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The fate of marine-derived nutrients: tracing $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ through oligotrophic freshwater and linked riparian ecosystems following salmon carcass analog additions

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Abstract

The oligotrophic condition of salmon bearing catchments in the Columbia River Basin is a potential limiting factor for the recovery of Pacific salmon (*Oncorhynchus* spp.). To address this issue, nutrient supplementation programs attempt to mitigate for reduced marine-derived nutrients (MDN). We examined the assimilation of MDN in the biota of tributaries of the Salmon River Basin, Idaho, USA, following the addition of salmon carcass analogs (SCA). We measured carbon (C) and nitrogen (N) stable isotopes from biofilm, macroinvertebrate, salmonid fish, and riparian vegetation samples and found significant ^{15}N enrichment and substantial assimilation of SCA material in all aquatic trophic levels and in riparian vegetation. Our results suggest that SCA are incorporated primarily through indirect pathways and provide a source of MDN to multiple trophic levels in freshwater and linked riparian ecosystems.

Introduction

In the Pacific Northwest (PNW), spawning salmon and steelhead (*Oncorhynchus* spp.), as well as Pacific Lamprey (*Lampetra tridentate*), primarily exhibit a semelparous lifecycle in which they rear in freshwater for a period as juveniles, out-migrate to the ocean where substantial growth occurs, and then complete an upstream migration to reproduce in their natal stream and subsequently die. While in the marine environment Pacific salmon accumulate ~95% of their body mass (Groot and Margolis 1991). In the ocean, where the vast majority of salmon biomass is accumulated, the heavier isotopes of C (^{13}C) and N (^{15}N) are more prevalent in marine organic material and are assimilated into salmon tissue (Chaloner et al. 2002; Kline et al. 1990).

Once Pacific salmon enter freshwater habitats they stop feeding and thus largely maintain the isotopic signature associated with the biomass accumulated in the marine environment (Kline et al. 1990). During the return migration anadromous fishes are essentially a vector transporting vast quantities of marine-derived C, N, and phosphorus (P) from the marine environment to inland freshwater and terrestrial ecosystems (Cederholm et al. 1999). Prior to death, salmon contribute ~30% of the N they deliver to freshwater habitats via excretion of metabolic waste products and release and deposition of gametes while spawning (Mathisen et al. 1988). Following spawning, the remaining nutrients contained in the body mass of salmon are released into the freshwater system by the natural decomposition of their carcass. The MDN that are

transported to freshwater ecosystems by anadromous fishes are subsequently assimilated into aquatic and terrestrial food webs by direct and indirect pathways (Arseniev 1996, Chaloner et al. 2002; Hicks et al. 2005, Koshino et al. 2013). The indirect pathway incorporates mineralized inorganic MDN within the base of the food chain (primary producers), which is extended to higher trophic levels from the bottom up; whereas the direct pathway incorporates organic nutrients by higher trophic levels via direct consumption of marine-derived material (i.e., salmon carcasses and eggs) (Kiernan et al. 2010).

While the migration of anadromous fishes historically provided substantial amounts of pulsed allochthonous subsidies (i.e., organic material and nutrients) to inland aquatic and terrestrial ecosystems, dramatic declines in contemporary anadromous fish abundance have disrupted this process (Gresh et al. 2000). In the Columbia River Basin, declining returns of Pacific salmon and other anadromous fishes are associated with reduced MDN subsidies (Alldredge et al. 2015). Because of factors such as habitat degradation and loss, commercial harvest, hatchery production, and hydro-system development, historically abundant anadromous fish returns have been eliminated entirely, or have declined dramatically across large spatial scales (Lichatowich 1999). Currently, it is estimated that spawning Pacific salmon and steelhead trout contribute just 6%-7% of the MDN historically delivered to PNW rivers and streams (Gresh et al. 2000). These subsidies likely contributed an ecologically meaningful amount of organic material and nutrients to freshwater and terrestrial ecosystems across the PNW (Cederholm et al. 1999).

In this study we utilized stable isotope analysis (SIA) of C and N as a tracer to investigate the potential assimilation of MDN into aquatic and terrestrial riparian habitats of four central Idaho streams following the addition of SCA. SCA was developed by Pearsons et al. (2007) and is specifically designed to fertilize streams. The analog is made from dried and pasteurized marine fishmeal; therefore, it is pathogen-free and intended to mimic the nutritional profile and natural decay rate of salmon carcasses, providing both a direct and indirect source of MDN to various trophic levels.

We selected streams based on their oligotrophic status and historical abundance of natural/wild Chinook Salmon (*Oncorhynchus tshawytscha*) populations. N is limited in central Idaho streams by a general scarcity of N fixing riparian species (Henderson et al. 1978) and it

has been estimated that 25%–50% of Idaho streams are nutrient-limited (Thomas et al. 2003). An evaluation of nutrient limitation conducted by Ebel et al. (2014) using nutrient diffusing substrata found that our study streams were primarily N limited with some co-limitation of N and P. Sanderson et al. (2009a) found similar results in experiments conducted throughout the Salmon River Basin that indicate N and P are typically limiting nutrients for aquatic primary production in these inherently oligotrophic systems.

We measured C and N stable isotopes among multiple trophic levels within freshwater ecosystems and adjacent riparian vegetation over the course of several seasons of SCA applications. SIA can be used to investigate energy pathways within freshwater and associated riparian habitats, to estimate the trophic position of organisms, and to evaluate the assimilation of available MDN subsidies into various trophic levels (Bilby et al. 1996; Post 2002; Kline et al. 2007). The stepwise enrichment of heavier isotopes along the food chain is used to identify food webs; however, SIA does have limitations, some of which are primarily related to variability in fractionation during metabolic processes (Focken and Becker 1998), thus potentially confounding results to estimate trophic position by virtue of a less predictable stepwise enrichment.

We tested whether SCA material was assimilated into the tissue of biofilm, macroinvertebrate, salmonid fish, and riparian vegetation within treatment segments of study streams using a multiple-before-after-control-impact (MBACI) experimental design. We hypothesized that components of the linked aquatic and terrestrial habitats in our Salmon River Basin study streams would demonstrate elevated $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures and associated estimates of proportional assimilation of MDN following the experimental addition of SCA over the course of a three-year study period.

Materials and Methods

Study Sites

We conducted our study in the Salmon River (SR) and Middle Fork Salmon River (MFSR) basins in central Idaho, USA (Fig. 1). Five of these streams: Elk Creek, Basin Creek, Panther Creek, Musgrove Creek, and Moyer Creek are in the SR Basin; and one stream, Cape Horn

Creek, is in the MFSR Basin. At ~1 448 river kilometers and 1 828 meters of elevation, this study area is located at one of the furthest inland migration points for Pacific salmon. These streams were chosen based on their historically high abundance of salmon populations, importance to the Shoshone-Bannock Tribes, and current oligotrophic status. Currently, Cape Horn Creek is the only stream that supports a sizeable naturally spawning population of Chinook Salmon, although contemporary abundance is highly depressed relative to historical conditions. For additional details regarding study stream physical, chemical, and biological characteristics, please see Ebel et al. (2014).

Experimental Design

We utilized a MBACI design to trace MDN through the food web following SCA additions by analyzing $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of benthic biofilms, benthic macroinvertebrates, salmonid fishes, and riparian vegetation. We delineated study streams into 6 km segments divided into a 3 km upstream control/reference segment and a 3 km downstream treatment segment (Fig. 2). Each segment was stratified into three 1 km stratum (upper, middle, and lower) for sampling purposes. Four treatment streams and two control streams were randomly chosen. The treatment streams, which include: Cape Horn Creek, Basin Creek, Panther Creek, and Moyer Creek received SCA additions in the downstream treatment segment and the two control streams: Elk Creek and Musgrove Creek did not receive SCA additions.

SCA Loading and Application Timeline

Of the four treatment streams, Cape Horn Creek and Panther Creek received a high SCA addition of 150 g m^{-2} of bank-full channel width and Basin Creek and Moyer Creek received a low SCA addition of 30 g m^{-2} of bank-full channel width. The loading quantity for low SCA streams was based on target stocking densities recommended by Wipfli et al. (2003) and the loading quantity for high SCA streams was based on estimates of salmon escapement made by Bilby et al. (2001). For comparison, we quantified natural stream loading rates (concentration x discharge x time) for C, N, and P from upstream reference segments during the treatment period (~30 days). We note that comparing our SCA treatments to annual stream loading rates would perhaps be most appropriate; however, we selected the short-term period due to a lack of annual stream-specific data. That said, our short-term comparisons highlight large natural loads of C relative to our SCA applications. To estimate natural loading rates, we used dissolved organic

carbon (DOC), dissolved inorganic carbon (DIC), total nitrogen (TN), and total phosphorus (TP) measures from the control (i.e., upstream) segment of study streams that received SCA additions. In high SCA streams, estimated natural loading (kg) of C, N, and P was 7 799, 81, and 11, respectively. In low SCA streams, estimated natural loading (kg) of C, N, and P was 20 622, 176, and 40, respectively. On average, high SCA streams received ~1 357 kg of C (18% of natural C load), 286 kg of N (488% of natural N load), and 57 kg of P (546% of natural P load) during each treatment application, while low SCA streams received 291 kg of C (2% of natural C load), 61 kg of N (37% of natural N load), and 12 kg of P (37% of natural P load). We also compared our SCA treatment loads to the mean N equivalent (3.04% wet mass) of 5.5 kg Chinook Salmon carcasses (Larkin and Slaney 1997). We estimate that Chinook Salmon carcass equivalents in high SCA streams was 400-739 carcasses per km, or 0.44 kg carcass material per m² of bank-full channel width. In low SCA streams, we estimate 98-146 Chinook Salmon carcasses per km, or 0.09 kg of carcass material per m² of bank-full channel width. SCA pellets were 9 mm in diameter and had a nutritional profile of ~43% C, 9% N and 1.8% P by mass. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the SCA were -20.0‰ and 10.9‰ respectively. For details regarding the development of SCA materials please see Pearsons et al. (2007).

We applied SCA in early August throughout the entire 3 km treatment segment of treatment streams, a time in which MDN would naturally begin to enter these systems by excretion and gamete expression from live salmon, as well as early post-spawning mortality. We sampled study streams in July of 2010, before SCA additions, to collect data for baseline/reference conditions. Treatments began in early August of 2010 and continued each successive August through 2012. We ceased SCA applications in 2012 for streams receiving high SCA and in 2013 for streams receiving low SCA. We sampled study streams before SCA additions in July and again ~30 days after SCA additions in September throughout the course of the study. We collected samples ~1 year after SCA additions in all study streams.

Benthic Biofilm Sampling

We sampled benthic biofilms at riffle habitat sites within each delineated stratum of both treatment and control segments of all study streams. We haphazardly selected three rocks and scrubbed biofilm with a fine bristled brush and rinsed with a wash bottle containing river water. The slurry of biofilm and river water was contained in a wash tub that we then transferred to a

500 ml graduated cylinder and agitated to facilitate a homogenous mixture. A subsample of the resulting slurry was filtered through a 0.45 μm pre-ashed glass fiber filter (GFF). We wrapped the GFF's containing the filtered biofilm samples in aluminum foil, put them on ice, and froze them within hours for storage.

Benthic Macroinvertebrate Sampling

We sampled benthic macroinvertebrates at riffle habitat sites within each delineated stratum of both treatment and control segments of all study streams. We utilized a customized sampler that had a surface area of 0.33 m^2 and a mesh size of 500 μm . We employed a method described by Taylor et al. (2001) using electroshocking techniques. We focused a localized field of direct current using a backpack electrofishing unit just above the substrate within the sampler for a standard period of 60 seconds. The electrical current induced benthic macroinvertebrates into the drift where we collected samples from the cod end cup of the sampler and transferred them to Whirl-Pak's filled with river water. We refrigerated samples for 24 hours to allow for gut evacuation and then froze samples for storage.

Salmonid Fish Sampling

We utilized standard electrofishing methods and employed a non-lethal sampling protocol for sampling threatened and endangered fishes. Salmonid fish fin tissue samples produce stable isotope measures consistent with muscle tissue samples and allow for non-lethal sampling (Sanderson et al. 2009b). In 2010 we collected fish fin tissue samples from salmonid fishes in all delineated stratum of both treatment and control segments of study streams before SCA additions. In 2012 we sampled the middle control and middle treatment strata of all streams before SCA additions and again ~1 month after SCA additions in streams receiving low SCA, as well as both control streams. We conducted one year post-treatment sampling in 2012 for streams that received high SCA and in 2013 for streams that received low SCA. We transferred fish fin tissue samples to a folded piece of weather resistant paper and stored them in a scale envelope.

Riparian Vegetation Sampling

We collected leaves from riparian vegetation in all strata of each study stream in late August of 2012. This sampling period was ~2 weeks after SCA additions in streams receiving low SCA and ~1 and 2 years after SCA additions in August 2011 and 2010, respectively. We collected samples from the dominant taxa of riparian vegetation present at each stratum. We clipped several leaves from each taxon with scissors and transferred samples to paper bags with forceps.

C and N Stable Isotope Analysis

SIA was conducted at the Interdisciplinary Laboratory for Elemental and Isotopic Analysis (ILEIA) at Idaho State University utilizing a Thermo Delta V Advantage Isotope Ratio Mass Spectrometer connected to Costech Elemental Combustion System 4010 (ECS 4010). We dried all samples prior to analysis. Preceding isotopic analysis, we acid fumigated biofilm samples following procedures outlined by Harris et al. (2001) for ~2 hours to remove any potential calcium carbonate that may confound $\delta^{13}\text{C}$ values, then dried in an oven at 60 °C. We ground biofilm, macroinvertebrate, and riparian vegetation samples to a powder using a porcelain mortar and pestle to assure homogeneity. We analyzed whole salmonid fin tissue samples due to the small mass of tissue available. Results are presented in the standard delta notation for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

Where, X is $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and R is the ratio of heavier to light isotopes ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are expressed using the unit, ‰ (parts per thousand or per mil); and the $\delta^{13}\text{C}$ values are relative to the Vienna Pee Dee Belemnite (VPDB) and the $\delta^{15}\text{N}$ values are relative to atmospheric N_2 (air).

Statistical Analysis

We considered samples collected from sites within stream segments as subsamples and used multiple sites to estimate mean response variable values for each stream segment. We used mean stream segment values for biofilm and macroinvertebrate stable isotope measures to facilitate comparisons between treatment and control streams (Kohler et al. 2012). We applied a MBACI design following that of Keough and Quinn (2000) to response variable data represented

by the difference between mean stream segment response variables (i.e., treatment segment mean minus control segment mean for treatment streams and downstream segment mean minus upstream segment mean for control streams) following Kohler et al. (2012). We used streams as replicates and analyzed a treatment level (TL: three levels- no SCA, low SCA, or high SCA) by period (i.e., before-after) interaction effect with multilevel model (MIXED) analysis of variance (ANOVA). The ANOVA model had two main fixed factors of interest: 1) SCA addition or treatment level (no SCA, low SCA, and high SCA) and 2) before-after SCA addition or period (BA: two levels, before and after) with streams (S) and year (Y) specified as random factors. The term of most interest was the treatment level (TL) by period (BA) interaction, which measured any relative change in response variables associated with SCA treatment.

We implemented our mixed models using lme4 (package version 1.1-10) in the R statistical platform version 3.1.2 (R Core Team 2015). We used restricted maximum likelihood estimates and Satterthwaite approximations to degrees of freedom for significance testing (Bates et al. 2015; Satterthwaite 1946). Sound statistical judgment and sensible biological interpretation, especially when conducting multiple tests, requires careful consideration to ‘experiment-wise’ or ‘family-wise’ error rates; however, utilization of traditional approaches that minimize type I error, such as Bonferroni or sequential Bonferroni procedures, come at the cost of increasing type II errors (Armstrong 2014; Verhoeven 2005). To control the false discovery rate (FDR) (i.e., the proportion of significant results that are actually false positives) we employed the Benjamini-Hochberg procedure and set the FDR at 10%. This procedure represents a practical and powerful approach to address multiple testing (Benjamini and Hochberg 1995) and has been recommended for ecological studies (Garcia 2004; Verhoeven 2005). Our FDR is intended to balance the risks of type I and type II errors while preserving the power of our tests. We chose a less conservative approach to controlling for type I errors (*a priori*) in an attempt to detect real effects where they exist. We recommend readers compare our statistical results with graphical representations of biological effect size to further inform interpretation. We evaluated normality and homogeneity of variance of modeled residuals visually with residual and fitted plots and statistically using Shapiro-Wilk and Breusch-Pagan tests.

Trophic Level Mixing Model

To estimate percent C and N MDN assimilation from SCA materials to various trophic levels we employed a mixing model utilizing the equation (after Johnston et al. 1997):

$$\text{MDN enrichment (\% X)} = 100 \times (\delta X_t - \delta X_c) / ((\delta X_a + (\text{TL} \times \delta X_e)) - \delta X_c)$$

Where δX_t is the isotopic ratio of the organism in areas enriched with SCA (treatment), δX_c is the stable isotope value of the organism in areas with no enrichment (control), δX_a is the stable isotope value of the SCA, δX_e is the isotope enrichment factor per trophic level (1‰ for $\delta^{13}\text{C}$ and 3‰ for $\delta^{15}\text{N}$ (Chaloner et al. 2002)), and TL is the correction factor for the trophic level of the organism (1 for shredders, primary consumer; 1.5 for filterers, omnivore; 2 for predaceous insects, secondary consumer; and 3 for salmonids (Chaloner et al. 2002)). We weighted TL values according to stream specific proportional representation of primary consumers, omnivores, and predatory insects from our benthic macroinvertebrate community assemblage data (Kohler et al. 2016; unpublished data).

We utilized the stable isotope mixing model SIAR (stable isotope analysis in R) version 4.0.2 (Parnell et al. 2010) package in the R statistical platform (R Core Team 2015) to estimate proportional dietary sources and to identify potential pathways (i.e., direct or indirect trophic transfer) for macroinvertebrate and fish organisms following SCA additions. This package takes data from target organism isotopes and fits a Bayesian model to their dietary habits (source isotopes) based upon a Gaussian likelihood (Parnell et al. 2010). The SIAR package incorporates sources of variability in model inputs such as trophic fractionation values (in our case 1‰ for $\delta^{13}\text{C}$ and 3‰ for $\delta^{15}\text{N}$ following Chaloner et al. 2002) and mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values ($\pm\text{SD}$) from dietary sources.

Results

Benthic Biofilms

Benthic biofilms were significantly enriched in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (treatment level by period interaction; $F = 3.133$, $p = 0.060$ and $F = 3.856$, $p = 0.035$, respectively) in the downstream treatment segments relative to upstream reference segments and control streams following SCA additions (Figs. 3a,c; 4c; Table 1). Biofilm $\delta^{15}\text{N}$ values were higher in treatment segments of

high SCA streams and only slightly elevated in treatment segments of low SCA streams (3.3‰ and 0.8‰, respectively). The calculated mean percent MDN assimilation (Table 2) was ~30% for C and 42% for N in high SCA streams; whereas 7% of C and 22% of N was assimilated in low SCA streams. For treatment level (no, low, and high) biofilm $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ mean, range, and variance estimates by year and period (before and after), please see Appendix Table A1 and A2, respectively.

Benthic Macroinvertebrates

Benthic macroinvertebrates were significantly enriched in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (treatment level by period interaction; $F = 2.706$, $p = 0.086$ and $F = 7.428$, $p = 0.003$, respectively) in the downstream treatment segments relative to upstream reference segments and control streams following SCA additions (Figs. 3*b,d*; 4*b,d*; Table 1). Macroinvertebrate $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were higher in treatment segments of high SCA streams (3.0‰ and 3.3‰, respectively) and elevated to a lesser extent in treatment segments of low SCA streams (1.3‰ and 2.0‰, respectively). Mean percent MDN assimilation (Table 2) was ~25% for C and 28% for N in high SCA streams; whereas ~15% of C and 17% of N were assimilated in low SCA streams. Based on the SIAR mixing model, macroinvertebrate proportional dietary sources were primarily (~90-95%) from indirect (i.e., ^{13}C and ^{15}N enriched biofilm and ^{15}N enriched riparian vegetation) pathways with minor contributions (~5-10%) from direct SCA consumption (Figs. 8*a,b*; Table 3). Leaf litter isotope values used in the SIAR mixing model were mean isotope values from all riparian vegetation taxa sampled in the treatment segment of each stream type (i.e., low SCA and high SCA). For treatment level (no, low, and high) benthic macroinvertebrate $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ mean, range, and variance estimates by year and period (before and after), please see Appendix Table A1 and A2, respectively.

Salmonid Fishes

Salmonid fish (analyzed as an aggregate) were ^{15}N enriched in the downstream treatment segments relative to upstream reference segments and control streams following SCA additions. Salmonid fish $\delta^{15}\text{N}$ isotopic signatures were higher in the treatment segments of high SCA streams by a mean value of 1.7‰ (Fig. 5*f*) and not different in the treatment segments of low SCA streams (Fig. 5*d*). Mean percent MDN assimilation (Table 2) was ~17% for C and 14% for

N in high SCA streams; whereas ~5% of C and 3% of N were assimilated in low SCA streams. Based on the SIAR mixing model, salmonid fish proportional dietary sources were primarily (~98%) from indirect (i.e., ^{13}C and ^{15}N enriched macroinvertebrates) pathways and secondarily (~2%) from direct SCA consumption (Figs. 8c,d; Table 3). For treatment level (no, low, and high) salmonid fish $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ mean, range, and variance estimates by year and period (before and after), please see Appendix Table A1 and A2, respectively.

Salmonid fish species specific analysis for each of our high SCA streams revealed isotopic enrichment in downstream treatment segments relative to upstream reference segments following SCA additions. In the downstream treatment segment of Cape Horn Creek (high SCA stream) there was no difference in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values for Chinook Salmon; however, Brook Trout (*Salvelinus fontinalis*) exhibited an elevated $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signature following SCA additions (Fig. 6b). In the downstream treatment segment of Panther Creek (high SCA stream) there was $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ enrichment of both Westslope Cutthroat Trout (*Oncorhynchus clarki lewisi*) and Bull Trout (*Salvelinus confluentus*) relative to the upstream control segments following SCA additions (Fig. 6d).

Riparian Vegetation

Riparian vegetation analysis indicated no difference in C and N isotope values for four of the five taxa sampled: birch (*Betula* spp.), aspen (*Populus tremuloides*), red alder (*Alnus rubra*) and red osier dogwood (*Cornus sericea*), with the exception of willow (*Salix* spp.). For willow, we found a large difference in $\delta^{15}\text{N}$ isotopic signatures within the treatment segments of high SCA streams, with a mean enrichment of 2.4‰, relative to upstream control segments and control streams (Fig. 7). For treatment level (no, low, and high) riparian vegetation (*Salix* spp.) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ mean, range, and variance estimates by year and period (before and after), please see Appendix Table A1 and A2, respectively.

Discussion

The utilization of inorganic fertilizers, salmon carcasses, and SCA to mitigate for the reduction and/or loss of MDN subsidies in streams with reduced or extirpated runs of anadromous fishes has been the focus of many studies (Perrin et al. 1987; Wipfli et al. 1998;

Kohler et al. 2008); however, relatively few studies have looked at the effects of SCA additions on the C and N isotopic signatures of biota in oligotrophic freshwater food webs (but see Kohler et al. 2012; Guyette et al. 2014; Marcarelli et al. 2014). We utilized SIA to trace MDN delivered by the addition of SCA to study stream habitats and found that these MDN subsidies were assimilated into multiple trophic levels. SCA was not only assimilated by aquatic organisms, but also by terrestrial vegetation in the adjacent riparian zone. The incorporation of MDN by macroinvertebrates and fish was found to be principally a result of indirect pathways, with minimal contribution by direct assimilation (i.e., consumption of SCA material). Effects were most apparent for streams receiving a high SCA loading of 150 g m^{-2} and for N than C. C and N stable isotope assimilation by aquatic and riparian ecosystems via SCA additions indicate that nutrients delivered to these oligotrophic systems were utilized in tissue generation of aquatic and terrestrial biota.

Benthic Biofilm Response

Benthic biofilm is composed of a gelatinous polysaccharide matrix that binds together algae, bacteria, and fungi in a tight internal nutrient cycling process between heterotrophs and autotrophs (Allan 1995). Biofilm biomass represents the foundation of the aquatic food web from which a bottom up trophic transfer of MDN can occur via remineralization (Kline et al. 2007) of MDN originating from SCA. Similar to the findings of Kohler et al. (2008 and 2012) and Marcarelli et al. (2014), we found ^{13}C enriched benthic biofilms only in streams treated with high SCA, whereas ^{15}N enriched benthic biofilms were found in streams treated with both low and high SCA. The different responses of C and N likely reflect pathways of nutrient incorporation. If via remineralization, then we would expect smaller contributions of C relative to N, as the loadings of C from SCA compared to background are much smaller for C than N. The calculation of assimilation rate (Table 2) assumes that incorporation of SCA is the only mechanism resulting in isotopic enrichment, and that there is no fractionation of either isotope during transfer from SCA to biofilm biomass. Such assumptions are untested in our system. It is possible that there is little fractionation of $\delta^{15}\text{N}$ during remineralization and uptake in these N limited systems; however, $\delta^{13}\text{C}$ increases under conditions of higher algal growth rates (Laws et al 1995). This kind of response may have occurred in high SCA streams. For example, Ebel et al. (2014) measured significant increases in whole-stream and benthic primary productivity and

metabolism in the same study streams following SCA additions. Regardless of the specific pathway, ^{13}C and ^{15}N enriched biofilm communities potentially provide ecologically meaningful, high-quality food resources that are subsequently available to a myriad of primary consumers (Verspoor et al. 2011).

Benthic Macroinvertebrate Response

Benthic macroinvertebrates represent an important ecological link between lower trophic levels and higher order consumers (Cederholm et al. 1999). Similar to our biofilm response, we found substantial isotopic enrichment of C and N in benthic macroinvertebrate samples collected from stream segments treated with SCA. Pathways of MDN incorporation into benthic macroinvertebrate communities may include direct feeding on SCA as well as indirect trophic transfer (Guyette et al. 2014; Bilby 1996). In our study, macroinvertebrates assimilated MDN material principally from indirect trophic transfer following SCA additions, as clearly revealed by the food web relationships (Fig. 5) and the SIAR mixing model (Fig's. 8a,c; Table 3). Biofilm (an autochthonous food source) and leaf litter (an allochthonous food source) contributed 30%-41% and 49%-65% of the proportional dietary sources consumed by macroinvertebrates, respectively. We found marginal evidence supporting the direct consumption of SCA by macroinvertebrates. Guyette et al. (2013) and Chaloner et al. (2002) utilized SCA and salmon carcasses (*Oncorhynchus* spp.) as treatments, respectively, and both determined that macroinvertebrates assimilated MDN by direct and indirect trophic transfer based upon the relative mode of feeding associated with individual FFG's. In addition to increased MDN isotopic enrichment, we found a substantial increase in benthic macroinvertebrate biomass and abundance (Kohler et al. 2016; unpublished data), as well as a short-term increase in macroinvertebrate drift abundance (Keeley et al. 2016) following SCA additions. Taken together, these findings suggest that macroinvertebrate MDN incorporation, combined with changes in abundance and biomass, appear to provide an important food resource available to stream dwelling fishes.

Salmonid Fish Response

Anadromous and resident salmonid fishes are often the focus of nutrient enrichment studies that attempt to better understand higher trophic level responses to experimental MDN subsidy

additions (Guyette et al. 2013, Kohler et al. 2012; Rinella et al. 2012). In our study, the isotopic enrichment of benthic macroinvertebrates indicates the assimilation of MDN following SCA additions. SCA subsidies likely contributed to an increased abundance and biomass of benthic macroinvertebrates (Kohler et al. 2016; unpublished data) available to stream dwelling fishes as forage. We conclude that salmonid fish communities within treatment segments of our study streams were enriched in ^{15}N from SCA based upon multiple lines of stable isotope evidence. The isotopic enrichment of salmonid fishes can be assessed in terms of contributions from both direct and indirect pathways. Pearsons et al. (2007) documented direct consumption of SCA material by fish and Guyette et al. (2013) suggest direct consumption based on growth rates of young of the year steelhead trout. Our findings (Figs. 8a,b; Table 3) indicate that macroinvertebrates contributed almost all of the MDN assimilation by fish in both low and high SCA streams and the direct consumption of SCA material was a minor pathway of incorporation. These results differ slightly from Collins et al. (2016) where 4-11% of consumption supporting trophic production of salmonid fishes was attributed to SCA. We infer that because macroinvertebrates, a primary prey item for stream dwelling salmonids, were ^{13}C and ^{15}N enriched, that the transfer of MDN to salmonids occurred primarily via predation on this food source. In a companion study, Keeley et al. (2016) documented a short-term increase in macroinvertebrate drift abundance and increases in net energy availability for salmonid fishes following SCA additions. Though we did not directly analyze fish growth in our study, Kohler et al. (2012) documented increased growth rates of salmonid fishes, Guyette et al. (2013) found enhanced growth and condition of juvenile Atlantic Salmon (*Salmo salar*), and Collins et al. (2016) documented increased growth rates and annual trout production following SCA additions. Kiffney et al. (2014) found Coho Salmon (*Oncorhynchus kisutch*) growth rates were positively related to increased drift macroinvertebrate biomass following the addition of SCA to experimental stream channels. The size of anadromous fishes at the time of outmigration to the ocean has been correlated with increased survival and is likely to increase fitness; however, potential changes in life history trajectories following nutrient enrichment applications should be closely monitored over multiple life cycles to better understand the possibility of unintended consequences (Ward and Slaney 1988).

As an example of unintended consequences, we note that before SCA additions in 2010 (Fig. 6a), Brook Trout and Chinook Salmon had similar baseline isotopic signatures; however,

following 2 years of high SCA additions in Cape Horn Creek, we observed (Fig. 6b) a greater ^{15}N enrichment in Brook Trout relative to Chinook Salmon. The implications of isotopically enriching and thus potentially benefiting a non-native/invasive species such as Brook Trout through nutrient mitigation efforts may have deleterious effects on ESA listed species (Compton et al. 2006). Achord et al. (2003) found that in Idaho streams the survival of Chinook Salmon parr was significantly lower in streams that had Brook Trout relative to those that did not and that in the presence of Brook Trout the relationship between Chinook Salmon parr survival and density disappeared (i.e., compensatory mortality). Managers should consider the potential that nutrient enhancement strategies aimed at salmon recovery may have adverse effects on target species if competition or other interactions with non-indigenous species are exacerbated by such actions. On the other hand, our study illustrates (Fig. 6) that native and threatened species were also ^{13}C and ^{15}N enriched following SCA additions (e.g., Cutthroat Trout and Bull Trout). We suggest that future nutrient mitigation efforts carefully consider issues such as fish assemblage structure and associated dynamics before adding MDN subsidies.

Riparian Vegetation Response

Riparian vegetation represents an important interface between aquatic environments and upland habitats. Similar to other studies, willow (*Salix* spp.) was the only genus that exhibited $\delta^{15}\text{N}$ enrichment. It is likely that MDN associated with SCA were carried into the hyporheic zone beneath the riparian area where they became available to the roots of willow (Naiman et al. 2002). The other four taxa sampled: birch (*Betula* spp.), aspen (*Populus tremuloides*), red alder (*Alnus rubra*), and redosier dogwood (*Cornus sericea*) did not exhibit a change in isotope values. Red alder is capable of utilizing fixed N such that its isotopic signature could be affected by that process. We have no clear explanation for the lack of an N stable isotope response for the other riparian vegetation taxa sampled. Further from the active stream channel and riparian area, studies have found that N enrichment in some plant species occurs up to 200 m from stream channels in salmon bearing catchments. This transport occurs by means of carcass displacement from scavengers and piscivorous predators (Helfield and Naiman 2001). SCA does not likely provide the potential for this type of bio-transport; therefore, MDN enrichment of riparian vegetation by SCA is assumed to be limited to those plants that have root systems connected to the stream channel and/or hyporheic zone.

It is likely that ^{15}N enriched riparian vegetation transfers MDN to terrestrial habitats through consumption by terrestrial herbivores (Cederholm et al. 1999) that are in turn potential prey items for many other terrestrial organisms (e.g., predatory insects, birds, bats, etc.) (Baxter et al. 2005). Enriched terrestrial components have the potential to once again enter the aquatic system as leaves senesce and herbivorous insects fall into aquatic habitats (Baxter et al. 2005). Additionally, the winged adult phase of enriched aquatic macroinvertebrates has the potential to further extend the terrestrial linkage by virtue of their mobility beyond the stream channel following emergence (Baxter et al. 2005). These reciprocal exchanges highlight the myriad ways that MDN influences both aquatic and terrestrial habitats across the PNW (Wipfli and Baxter 2010).

Ecological Considerations

The response of aquatic and linked terrestrial riparian ecosystems to nutrient enhancement via SCA appears to have both short and long-term (i.e., legacy) effects. Guyette et al. (2014) found that longer lived macroinvertebrates (e.g. *Pteronarcys*), as well as age one Atlantic Salmon were ^{13}C and ^{15}N enriched almost one year after SCA additions. These findings are similar to our study with respect to detections of elevated $\delta^{15}\text{N}$ in salmonid fish and riparian vegetation (i.e., willow) samples collected ~1 year after 2 years of SCA additions. Regardless of how long or short-term the ecosystem response to SCA additions may be, it is clear that MDN is assimilated into multiple trophic levels and likely not only increases the abundance and biomass of stream biota (Kohler et al. 2008; 2012), but also provides high-quality prey items for threatened and endangered salmonids at a time when growth and lipid storage is crucial for overwinter survival (Bilby et al. 1996; Kiffney et al. 2014). Despite these conclusions, we caution that nutrient subsidies provided to habitats with non-native species have the potential to exacerbate negative interactions with target organisms (e.g., ESA listed species), and thus undermine otherwise well-intentioned recovery efforts.

The ecosystems of salmon bearing catchments in the PNW evolved over long periods of time with abundant annual returns of wild salmon and other anadromous fishes. These pulsed, annual returns modified physical habitats and influenced ecological processes (Moore et al. 2004), while simultaneously delivering substantial amounts of organic material and nutrients from the marine environment to inland aquatic and terrestrial habitats (Wipfli and Baxter 2010). Supplementing

aquatic systems with inanimate SCA or carcasses cannot replace the important ecological functions provided by anadromous fishes. For example, live spawning salmon have profound influences on many physical and functional aspects of aquatic habitats during redd construction and associated spawning activities (Moore et al. 2004). In this context, nutrient mitigation efforts should not be thought of as a replacement for MDN delivered by naturally spawning fishes. However, highly depressed or extirpated returns of Pacific salmon, steelhead trout, and Pacific Lamprey in the contemporary setting may necessitate various nutrient enrichment strategies as an interim management tool to provide a mechanism to stimulate freshwater productivity and potentially increase the carrying capacity of oligotrophic systems until meaningful recovery efforts are achieved (Anders and Ashley 2007). For example, in the Elwa River, Tonra et al. (2015) found that shortly after the removal of the Elwa Dam, salmon recolonized formerly obstructed spawning habitat and delivered MDN subsidies to upstream freshwater and associated terrestrial habitats. Findings from this study suggest the American dipper (*Cinclus mexicanus*) acquired 10%-36% of their N from MDN following dam removal. Restoring connectivity to the migration corridors of anadromous fishes can have profound cascading effects on recipient ecosystems where pulsed MDN subsidies have the potential to stimulate productivity and facilitate the reciprocal exchange of nutrients from linked marine, aquatic, and terrestrial ecosystems (Wipfli and Baxter 2010). Elwa River dam removal demonstrates that when given the opportunity, Pacific salmon can quickly recolonize habitats and help to restore natural ecological processes.

Given the duration of diminishing salmon returns and thus the associated reduction/loss of MDN in inherently nutrient poor systems over the last century, the long-term and potentially synergistic effects of nutrient enhancement cannot be understood through short-term studies. We suggest that future studies examine the potential for legacy effects and better determine the consequences, some of which may be unintended, that sustained MDN subsidies might have on recipient ecosystems. Successful efforts to improve habitat conditions and associated fish production will ultimately depend upon restoring the form and function of high quality habitats that support abundant anadromous fish populations across the PNW.

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682 **Fig. 1** Study area map showing six experimental study streams in the upper Salmon River Basin
 683 (Elk Creek, Basin Creek, Panther Creek, Musgrove Creek, and Moyer Creek) and Middle Fork
 684 Salmon River Basin (Cape Horn Creek). Circles represent the 6 km study segments of each
 685 study stream.

Fig. 2 Diagram of the study design illustrating segment level sampling locations in control streams (no SCA) and treatment streams (low SCA and high SCA). Stable isotope samples were collected from randomly chosen riffle habitat and riparian zone sites within each: upper, middle, and lower stratum in the 3 km upstream and 3 km downstream segments.

Fig. 3 Global magnitude of change (treatment – control) mean values (± 1 SE) for: $\delta^{13}\text{C}$ (a and b); and $\delta^{15}\text{N}$ (c and d) stable isotopes relative to treatment levels for biofilm (a and c) and macroinvertebrates (b and d) for periods after SCA addition. Open circles represent control streams (no SCA), light shaded circles represent low SCA treatment streams, and dark shaded circles represent high SCA treatment streams. Dashed lines represent periods before SCA addition and solid lines represent periods after SCA addition.

Fig. 4 Magnitude of change (treatment – control) mean values (± 1 SE) by sampling period for: biofilm (*a* and *c*) and macroinvertebrate (*b* and *d*) $\delta^{13}\text{C}$ (*a* and *b*) and $\delta^{15}\text{N}$ (*c* and *d*) stable isotope signatures. Open circles represent control streams (no SCA), light shaded circles represent low SCA treatment streams, and dark shaded circles represent high SCA treatment streams.

Fig. 5 Global stable isotope biplots for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ mean values (± 1 SE) for all trophic levels evaluated for 3 different SCA treatment levels: no SCA (a and b), low SCA (c and d), and high SCA streams (e and f). Trophic level codes are: B, biofilm; M, macroinvertebrate; F, fish; SCA, salmon carcass analog; and W, willow. On the left side are data from before SCA additions (a, c, and e) and on the right side are data from after SCA additions (b, d, and f). Open circles

represent control segments and closed circles represent treatment segments. Although closed circles are used in biplots representing control streams, those stream segments did not receive SCA additions and the use of closed circles are out of convention to distinguish between upstream and downstream segments.

Fig. 6 Biplots for salmonid by species $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ mean values (± 1 SE) from: Cape Horn Creek (a and b) and Panther Creek (c and d), both high SCA streams. This period represents data collected in 2010 before SCA additions and in 2012 after SCA additions. Salmonid species codes are: BRK, Brook Trout (*Salvelinus fontinalis*); CH, Chinook Salmon (*Oncorhynchus tshawytscha*); BT, Bull Trout (*Salvelinus confluentus*); and CT, Westslope Cutthroat Trout (*Oncorhynchus clarki lewisi*). On the left side are data from before SCA additions (a and c) and

on the right side are data from after SCA additions (*b* and *d*). Open circles represent control segments and closed circles represent treatment segments.

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Fig. 7 Biplots for willow (*Salix* spp.) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ mean values (± 1 SE). This period represents samples collected in 2012, ~2 weeks after the addition of SCA in low SCA streams and ~1 year after the cessation of treatments in high SCA streams. Open circles represent upstream control segments and closed circles represent downstream treatment segments. Although closed circles are used in biplots representing control streams, those stream segments did not receive SCA additions and the use of closed circles are out of convention to distinguish between upstream and downstream segments.

Fig. 8 Proportional dietary sources for: macroinvertebrate (*a* and *b*) and fish (*c* and *d*) after SCA additions. Black bars represent the SCA dietary source, dark grey bars represent the biofilm dietary source, and light grey bars represent the riparian vegetation and macroinvertebrate dietary sources. The left side is low SCA streams (*a* and *c*) and the right side is high SCA streams (*b* and *d*).

Table 1. Analysis of variance (ANOVA) model statements and results for effects of salmon carcass analog (SCA) treatment level (no SCA, low SCA, and high SCA: *ANOVA_Model_TL*) and interactions on biofilm and macroinvertebrate (Macro) nitrogen (N) and carbon (C) stable isotope variables. *ANOVA_Model_TL <- lmer (response_var ~ Treatment_Level + Period + (I|Stream) + (I|Year) + Treatment_Level:Period, data = Biofilm or Macro, REML = TRUE)*.

Trophic Level	Dependent variable	Effect	Num. d.f.	Den. d.f.	F-value	Pr-(>F)	Benjamini-Hochberg critical value
Biofilm	$\delta^{13}\text{C}$	TL	2	3.069	1.939	0.285	
		BA	1	26.092	4.458	0.044	
		TL x BA	2	26.091	3.133	0.060	0.075
Biofilm	$\delta^{15}\text{N}$	TL	2	2.882	1.809	0.310	
		BA	1	23.788	17.224	<0.001	
		TL x BA	2	24.183	3.856	0.035	0.050
Macro	$\delta^{13}\text{C}$	TL	2	3.162	1.242	0.400	
		BA	1	25.085	3.819	0.062	
		TL x BA	2	25.095	2.706	0.086	0.100
Macro*	$\delta^{15}\text{N}$	TL	2	28.786	19.059	<0.001	
		BA	1	28.810	5.082	0.032	
		TL x BA	1	28.786	7.428	0.003	0.025

Bold indicates statistically significant differences using a 10% FDR and a Benjamini-Hochberg (1995) procedure to determine critical values for the TL by BA interaction term. *P* values less than the corresponding Benjamini-Hochberg critical value are considered significant. All variables met assumptions of normality and homogeneity of variance (HOV). *Macro $\delta^{15}\text{N}$ was log (plus a constant) transformed prior to analyses.

Table 2. Percent MDN assimilation of biofilm, macroinvertebrate, and fish.

Loading	Trophic Level	$\delta^{15}\text{N}$ % MDN	SE	$\delta^{13}\text{C}$ % MDN	SE
Low	Biofilm	22	4.6	7	0.6
Low	Macroinvertebrate	17	4.7	15	6.6
Low	Fish	3	2.9	5	2.9
High	Biofilm	42	15.0	30	19.6
High	Macroinvertebrate	28	5.4	25	6.0
High	Fish	14	4.1	17	15.8

Table 3. Proportional dietary sources (mean (SD)) for macroinvertebrate and fish consumers following SCA additions.

Loading	Trophic Level	SCA_source	Rip Veg_source	Biofilm_source	Macro_source
Low	Macro	0.10 (0.07)	0.49 (0.11)	0.41 (0.17)	NA
Low	Fish	0.02 (0.01)	NA	NA	0.98 (0.01)
High	Macro	0.05 (0.05)	0.65 (0.11)	0.30 (0.12)	NA
High	Fish	0.02 (0.01)	NA	NA	0.98 (0.01)

*Trophic level denotes target organism and source denotes dietary sources where SCA is salmon carcass analog, Rip Veg is riparian vegetation, Biofilm is benthic biofilm, Macro is benthic macroinvertebrate, and NA is not applicable.

1 Appendix A

2 **Table A1.** Mean $\delta^{13}\text{C}$ (‰) values, range, and variance estimates ($\pm 1\text{SD}$) by year and period
 3 (before and after) for all study streams analyzed by loading quantity. Loading is the SCA
 4 quantity; trophic is the trophic level of biological samples; upstream is the control segment of
 5 each stream and downstream is the treatment segment of each stream. Streams that received no
 6 loading are control streams that did not receive SCA additions.

Loading	Trophic	Year	Period	Upstream		Downstream	
				$\delta^{13}\text{C}$ mean and range	SD	$\delta^{13}\text{C}$ mean and range	SD
No	Biofilm	2010	Before	-28.61 ((-29.36) – (-27.87))	1.05	-27.81 ((-28.90) – (-26.72))	1.05
No	Biofilm	2010	After	-29.98 ((-31.12) – (-28.84))	1.61	-28.79 ((-28.80) – (-28.77))	1.61
No	Biofilm	2011	Before	-30.19 ((-30.85) – (-29.54))	0.93	-29.44 ((-29.51) – (-29.37))	0.93
No	Biofilm	2011	After	-31.07		-29.39 ((-29.70) – (-29.08))	0.43
No	Biofilm	2012	Before	-30.81 ((-30.86) – (-30.76))	0.07	-29.26 ((-29.56) – (-28.97))	0.07
No	Biofilm	2012	After	-30.12 ((-31.07) – (-29.49))	1.11	-26.69 ((-27.73) – (-25.65))	1.48
Low	Biofilm	2010	Before	-26.95 ((-28.66) – (-27.39))	0.90	-28.02 ((-27.64) – (-26.26))	0.90
Low	Biofilm	2010	After	-25.65 ((-27.41) – (-25.51))	1.34	-26.46 ((-26.68) – (-24.63))	1.34
Low	Biofilm	2011	Before	-26.50 ((-26.51) – (-26.50))	0.00	-25.57 ((-26.07) – (-25.06))	0.00
Low	Biofilm	2011	After	-28.16 ((-27.88) – (-25.01))	2.03	-25.95 ((-28.38) – (-26.89))	1.05
Low	Biofilm	2012	Before	-26.92 ((-29.62) – (-24.21))	3.83	-28.23 ((-28.25) – (-28.21))	3.83
Low	Biofilm	2012	After	-23.53 ((-25.59) – (-21.46))	2.92	-24.65 ((-25.44) – (-23.87))	1.11
Low	Biofilm	2013	Before	-24.49 ((-27.01) – (-21.57))	2.08	-24.99 ((-27.06) – (-23.89))	1.23
High	Biofilm	2010	Before	-27.90 ((-29.02) – (-26.78))	1.58	-28.86 ((-29.49) – (-28.23))	1.58
High	Biofilm	2010	After	-30.28 ((-30.38) – (-30.19))	0.13	-25.77 ((-29.63) – (-21.90))	0.13
High	Biofilm	2011	Before	-28.14 ((-28.68) – (-27.61))	0.76	-28.17 ((-28.56) – (-27.78))	0.76
High	Biofilm	2011	After	-30.39 ((-31.70) – (-29.09))	1.85	-28.75 ((-30.05) – (-27.44))	1.84
High	Biofilm	2012	After	-29.88 ((-30.38) – (-29.37))	0.71	-29.47 ((-29.94) – (-29.00))	0.71
No	Macro	2010	Before	-30.10 ((-31.60) – (-28.60))	2.12	-29.28 ((-30.36) – (-28.21))	1.52
No	Macro	2010	After	-30.67 ((-30.91) – (-30.54))	0.19	-29.93 ((-30.63) – (-29.22))	0.99
No	Macro	2011	Before	-30.35 ((-33.53) – (-27.17))	4.50	-28.34 ((-30.75) – (-25.92))	3.41
No	Macro	2011	After	-31.24 ((-31.60) – (-30.88))	0.51	-29.11 ((-30.15) – (-28.08))	1.47
No	Macro	2012	Before	-31.47 ((-32.00) – (-30.95))	0.74	-30.02 ((-30.88) – (-29.16))	1.22
No	Macro	2012	After	-31.47 ((-31.50) – (-31.44))	0.05	-29.77 ((-30.52) – (-29.01))	1.07
Low	Macro	2010	Before	-28.63 ((-30.30) – (-26.97))	2.35	-27.74 ((-28.44) – (-27.04))	0.99
Low	Macro	2010	After	-27.53 ((-28.68) – (-26.38))	1.62	-26.67 ((-27.21) – (-26.13))	0.76
Low	Macro	2011	Before	-30.43 ((-31.88) – (-28.98))	2.05	-27.84 ((-30.16) – (-25.52))	3.28
Low	Macro	2011	After	-27.00 ((-27.16) – (-26.83))	0.23	-25.44 ((-26.86) – (-24.02))	2.01
Low	Macro	2012	Before	-27.87 ((-29.79) – (-25.94))	2.72	-28.00 ((-29.13) – (-26.88))	1.59
Low	Macro	2012	After	-26.82 ((-28.60) – (-25.04))	2.52	-25.47 ((-27.06) – (-23.89))	2.24
Low	Macro	2013	Before	-25.33 ((-27.32) – (-22.28))	0.90	-24.97 ((-25.62) – (-23.86))	0.68
High	Macro	2010	Before	-32.28 ((-33.06) – (-31.49))	1.11	-31.69 ((-31.82) – (-31.56))	0.18
High	Macro	2010	After	-31.24 ((-31.35) – (-31.14))	0.15	-28.91 ((-29.67) – (-27.50))	0.96
High	Macro	2011	Before	-33.30 ((-33.98) – (-32.61))	0.97	-30.18 ((-30.45) – (-29.90))	0.39
High	Macro	2011	After	-30.92 ((-31.10) – (-30.75))	0.25	-27.34 ((-27.56) – (-27.11))	0.32
High	Macro	2012	After	-33.02 ((-33.73) – (-32.30))	1.01	-32.05 ((-33.05) – (-31.04))	1.42
No	Fish	2012	After	-27.65 ((-27.75) – (-27.54))	0.15	-26.44 ((-27.01) – (-25.87))	0.81
Low	Fish	2010	Before	-24.58 ((-25.04) – (-24.12))	0.65	-24.39 ((-24.52) – (-24.27))	0.18

Low	Fish	2012	After	-24.96 ((-26.02) – (-23.90))	1.50	-24.53 ((-25.31) – (-23.75))	1.11
High	Fish	2010	Before	-28.22 ((-28.40) – (-28.04))	0.26	-27.28 ((-28.18) – (-26.38))	1.27
High	Fish	2012	After	-28.56 ((-28.67) – (-28.45))	0.15	-26.65 ((-28.38) – (-24.93))	2.44
No	Willow	2012	After	-28.31 ((-29.39) – (-27.10))	1.01	-28.33 ((-29.05) – (-27.43))	0.58
Low	Willow	2012	After	-27.67 ((-28.56) – (-26.76))	0.68	-28.65 ((-30.98) – (-25.76))	1.79
High	Willow	2012	After	-28.86 ((-30.11) – (-28.18))	0.67	-28.95 ((-30.28) – (-27.63))	0.67

7

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Table A2. Mean $\delta^{15}\text{N}$ (‰) values, range, and variance estimates ($\pm 1\text{SD}$) by year and period (before and after) for all study streams analyzed by loading quantity. Loading is the SCA quantity; trophic is the trophic level of biological samples; upstream is the control segment of each stream and downstream is the treatment segment of each stream. Streams that received no loading are control streams that did not receive SCA additions.

Loading	Trophic	Year	Period	Upstream		Downstream	
				$\delta^{15}\text{N}$ mean and range	SD	$\delta^{15}\text{N}$ mean and range	SD
No	Biofilm	2010	Before	3.42 (3.17 – 3.68)	0.36	2.82 (2.64 – 2.99)	0.25
No	Biofilm	2010	After	2.11 (0.57 – 3.65)	2.18	2.70 (2.16 – 3.61)	1.02
No	Biofilm	2011	Before	2.92 (2.05 – 3.80)	1.24	2.56 (2.05 – 3.07)	0.72
No	Biofilm	2011	After	4.83		3.90 (2.02 – 5.79)	2.67
No	Biofilm	2012	Before	1.27 (-0.64 – 3.17)	2.70	2.64 (0.60 – 4.67)	2.88
No	Biofilm	2012	After	2.35 (0.83 – 3.36)	1.79	1.94 (1.01 – 2.87)	1.31
Low	Biofilm	2010	Before	4.79 (3.79 – 4.49)	0.49	4.14 (3.88 – 5.69)	1.28
Low	Biofilm	2010	After	3.85 (0.69 – 3.32)	1.86	2.01 (3.19 – 4.51)	0.93
Low	Biofilm	2011	Before	4.02 (3.84 – 4.20)	0.25	4.42 (4.21 – 4.63)	0.29
Low	Biofilm	2011	After	3.05 (2.66 – 4.27)	1.13	4.29 (2.13 – 5.92)	2.68
Low	Biofilm	2012	Before	2.07 (1.99 – 2.14)	0.10	3.37 (3.34 – 3.39)	0.04
Low	Biofilm	2012	After	1.49 (0.50 – 2.49)	1.41	4.48 (3.30 – 5.67)	1.67
Low	Biofilm	2013	Before	3.28 (1.83 – 5.28)	1.41	2.80 (2.09 – 3.31)	0.42
High	Biofilm	2010	Before	2.76 (1.26 – 4.26)	2.13	4.57 (3.89 – 5.25)	0.96
High	Biofilm	2010	After	2.29 (1.19 – 3.38)	1.55	5.08 (4.98 – 5.19)	0.15
High	Biofilm	2011	Before	4.19 (4.01 – 4.38)	0.26	3.99 (3.69 – 4.29)	0.43
High	Biofilm	2011	After	3.41 (3.34 – 3.47)	0.09	7.27 (5.65 – 8.89)	2.29
High	Biofilm	2012	Before	2.99 (2.33 – 3.65)	0.94	2.75 (2.53 – 2.97)	0.31
No	Macro	2010	Before	4.11 (3.23 – 4.99)	1.25	3.94 (3.39 – 4.71)	0.77
No	Macro	2010	After	3.84 (2.48 – 5.19)	1.92	3.97 (2.54 – 5.40)	2.02
No	Macro	2011	Before	3.85 (2.34 – 5.36)	2.14	4.26 (3.11 – 5.71)	1.63
No	Macro	2011	After	4.31 (2.82 – 5.79)	2.10	4.22 (2.95 – 5.48)	1.79
No	Macro	2012	Before	4.36 (3.95 – 4.76)	0.57	4.61 (3.90 – 5.75)	1.01
No	Macro	2012	After	4.50 (3.32 – 5.67)	1.66	3.46 (2.56 – 4.37)	1.28
Low	Macro	2010	Before	4.60 (3.57 – 5.63)	1.46	5.01 (3.89 – 6.32)	1.58
Low	Macro	2010	After	3.91 (2.14 – 5.68)	2.50	5.61 (4.56 – 6.66)	1.49
Low	Macro	2011	Before	4.22 (3.55 – 4.89)	0.95	5.03 (3.64 – 7.05)	1.97
Low	Macro	2011	After	4.67 (3.25 – 6.08)	2.00	6.31 (5.84 – 6.78)	0.66
Low	Macro	2012	Before	5.34 (4.34 – 6.33)	1.41	5.51 (4.56 – 7.62)	1.34
Low	Macro	2012	After	4.01 (2.12 – 5.90)	2.67	6.61 (5.50 – 7.72)	1.57
Low	Macro	2013	Before	4.98 (3.96 – 6.06)	0.90	5.46 (3.94 – 6.69)	1.14
High	Macro	2010	Before	3.34 (3.07 – 3.60)	0.38	3.46 (3.31 – 3.89)	0.21
High	Macro	2010	After	3.88 (3.35 – 4.42)	0.75	5.84 (5.27 – 6.41)	0.80
High	Macro	2011	Before	3.64 (3.14 – 4.14)	0.71	5.36 (5.15 – 6.63)	0.30
High	Macro	2011	After	3.64 (3.44 – 3.83)	0.28	8.21 (8.10 – 8.32)	0.16
High	Macro	2012	Before	3.97 (3.78 – 4.17)	0.27	5.26 (4.81 – 6.00)	0.64
No	Fish	2012	After	7.58 (6.50 – 8.66)	1.53	7.80 (7.13 – 8.47)	0.94
Low	Fish	2010	Before	8.77 (8.46 – 9.09)	0.44	9.06 (8.76 – 9.37)	0.43
Low	Fish	2012	After	8.46 (7.54 – 9.39)	1.31	8.70 (8.18 – 9.23)	0.74
High	Fish	2010	Before	7.86 (7.58 – 8.14)	0.39	8.00 (7.62 – 8.39)	0.55

High	Fish	2012	After	7.79 (7.69 – 7.90)	0.15	9.54 (8.95 – 10.12)	0.83
No	Willow	2012	After	-0.41 ((-2.22) – 1.12)	1.48	0.08 ((-1.03) – 1.65)	1.11
Low	Willow	2012	After	0.23 ((-2.52) – 1.62)	1.50	0.87 ((-0.92) – 2.28)	1.15
High	Willow	2012	After	-0.47 ((-1.50) – 0.19)	0.62	1.96 (0.05 – 3.95)	1.35

13

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