipitation within the study region as a function of year day over the 2008 – 2011 study period. The Beaver Creek SWC represents the typical SWC trends expected within this watershed whereas the Banner Creek SWC was progressively influenced by beaver dams. The Natural Resource Conservation Service (NRCS) weather station precipitation reported for Mores Creek Summit and Graham Guard Station represent expected precipitation rates at the Little Beaver Creek/Banner Creek and Trail/Beaver Creek sites, respectively.

Figure 2. The mean daily high and low atmospheric temperatures, as a function of year day, collected within the carcass treated riparian areas of Little Beaver Creek and Trail Creek over the study period of 2008 – 2011.

Figure 3. The mean $\delta^{15}N$ of common horsetail (*Equisetum arvense*) growing near the stream edge, as a function of year day over three years from August 2008 to August 2011. Anadromous fish carcasses were added to the treatment streams the first week of August in 2008, 2009, and 2010. These fish carcass additions are denoted by the vertical arrows. Error bars are standard error. The * indicates a significant difference at $\alpha=0.05$ in $\delta^{15}N$ between the treated and control horsetail within sampling intervals. The black dot represents a significant difference at $\alpha=0.05$ in $\delta^{15}N$ between the pretreatment and post treatment values in 2008.

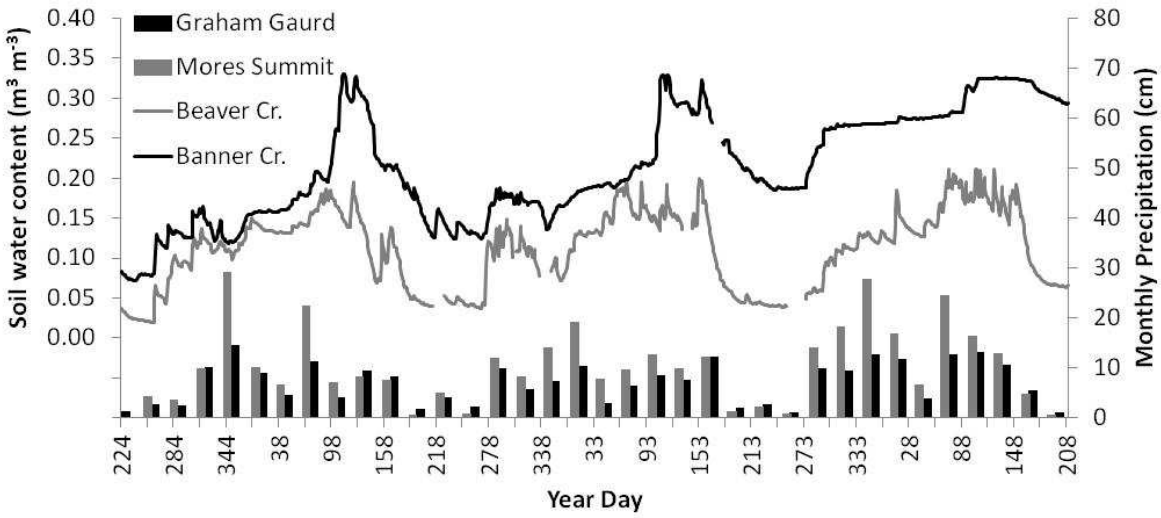
Figure 4. A) The pooled mean foliar $\delta^{15}N$ of common horsetail (*Equisetum arvense*) in riparian plots treated in 2008 and 2009 as a function of mean foliar %N and B) The pooled mean foliar $\delta^{15}N$ of horsetail from the 2008 and 2009 plots prior to treatment and one year post treatment in relation to horsetail foliage collected from these plots in 2011. The two year post treatment values derive from the 2009 treatment plots whereas the three year post treatment derives from the 2008 treatment plots. Error bars are standard error of the mean. The * indicates a significant difference at $\alpha=0.05$ in $\delta^{15}N$ between the treated and control horsetail.

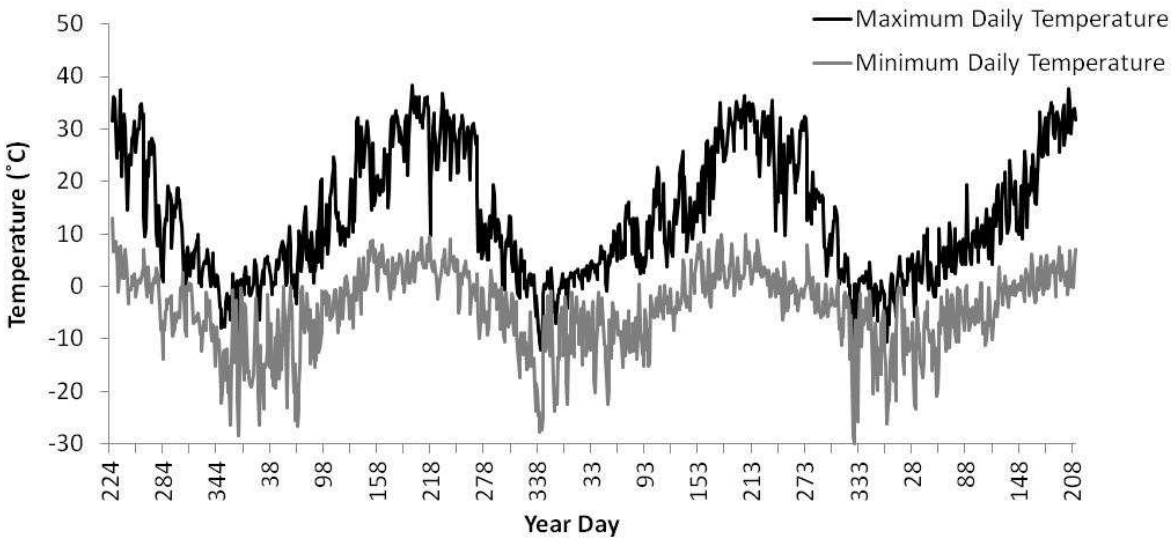
Figure 5. A) The pooled mean foliar $\delta^{15}N$ of conifer seedlings as a function of mean foliar %N in association with the deposition of anadromous fish carcasses within riparian forests in 2008, 2009, and 2010. B) The mean foliar $\delta^{15}N$ of the one, two, and three year post treatment foliage from the seedlings treated in 2008 and 2009. The one and two year values are pooled values from 2008 and 2009 whereas the three year foliage only derives from the 2008

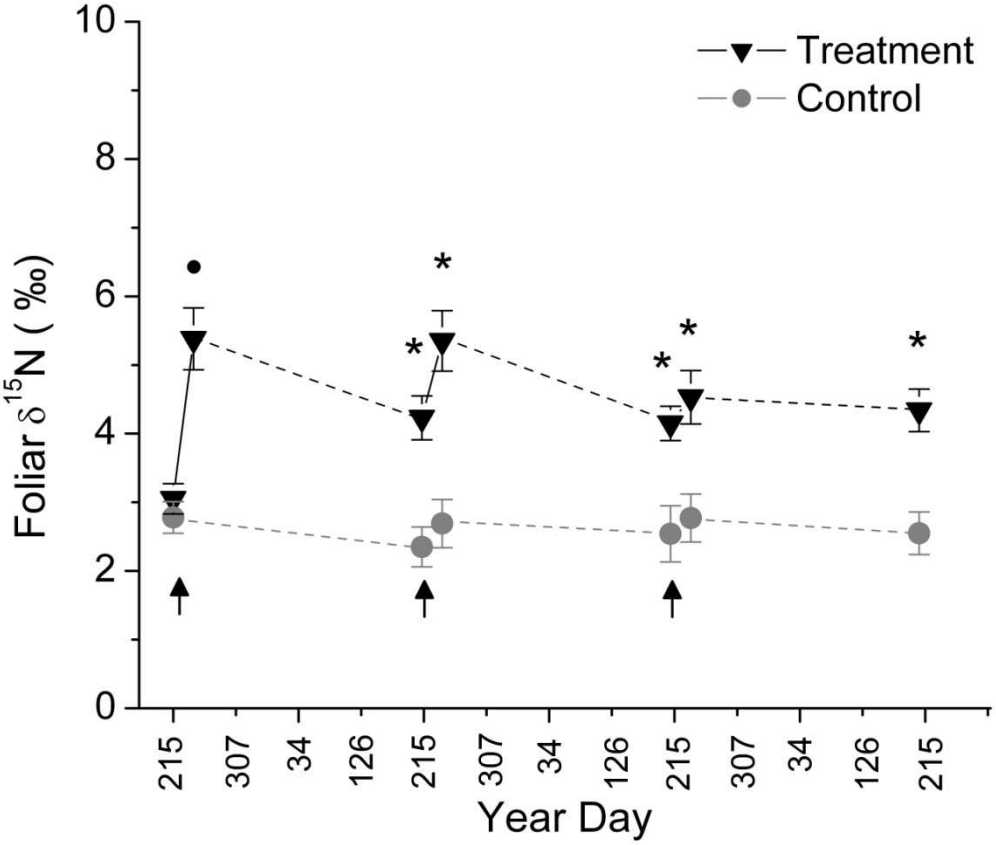
treatment seedlings. Error bars are standard error of the mean. The * indicates a significant difference at α=0.05 between the treated and control seedlings.

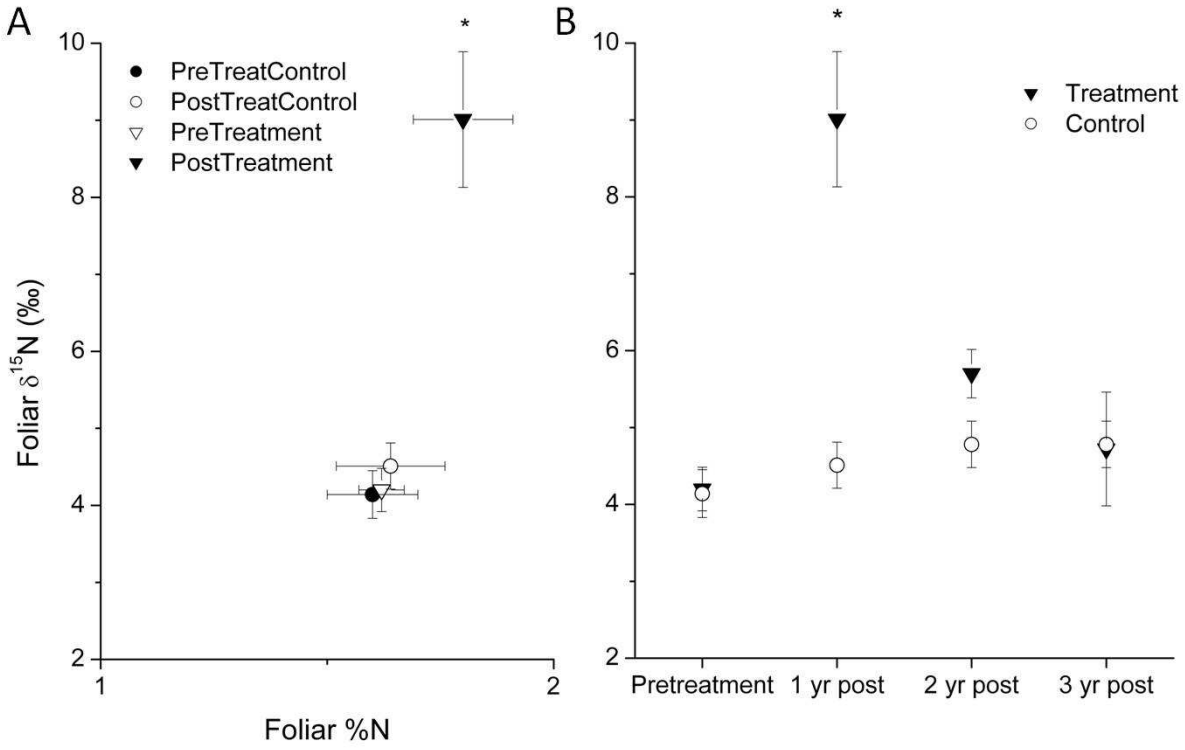
Figure 6. The mean foliar C:N ratios of control and treated conifer seedlings prior to the addition of anadromous fish carcasses and one year following the amendment Error bars are standard error of the mean. The letters a, b, and c indicate significant differences between similarly labeled observations at α=0.05.

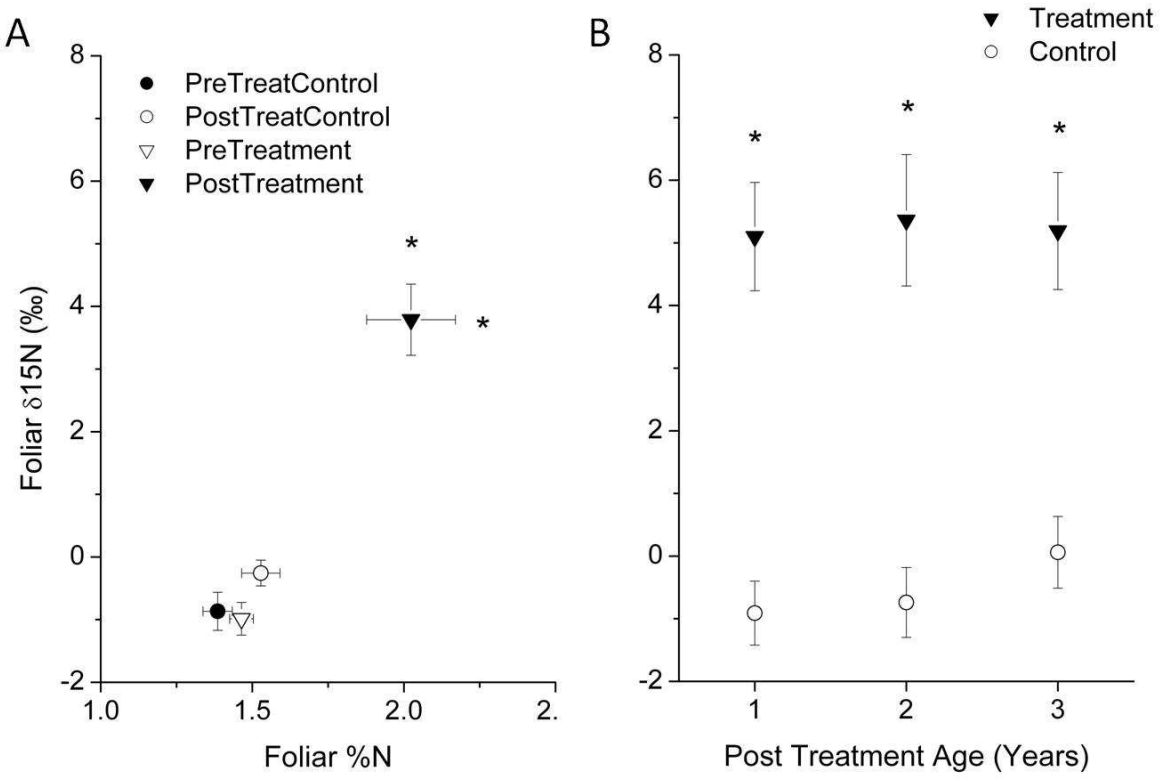
Figure 7. The soil $\delta^{15}N$ of the 2009 riparian plot soils as a function of year day. Error bars are standard error of the mean. The initial values are the pretreatment values. The * indicates a significant difference at α=0.05 in $\delta^{15}N$ between the soils of the treated and control plots. 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.

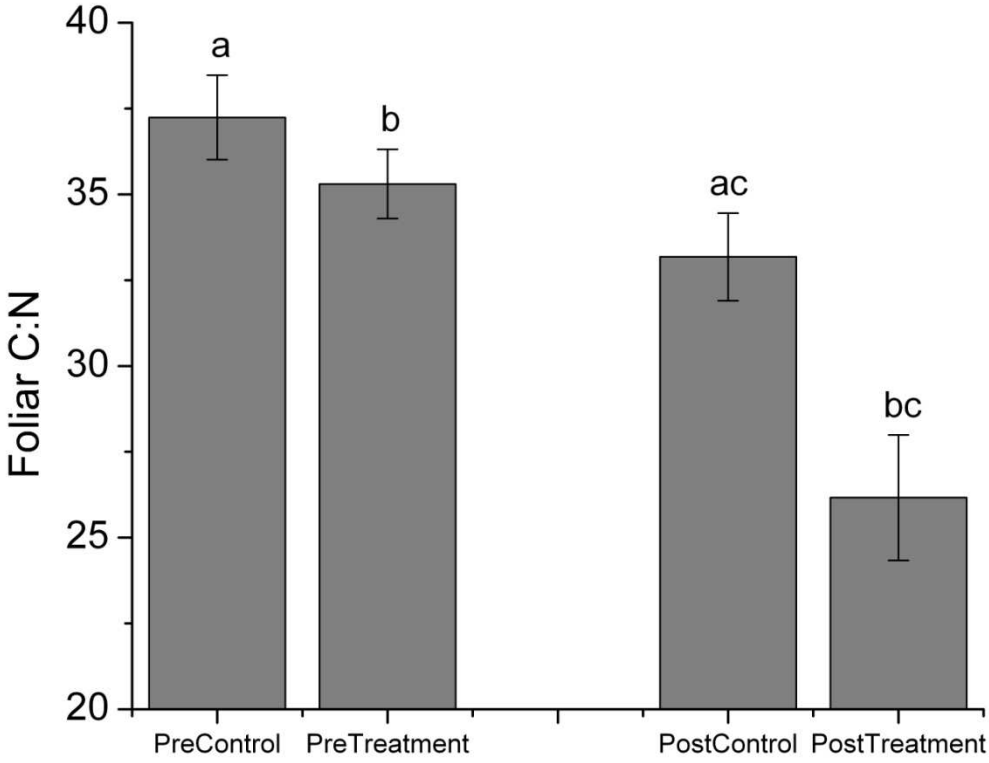

Graham Gaurd
Mores Summit
Beaver Cr.
Banner Cr.
Soil water content (m³ m⁻³)
Monthly Precipitation (cm)
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
80
70
60
50
40
30
20
10
0
224
284
344
38
98
158
218
278
338
33
93
153
213
273
333
28
88
148
208
Year Day

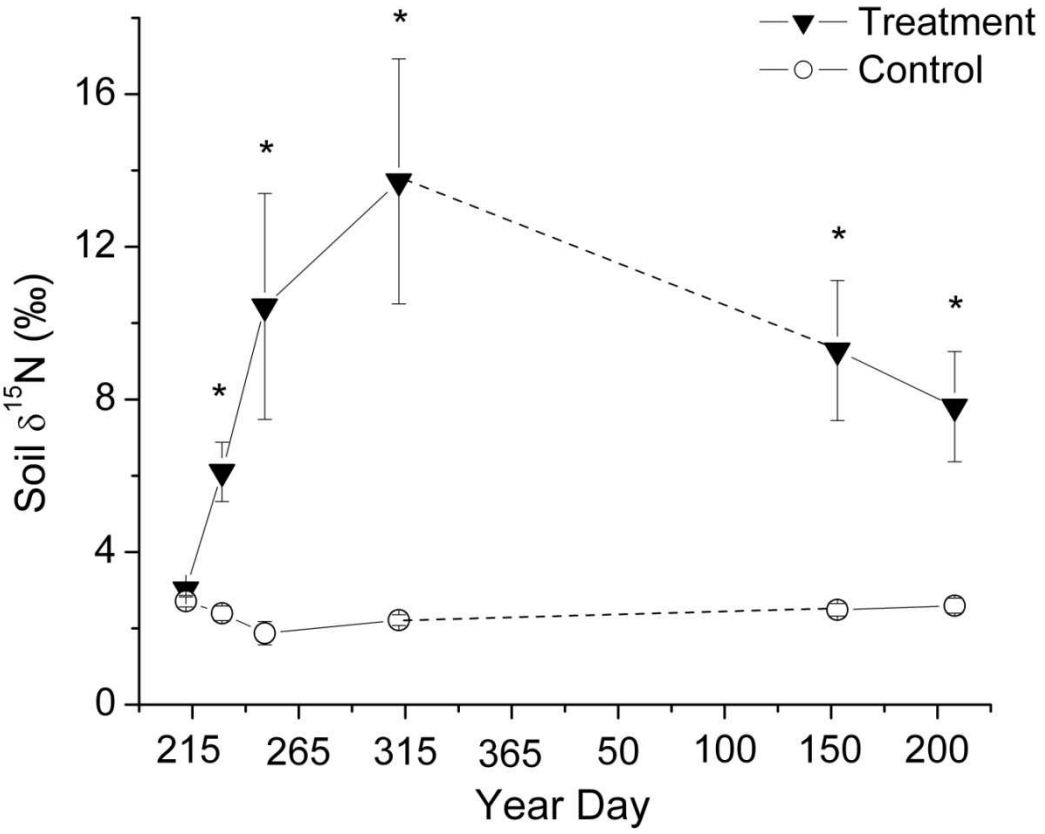

Maximum Daily Temperature
Minimum Daily Temperature
Temperature (°C)
50
40
30
20
10
0
-10
-20
-30
224
284
344
38
98
158
218
278
338
33
93
153
213
273
333
28
88
148
208
Year Day


Treatment
Control
Foliar δ15N (‰)
Year Day
0
2
4
6
8
10
215
307
34
126
215
307
34
126
215
307
34
126
215


A
B
PreTreatControl
PostTreatControl
PreTreatment
PostTreatment
Treatment
Control
Foliar δ15N (‰)
Foliar %N
Pretreatment
1 yr post
2 yr post
3 yr post
*
*


A
PreTreatControl
PostTreatControl
PreTreatment
PostTreatment
Foliar δ15N (‰)
Foliar %N
B
Treatment
Control
Post Treatment Age (Years)


40
35
30
25
20
Foliar C:N
a
b
ac
bc
PreControl
PreTreatment
PostControl
PostTreatment


Treatment
Control
Soil δ15N (‰)
0
4
8
12
16
215
265
315
365
50
100
150
200
Year Day
*
*
*
*
*