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## Nutrient additions to mitigate for loss of Pacific salmon: consequences for stream biofilm and nutrient dynamics

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**Abstract.** Mitigation activities designed to supplement nutrient and organic matter inputs to streams experiencing decline or loss of Pacific salmon typically presuppose that an important pathway by which salmon nutrients are moved to fish (anadromous and/or resident) is via nutrient incorporation by biofilms and subsequent bottom-up stimulation of biofilm production, which is nutrient-limited in many ecosystems where salmon returns have declined. Our objective was to quantify the magnitude of nutrient incorporation and biofilm dynamics that underpin this indirect pathway in response to experimental additions of salmon carcasses and pelletized fish meal (a.k.a., salmon carcass analogs) to 500-m reaches of central Idaho streams over three years. Biofilm standing crops increased 2–8-fold and incorporated marine-derived nutrients (measured using <sup>15</sup>N and <sup>13</sup>C) in the month following treatment, but these responses did not persist year-to-year. Biofilms were nitrogen (N) limited before treatments, and remained N limited in analog, but not carcass-treated reaches. Despite these biofilm responses, in the month following treatment total N load was equal to 33–47% of the N added to the treated reaches, and N spiraling measurements suggested that as much as 20%, but more likely 2–3% of added N was taken up by microbes. Design of biologically and cost-effective strategies for nutrient addition will require understanding the rates at which stream microbes take up nutrients and the downstream distance traveled by exported nutrients.

**Key words:** biofilm; Idaho; mitigation; nutrient limitation; nutrient uptake; *Oncorhynchus* spp.; Pacific salmon; stable isotopes.

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### INTRODUCTION

Transfers of organisms, energy, nutrients and organic matter that cross ecosystem boundaries, termed subsidies, can have broad consequences for recipient ecosystems and food webs (Polis et al. 2004, Baxter et al. 2005, Marcarelli et al. 2011). Fish movement and migrations are an important mechanism for subsidy delivery and disturbance in aquatic ecosystems (Flecker et al. 2010), and an

archetypal example is the migration of Pacific salmon (*Oncorhynchus* spp.). Because of their anadromous life history, Pacific salmon are vectors of nutrient and energy subsidies from marine to freshwater-riparian ecosystems (Naiman et al. 2002). Returning adult salmon release organic and inorganic nutrients via excretion and metabolic wastes, spawning, redd building, and carcass decomposition (Moore et al. 2007, Tiegs et al. 2011). Some of these nutrients are assimilated

lated by stream autotrophs (algae and cyanobacteria; Johnston et al. 2004, Chaloner et al. 2007, Verspoor et al. 2010), may stimulate rates of primary production and microbial respiration (Holtgrieve and Schindler 2011, Rüegg et al. 2011, Levi et al. 2013), and may support secondary consumers such as aquatic invertebrates (Wipfli et al. 1998, Lessard et al. 2009) and fishes, including juvenile salmon (Bilby et al. 1996, Larkin and Slaney 1997).

In regions of the Pacific Northwest, runs of Pacific salmon have dramatically declined over the past century due to a combination of threats from harvest, hydroelectric dam construction and operation, habitat degradation, and hatchery operations (Lichatowich 1999). The decline of Pacific salmon has fueled concern about declining ecosystem productivity, termed oligotrophication, following loss of material and nutrient subsidies (Stockner et al. 2000). For example, in the headwaters of the Snake and Salmon Rivers in central Idaho, dramatic declines in salmon returns (McClure et al. 2003) and continued outmigration of juvenile fish (Scheuerell et al. 2005, Kohler et al. 2013), coupled with extremely low geologic, atmospheric, and anthropogenic nutrient sources, have been linked to very low background nutrient concentrations and consistent nutrient limitation of stream biofilms (Thomas et al. 2003, Marcarelli and Wurtsbaugh 2007, Sanderson et al. 2009). There is concern that reduced nutrient returns have led to decreases in primary and secondary production, in turn causing bottom-up limitation of juvenile salmon production. This oligotrophication has been hypothesized to lead to a continued downward trend in salmon populations (Stockner et al. 2000, Achord et al. 2003). In this region and others, nutrient additions to mitigate for and restore natural salmon runs have been widely considered and implemented (Hyatt et al. 2004, Compton et al. 2006).

The practice of nutrient mitigation typically presupposes that an important pathway by which salmon nutrients are transferred to fish (anadromous and/or resident) is via bottom-up stimulation of biofilm production, which is nutrient-limited in many ecosystems where salmon returns have declined (Thomas et al. 2003, Sanderson et al. 2009). Subsequently, it is generally expected that an increase in biofilm

production or standing crop would lead to bottom-up stimulation of invertebrate production, and ultimately increase food availability for juvenile fish (Claeson et al. 2006). Indeed, studies have demonstrated that additions of salmon carcasses or other fish-derived materials for mitigation can increase standing crops of biofilms and invertebrates (Claeson et al. 2006, Kohler et al. 2012) and growth, production and body condition of resident salmonids (Wipfli et al. 2004, Kohler et al. 2012). Yet, in many of these experiments, it is unclear whether increases in fish and invertebrate production were due to direct (consumption of mitigation material) or indirect (nutrient stimulation of biofilm) pathways (Kiernan et al. 2010). Other studies have demonstrated limited responses of biofilm standing crop or productivity in response to both naturally spawning salmon and additions of salmon materials for mitigation (Ambrose et al. 2004, Janetski et al. 2009), raising questions regarding the responsiveness and efficiency of the biofilm pathway for nutrient mitigation.

The extent to which nutrient uptake and subsequent production by stream microbes (i.e., algae, bacteria and fungi), including those that comprise biofilms, respond to salmon nutrient mitigation practices is rarely studied, but has important implications for understanding ecological responses to mitigation and for planning better mitigation programs. Although influential whole-stream nutrient addition experiments have indicated that microbes have the capacity to take up excess nutrients and increase their standing crop or production (Peterson et al. 1993, Suberkropp et al. 2010), at some point this capacity may become saturated or lose efficiency (Earl et al. 2006, O'Brien et al. 2007). Microbial production may only increase until some other top-down or bottom-up factor, such as light, grazing or disturbance becomes limiting or constraining (Hill 1996, Peterson 1996, Steinman 1996), as others have observed in response to both naturally spawning salmon and additions of salmon carcasses to streams (Ambrose et al. 2004, Janetski et al. 2009). Nutrients added in excess of microbial uptake capacity may be transported to reaches that are not a target for mitigation, wasting valuable mitigation dollars. Direct measurements of nutrient uptake and limitation have been proposed to improve planning of stream

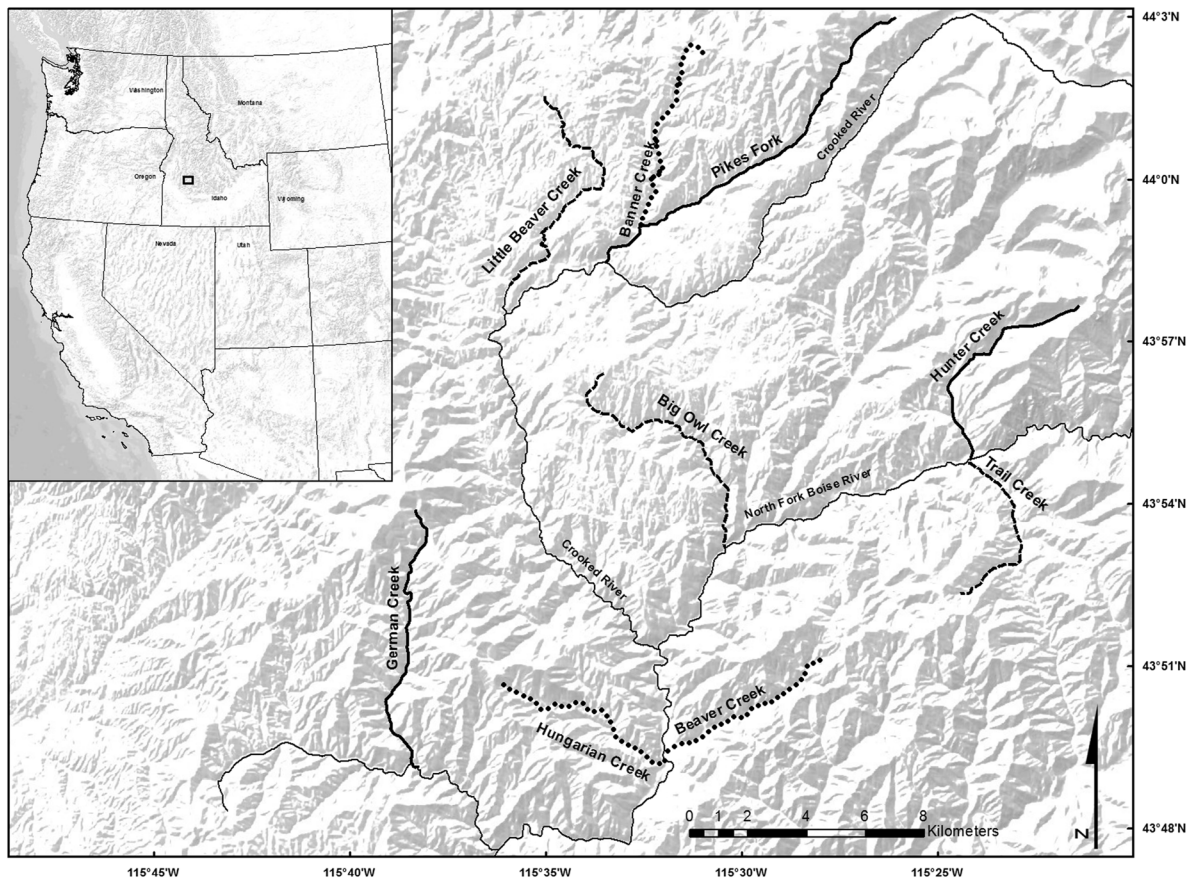


Fig. 1. Map of the experimental streams and study area in the North Fork Boise River Basin. Dotted lines indicate control streams, bold solid lines indicate analog-treated streams, and dashed lines indicate carcass-treated streams. Inset shows the location of the study area in the western United States.

nutrient mitigation measures (Thomas et al. 2003), but have been rarely utilized in this context.

Here, we describe results from a three-year study examining stream biofilm and nutrient responses to nutrient additions in central Idaho streams, where salmon returns have been eliminated for over a century. We conducted experimental additions of two different forms of salmon materials typically used for mitigation (pasteurized salmon carcasses and pelletized fish meal known as salmon carcass analog), and monitored short (weeks to months) and long (annual)-term responses of stream biofilms in terms of standing crop, stable isotope composition and nutrient limitation. In addition, we quantified nutrient loads and whole-stream nutrient uptake to estimate ecosystem-level

responses to nutrient additions. The ultimate goal of this study was to understand the extent to which biofilms may incorporate nutrients from treatment materials into stream food webs and ecosystems, and how much of the nutrients added for mitigation may be transported out of target mitigation reaches following treatment.

## METHODS

### Study area

This study was conducted in nine first- to third-order streams located in the North Fork Boise River drainage in central Idaho (Fig. 1, Table 1). This 980 km<sup>2</sup> drainage is a tributary of the Snake River, ranges in elevation from 1060 to 2990 m a.s.l., and is entirely contained within the Boise National Forest. The drainage is located on



Table 1. Characteristics of the streams included in this study.

Treatment	Stream	Drainage area (km <sup>2</sup> )†	Median particle size (mm)‡	Q (L/s)§	Volume in pools (%)¶	Volume in riffles and runs (%)¶	Large woody debris abundance (no./100 m)
Control	Banner	23	30	66.4	47	53	0.0
	Beaver	15	45	32.7	0	100	8.7
	Hungarian	11	15	52.4	5	95	55.3
Carcass	Big Owl	18	20	39.5	22	78	2.9
	Little Beaver	6	10	26.9	35	65	8.0
	Trail	20	75	66.5	8	92	17.2
Analog	German	23	55	109.8	20	80	6.8
	Hunter	16	60	60.4	12	88	0.0
	Pikes Fork	28	30	61.2	22	78	19.5

Note: Methods described in detail in Collins (2014).

† Area above the downstream end of the study reaches, determined using USGS StreamStats, <http://water.usgs.gov/osw/streamstats/>

‡ Estimated from 100 point counts in the downstream 100 m of the study reaches.

§ Q = Stream discharge, averaged across all measurements in the stream between 2008 and 2011.

¶ Riffle and run volume determined in the downstream 100 m of the study reach in association with annual electrofishing surveys.

the Idaho Batholith, a large geologic formation in central Idaho comprised primarily of granites, resulting in very low inputs of geologic nutrients. This region also experiences some of the lowest atmospheric nutrient deposition rates in the United States (NADP 2012; <http://nadp.sws.uiuc.edu/nadp/useConditions.aspx>), resulting in nutrient-poor, low conductivity, poorly buffered surface water. The annual hydrograph of the North Fork Boise River is dominated by a spring snowmelt pulse peaking in late May, followed by a prolonged baseflow period beginning in mid-late July. Although anadromous fish including spring Chinook salmon (*O. tshawytscha*) and steelhead (*O. mykiss*) were historically abundant in this tributary of the Snake River (NWPCC 2004), anadromous fish runs have been eliminated for over a century by the construction of three dams between 1906 and 1915. Oligotrophication is a concern in this drainage because of potential effects on resident, native redband trout (*O. mykiss*), migratory populations of threatened bull trout (*Salvelinus confluentus*), and a suite of terrestrial wildlife that may be affected, directly and indirectly, by aquatic-derived productivity. Hillslope vegetation consists of mixed conifer forests, some areas of which experienced mixed severity fire in 1994 (Dunham et al. 2007). Riparian zones are dominated by willows (*Salix* spp.), red-osier dogwood (*Cornus sericea*), and tall grasses (predominantly *Festuca* spp.); although alder (*Alnus* sp.) is commonly found along some streams in the region, we selected stream reaches

without dense accumulations of alder to facilitate the use of isotopes to detect nutrient transfers. Wetlands are rare in these watersheds, but one stream (Banner Creek) was the site of significant beaver (*Castor canadensis*) activity that increased over the duration of the study. Anthropogenic land use impacts in the drainage are limited, but include grazing, dispersed recreation, and legacy mining effects.

### Experimental design

We included two salmon materials commonly used for nutrient mitigation in our experiment: pasteurized salmon carcasses and salmon carcass analog. We chose these two materials because they are the most realistic mimics of material delivered by naturally spawning salmon including carbon (C), nitrogen (N), phosphorus (P), trace metals and other micronutrients, and because they are being used for nutrient mitigation projects elsewhere in the Columbia River basin (e.g., Compton et al. 2006, Kohler et al. 2012).

Steelhead and Chinook salmon carcasses were obtained from Dworshak and Rapid River fish hatcheries. Because of concerns that transporting salmon carcasses for mitigation may facilitate the spread of fish disease (Compton et al. 2006), all salmon carcasses were frozen for storage then pasteurized by heating until the internal head temperature reached 60°C for 20 minutes. The freezing and heating was implemented to kill a suite of fish pathogens, particularly infectious

hematopoietic necrosis (IHN) virus and whirling disease (*Myxobolus cerebralis*; Noga 2000), and is required by the State of Idaho and US Fish and Wildlife Service for translocated carcasses; therefore this treatment was representative of techniques used in nutrient mitigation projects. A terrestrial decomposition experiment showed that treated salmon carcasses lost more weight and consequently slightly more C and N during the first 24 hours compared to unpasteurized carcasses, but there were no long-term differences in nutrient form, nutrient loss, or decomposition rate between pasteurized and unpasteurized carcasses (Wheeler et al. 2014).

Because of the difficulty handling and transporting fish carcasses, salmon carcass analog (hereafter, termed ‘analog’) is an increasingly popular alternative used in nutrient mitigation projects in the Pacific Northwest, including central Idaho (Kohler et al. 2008, Ebel 2012, Kohler et al. 2012). Analog is manufactured from pasteurized fish meal so it is pathogen-free and contains nutrient content similar to salmon carcasses (Pearsons et al. 2007). Several studies have shown that analog is incorporated by stream producers and consumers (Wipfli et al. 2004, Kohler et al. 2012).

We selected 500-m-long reaches in nine different streams (Fig. 1) for inclusion in the study that had typical stream characteristics for the region (e.g., discharge, benthic substrate; Table 1). We assigned three treatments (salmon carcass, salmon carcass analog, untreated control) to the nine streams following a stratified random design; we classified streams into three groups according to slope and valley form characteristics, then randomly assigned the three treatments within each group to insure that each treatment group included streams with a range of physical characteristics. We based carcass application rates on a target of 0.5 salmon carcasses/m<sup>2</sup> of wetted stream channel, which was estimated to be at the high end of the reported historical data (IDFG 1985), and roughly a midpoint in the range over which stream biota respond the most to nutrient subsidies from salmon (Wipfli et al. 1998). We targeted analog treatment rates to match P application rate from the salmon carcass treatment at 5.5 g P/m<sup>2</sup>. Differences in N content of carcasses and analog resulted in an N application rate of 27 g N/m<sup>2</sup> to analog-treated

streams and 50 g N/m<sup>2</sup> to carcass-treated streams. Treatment materials were applied annually to the same reaches during the first week of August 2008–2010. The timing of treatment coincided with baseflow discharge and with early spawning by natural spring Chinook salmon in regional streams where migrations have not been blocked (Isaak and Thurow 2006). Carcasses were distributed haphazardly in the stream channel along the entire 500-m long study reach and were not staked down. Mesh fences installed in year 1 revealed that no carcasses were washed downstream in the week following treatment; therefore fences were not placed in following years. Analog pellets were placed in water-permeable bags and allowed to saturate within the stream before distributing along the stream bottom to minimize export in the water column. Both analog and carcass treatment materials were still visible up to 6-weeks following treatment application. Crews walked along untreated control streams to mimic the disturbance to treated streams during treatment deployment.

### Biofilm responses

*Standing crop.*—To monitor the responses of stream microbes to treatment materials, we sampled biofilm standing crop before, two weeks following and six weeks following treatment application in 2008, before and one month following treatment applications in 2009 and 2010, and one year following the final treatment application in 2011 (eight total sampling periods). Samples were collected at seven random locations within the downstream 250 m of each treatment reach. Biofilms were scrubbed with a small brush from three rocks at each location, combined into approximately 500 mL of water, and subsamples of the resultant slurry were filtered through pre-combusted 0.7 µm GF/F filters for analysis. Filters were placed on dry ice until frozen for storage. Rock area was determined by tracing the planar rock area onto paper, weighing the cutout and applying a paper weight-to-surface area regression (Bergey and Getty 2006). Standing crop of biofilms was estimated as chlorophyll *a* spectrophotometrically and ash-free dry mass (AFDM) via combustion at 550°C using standard methods (APHA 2005).

Benthic biofilm responses to treatment materi-

als across all years were analyzed using two-way, repeated measures analysis of variance (RMANOVA) with treatment and sampling period as fixed factors, stream as the repeated subject and chlorophyll *a* and AFDM as response variables. RMANOVA was selected because it accounts for both temporal autocorrelation and random differences among streams. For these and all following ANOVA analyses, response variables were log transformed when necessary to meet assumptions, differences among treatments were determined when appropriate using post-hoc Tukey tests for significant factors or interactions, and significance was considered at  $\alpha = 0.05$ . To evaluate year-to-year differences in benthic biofilm standing crop caused by treatments (e.g., annual increases or decreases prior to treatment application), we also compared chlorophyll *a* and AFDM responses from only the four pre-treatment periods in 2008–2011 using RMANOVA with treatment and sampling period as fixed factors and stream as the repeated subject. All statistical analyses were conducted using SAS 9.2 (SAS Institute, Cary, North Carolina, USA).

*Isotope composition.*—We analyzed biofilms for isotope composition of carbon and nitrogen after treatment application in all streams in 2008 and 2009, and before treatment application in 2009. Biofilm samples for isotope analysis were collected as described for standing crop. In the lab, filters were defrosted and dried at 60°C. Biofilm material was scraped from the filters, homogenized with a mortar and pestle, and 1.5 mg of material was weighed into aluminum tins and encapsulated for analysis. We also analyzed samples of treatment materials for comparison; because these materials were composed of marine-derived fish tissue they had a distinct isotopic signature compared to freshwater nutrient sources. Samples were analyzed for  $^{13}\text{C}$  and  $^{15}\text{N}$  on an Elemental Combustion System 4010 interfaced to a Delta V advantage mass spectrometer (Thermo Electron Corporation, Waltham, Massachusetts, USA) operated by the Center for Archaeology, Materials and Applied Spectroscopy at Idaho State University. Isotope values are expressed as stable isotope ratios of N and C as:

$$\delta^{15}\text{N} \text{ or } \delta^{13}\text{C}(\text{‰}) = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 100$$

where *R* is the ratio of heavy to light isotope

( $^{15}\text{N}/^{14}\text{N}$  or  $^{13}\text{C}/^{12}\text{C}$ ).  $R_{\text{standard}}$  was determined from accepted standards (PDB for C, atmospheric air for N). Responses of both  $^{15}\text{N}$  and  $^{13}\text{C}$  composition of biofilm were analyzed using a two-way multivariate analysis of variance (MANOVA) with treatment and sampling period as fixed factors.

*Nutrient limitation.*—Nutrient limitation of biofilms was determined following treatment application in 2008 using nutrient diffusing substrates (NDS; Tank et al. 2006, Marcarelli and Wurtsbaugh 2007). Plastic vials measuring 37-mL were filled with nutrient-enriched 2% agar and capped with a 2.6-cm diameter fritted glass disk (Leco, St. Joseph, Michigan, USA). Nutrients contained in the agar diffused out through the glass disk, which served as the substrate for biofilm attachment and growth. Four nutrient amendments (control with no added nutrients, N-enriched, P-enriched and N + P-enriched) were used; N and P were added in a 16:1 ratio as 0.8 mol N/L as  $\text{NaNO}_3$  and/or 0.05 mol P/L as  $\text{KH}_2\text{PO}_4$  (Marcarelli and Wurtsbaugh 2007). Six replicates of each treatment were randomly distributed on an aluminum rack and deployed immediately following the treatment application at the downstream end of the treatment reach. At the conclusion of a three-week incubation period NDS were collected, immediately placed into plastic bags, and transported on ice until frozen for storage. Standing crop of biofilms on the entire glass disks was estimated as chlorophyll *a*, followed by AFDM analysis. Previous analyses have demonstrated that accurate chlorophyll *a* and AFDM values can be obtained from the same samples using this approach (Davis et al. 2001).

Nutrient limitation responses were analyzed using three-way ANOVA with treatment, N amendment and P amendment as fixed factors, chlorophyll *a* or AFDM as response variables, and including stream as a random factor to account for among-stream variation in physical and chemical conditions. It is the convention for nutrient limitation bioassays to analyze N and P as independent factors to allow evaluation of interactions and possible co-limitation effects (Tank et al. 2006); evaluating interactions among the NDS nutrient amendments and the treatment materials allowed evaluation of whether treatment materials changed the nutrient limitation status of the biofilms (e.g., Mineau et al. 2011). To

graphically compare NDS responses among treatments, we calculated the response ratio of each treatment as  $\ln(\text{treatment/control})$ , such that control values equal zero, a stimulation response is positive, and a suppression response is negative (Tank et al. 2006).

#### *Streamwater nutrient responses*

*Nutrient concentrations and loads.*—Samples were collected at the downstream end of the treatment reaches in all study streams before and after treatment addition in 2008–2010 for analysis of total and dissolved nutrient loads [total N (TN), total dissolved N (TDN), nitrate-N, total P (TP), total dissolved P (TDP), and soluble reactive phosphate (SRP)]. Samples were collected on 5 dates in 2008 (immediately before and 1 day, 1 week, 2 weeks, and 6 weeks following), 3 dates in 2009 (immediately before and 1 day and 6 weeks following) and 3 dates in 2010 (immediately before, 2 days and 4 weeks following). Ammonium-N samples were collected at the same sites and dates in 2010 only. Water for dissolved nutrients was filtered streamside through a 0.45  $\mu\text{m}$  membrane filter; water was not filtered for total nutrient samples. Samples for all constituents except ammonium-N were placed on ice until frozen for storage. Ammonium-N concentrations were determined within 6 hours following collection using fluorometric analysis (Holmes et al. 1999; following modifications of Taylor et al. 2007) with an AquaFluor handheld fluorometer (Turner Designs, Sunnyvale, California, USA). All other constituents were analyzed using an Astoria-Pacific auto analyzer (Astoria-Pacific, Clackamas, Oregon, USA). A persulfate digestion was first applied to TDN, TDP, TN and TP samples to convert all N to nitrate-N and P to SRP (Valderama 1981). Nitrate-N (+nitrite-N) was analyzed via cadmium reduction (Nydahl 1976) and SRP via the ascorbic acid colorimetric method (APHA 2005).

To estimate the quantity of added nutrients that might have been transported in stream flow from the study streams, we calculated daily loads and also integrated across the month following treatment to estimate a total exported load. Daily loads were estimated by multiplying nutrient concentrations by discharge and scaling to 24 hours. Discharge was measured on or within a few days of water sample collection, provided

that flow conditions were similar, by establishing cross-sections where channel width, depth and velocity were measured at 10–15 intervals. Water velocities were measured using a Flo-mate flow meter (Hach/Marsh-McBirney, Frederick, Maryland, USA). Daily load responses were analyzed using RMANOVA with treatment and sampling period as fixed factors and stream as the repeated subject. To estimate how much of the nutrients added might have been exported during the post-treatment period within which we also measured biological responses, we calculated loads of total N and P and total dissolved N and P for the 28-day period following treatment application. We limited this analysis to 2008, when we had the most temporally detailed record of post-treatment water chemistry, and 28 days to match the duration of post-treatment response monitoring common across all study years. Discharge, TN, TP, TDN and TDP were interpolated between measurement dates. For each day, nutrient concentration was multiplied by discharge and scaled to a daily load; daily loads were then summed over the 28-day period and are reported as kg/month. Monthly nutrient loads were compared among treatments using one-way ANOVA.

In 2009 and 2010, we also conducted longitudinal nutrient sampling by collecting samples for ammonium-N, nitrate-N and SRP at 50–100 m intervals along a subset of study reaches in association with nutrient uptake measurements (*Whole-stream nutrient uptake* below; Table 2). Responses of nutrient concentrations along study reaches were analyzed using analysis of covariance (ANCOVA) with treatment as a fixed factor and distance along reach as a covariate.

*Whole-stream nutrient uptake.*—We conducted short-term additions of ammonium-N and nitrate-N to determine rates of whole-stream nutrient uptake via nutrient spiraling techniques (Webster and Valett 2006). Due to the time and sample-intensive nature of these measurements, we were unable to replicate them across all streams on all study dates, but we did compare uptake of ammonium-N and nitrate-N in six of the nine study streams post-treatment in 2009 to evaluate whether treatments altered DIN uptake rates in the short-term following treatments. We also performed additions of nitrate-N in a trio of representative streams (one in each treatment



Table 2. Results from short-term additions of ammonium-N and nitrate-N.

Nutrient	Date	Site	Treatment	Q (L/s)	Bkgd. conc. ( $\mu\text{g N/L}$ )	EF	$V_f$ (mm/s)	$U$ ( $\text{mg}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ )
Pre-treatment Nitrate-N	Jul 2009	Beaver	Control	39	56.1	1.8	0.021	4.26
		Hunter	Analog	99	3.2	6.1	0.013	0.16
		Trail	Carcass	143	14.8	1.8	0.107	5.69
	Jul 2010	Beaver	Control	44	82.8	1.8	0.011	3.39
		Hunter	Analog	78	4.9	8.0	0.028	0.50
		Trail	Carcass	104	12.2	3.6	0.042	1.83
Post-treatment Nitrate-N	Aug 2009	Beaver	Control	26	56.1	1.7	0.007	1.38
		Banner	Control	48	2.1	3.7	0.036	0.27
		Hunter	Analog	38	9.0	2.8	0.003	0.11
		Pikes Fork	Analog	66	3.0	2.1	0.017	0.19
		Trail	Carcass	71	11.9	1.5	...	...
		Little Beaver	Carcass	20	2.5	3.3	0.071	0.63
		Beaver	Control	26	4.0	1.7	0.064	0.91
		Banner	Control	48	3.1	2.5	0.060	0.66
		Hunter	Analog	38	19.6	1.5	0.011	0.79
	Aug 2009	Pikes Fork	Analog	66	17.1	1.3	0.083	5.07
		Trail	Carcass	71	24.9	1.3	...	...
		Little Beaver	Carcass	20	46.3	1.2	...	...

Notes: Q = stream discharge, measured on the day of the nutrient injection; Bkgd. conc. = background concentration of nutrients before short-term enrichment; EF = enrichment factor, calculated as enrichment/background concentration;  $V_f$  = uptake velocity;  $U$  = uptake rate. An ellipsis (...) indicates uptake was not detectable due to background enrichment along the study reach.

type and a control) pre-treatment in 2009 and 2010 to evaluate potential interannual changes in nutrient uptake due to treatment effects.

Nutrient uptake was measured by conducting short-term nutrient additions in the 500-m long treatment reaches. In one stream (Trail Creek), the reach was shortened to 400m due to a tributary entering the stream ca. 80 m above the bottom of the reach. We first collected water samples for background nutrient concentrations at 6–7 locations along the treatment reaches. We then added solutions of the nutrient of interest (nitrate-N as  $\text{NaNO}_3$  or ammonium-N as  $\text{NH}_4\text{Cl}$ ) to the upstream end of the treatment reaches at a rate of 100 mL/min. Nitrate-N and ammonium-N releases were always conducted separately. Our target enrichment factor (EF, enrichment/background nutrient concentrations) was 1.3 to 1.5, however the actual enrichment factor ranged from 1.2 to 8.0 due to uncertainty in background concentrations on some study dates (Table 2). Along with the nutrient of interest, we simultaneously added the non-reactive tracer rhodamine WT to account for water movement via hyporeic exchange or groundwater recharge. The target concentration for rhodamine was 8  $\mu\text{g/L}$ , which is below visual detection in water, and rhodamine WT concentrations were measured on filtered water samples using an AquaFluor

handheld fluorometer (Turner Designs, Sunnyvale, CA). Once the conservative tracer had reached plateau at the downstream end of the study reach (approximately 2.5 hrs), water samples were collected at the same locations where background concentrations had been sampled prior to start of the release. Samples for ammonium-N and nitrate-N were stored and/or analyzed as described.

Nutrient spiraling parameters were determined by plotting the natural log of the background-corrected plateau nutrient concentrations divided by the conservative tracer concentrations vs. distance from upstream end of the reach. These data were then fit with linear regression, and the uptake length ( $S_w$  in m), or distance traveled in dissolved inorganic form before being removed from solution, was calculated as the inverse of the slope. Because  $S_w$  is strongly influenced by stream discharge and water velocity, we also calculated uptake velocity ( $V_f$  in mm/s), or the velocity at which a nutrient moves towards the location of immobilization, and uptake rate ( $U$ , in  $\text{mg}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ ), which describes the flux of a nutrient to the streambed over a given time. All calculations followed Webster and Valett (2006).  $V_f$  is useful for comparing nutrient dynamics among streams of different sizes (Davis and Minshall 1999), where-

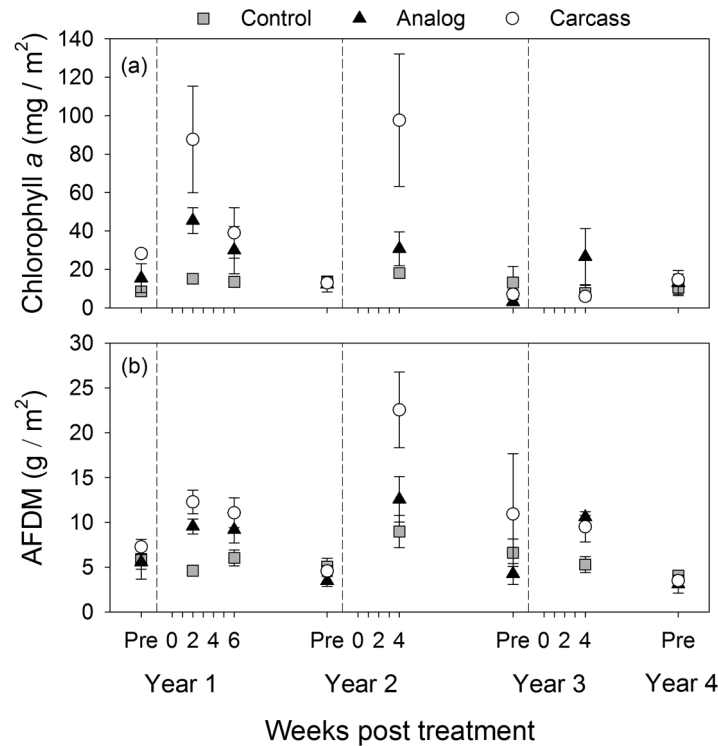


Fig. 2. (a) Chlorophyll *a* and (b) ash-free dry mass (AFDM) significantly increased 2–6 weeks post treatment in all years for analog treatments, and in years one and two for carcass treatments. We observed no year-to-year increases in either measure of biofilm standing crop when comparing the pre-treatment sampling periods only. Vertical dashed lines represent treatment application in each year. Error bars are  $\pm 1$  SE,  $n = 3$  for each point.

as *U* can be used to generate estimates of nutrient flux that can be compared to treatment application rates.

## RESULTS

### Biofilm responses

**Standing crop.**—Biofilms on natural rock substrates in the treatment streams responded strongly to treatment materials by increasing standing crops up to six weeks following treatment application, but these effects did not translate into year-to-year increases. Both chlorophyll *a* and AFDM significantly increased 2–8-fold up to 6 weeks post-treatment in years one and two for both carcass and analog treatments (Fig. 2). In year 3, both chlorophyll *a* and AFDM increased 4-weeks post treatment in analog treatments but not carcass treatments (Fig. 2). Overall, we observed significant interactions between treatment and time period for both AFDM and chlorophyll *a* (two-way RMANOVA

for chlorophyll *a* treatment  $\times$  period,  $F_{14,41} = 2.2$ ,  $p = 0.028$ ; for AFDM treatment  $\times$  period,  $F_{14,41} = 2.3$ ,  $p = 0.017$ ).

Across all four years, we observed no inter-annual increase in biofilm standing crop due to treatment additions, as assessed by comparing the pre-treatment samples across years (Fig. 2). For chlorophyll *a*, there was a significant interaction between treatment and year (two-way RMANOVA treatment  $\times$  period,  $F_{6,18} = 3.0$ ,  $p = 0.033$ ), with pre-treatment concentrations being higher in carcass and analog streams vs. control streams in year 1, but similar among all streams in years 2–4. For AFDM, there was a significant effect of period (two-way RMANOVA period,  $F_{3,18} = 4.0$ ,  $p = 0.024$ ), with the highest pre-treatment AFDM standing crops observed in years 1 and 3.

**Isotope composition.**—Because they were derived from marine fish tissue, both of the treatment materials exhibited enriched  $\delta^{15}\text{N}$  and

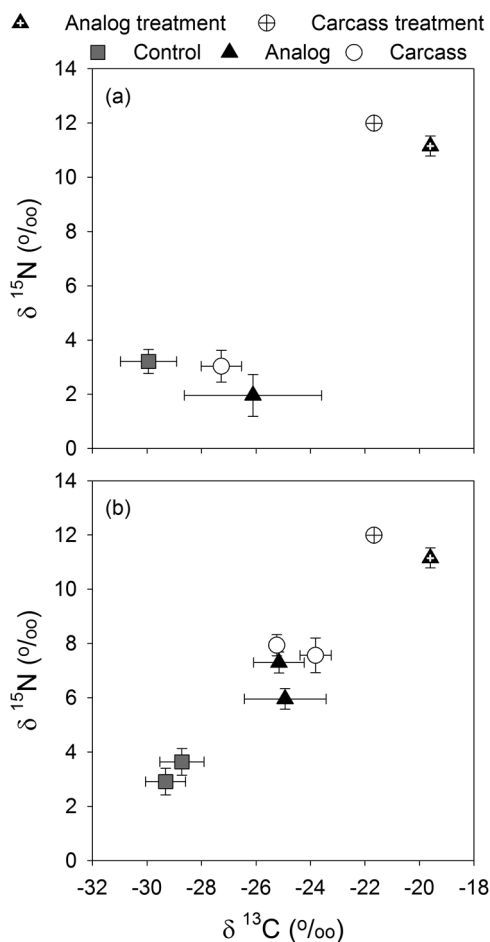


Fig. 3. Stable isotope signatures of biofilms were similar in all study streams (a) pre-treatment in 2009, but were significantly enriched in both  $^{15}\text{N}$  and  $^{13}\text{C}$  in both analog and carcass-treated streams (b) post-treatment in 2008 and 2009. On both the average isotope signatures of the carcass and analog materials added during the experiment are shown. Error bars are  $\pm 1$  SE,  $n = 3$  for each point.

$^{13}\text{C}$  relative to biofilms in control streams, with mean  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of  $12.0 \pm 0.2\text{‰}$  ( $\pm$ SE, here and throughout) and  $-21.7 \pm 0.1\text{‰}$  for carcasses and  $11.2 \pm 0.4\text{‰}$  and  $-19.6 \pm 0.1\text{‰}$  for analog (Fig. 3). In contrast, mean  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of biofilms in the control streams across all sampling dates were  $3.3 \pm 0.3\text{‰}$  and  $-29.3 \pm 0.5\text{‰}$ , respectively. Following treatment application in both years 1 and 2, biofilm isotope composition became more enriched in both carcass and analog streams (mean  $\delta^{15}\text{N}$  ranging from 6.0‰ to 7.9‰

and  $\delta^{13}\text{C}$  from  $-25.3\text{‰}$  to  $-23.8\text{‰}$ ), but remained less enriched than the treatment material (Fig. 3b). Two-way MANOVA showed a significant interaction effect of both treatment and sampling period on  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  (Wilks' Lambda = 0.024;  $F_{8,34} = 4.4$ ,  $p = 0.001$ ). Pre-treatment in year 2,  $\delta^{15}\text{N}$  was similar among treatment and control streams, while  $\delta^{13}\text{C}$  remained slightly enriched in treatment vs. control streams (Fig. 3a); post-hoc tests following MANOVA revealed that these differences were not significant.

**Nutrient limitation.**—NDS incubations indicated that nutrient limitation of autotrophs, but not the entire biofilm assemblage, varied between streams treated with the two treatment materials. Chlorophyll *a*, which indicates responses by algae and cyanobacteria in the biofilms, was primarily N limited on NDS in control and analog streams, but not carcass streams (Fig. 4a; three-way ANOVA, treatment  $\times$  N,  $F_{2,198} = 14.1$ ,  $p < 0.001$ ; no significant three-way or treatment  $\times$  P interactions). P additions suppressed chlorophyll *a* across all treatment streams ( $F_{1,198} = 20.6$ ,  $p < 0.001$ ). In contrast, AFDM, which integrates all autotrophs and heterotrophs in the biofilms, was significantly suppressed by P, but the degree of suppression varied among treatments (Fig. 4b; three-way ANOVA, treatment  $\times$  P,  $F_{2,198} = 4.6$ ,  $p = 0.01$ ; no significant three-way or treatment  $\times$  N interactions).

### Streamwater nutrient responses

**Nutrient fluxes.**—We observed short-term increases in total dissolved and total N and P loads within two weeks following treatment applications in all three years, although the magnitude and timing of responses varied among nutrients and years (Figs. 5 and 6). For example, in year one TN load increased on average 2.6-fold in analog and 3.8-fold in carcass-treated streams in the few days post treatment compared to 1.9-fold in the control streams; TDN increased 3.7-fold in analog, 4.2-fold in carcass, and 1.5-fold in control streams during the same time period (Fig. 5b, c). Even larger increases in TN and TDN were observed in only the carcass and analog treated streams during the few days post-treatment in year 2, but neither increased following treatment in year 3 (Fig. 5b, c). Loads of both TDN and TN were significantly related to both treatment and sampling date, as indicated by two-way RMA-

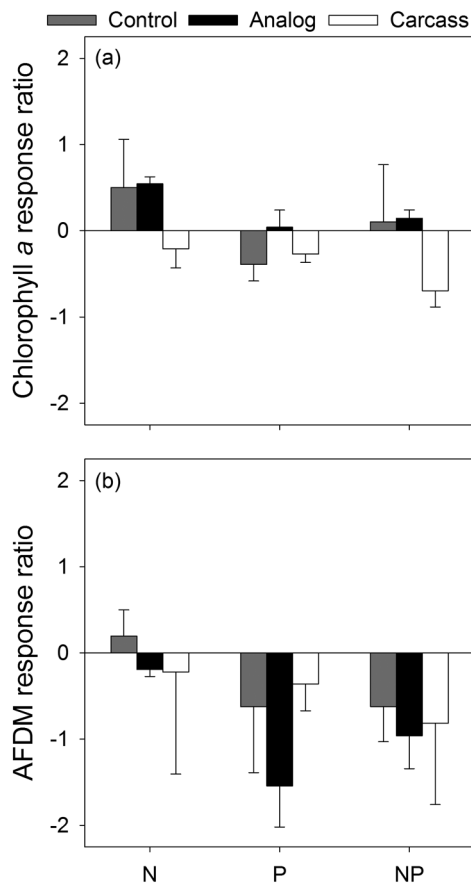


Fig. 4. Response ratios of biofilms growing on nutrient diffusing substrates showed that (a) chlorophyll *a* was primarily N limited in control and analog streams, but not carcass streams, and (b) ash-free dry mass (AFDM) was significantly suppressed by P across all treatments. Bars show mean and 1 SE,  $n = 3$  for each bar.

NOVA (TN treatment  $\times$  period,  $F_{20,57} = 1.9$ ,  $p = 0.03$ ; TDN treatment  $\times$  period,  $F_{20,59} = 3.4$ ,  $p = 0.0001$ ). Nitrate-N showed similar patterns as TDN, and also responded significantly to treatment and time in combination (Fig. 5a; treatment  $\times$  period,  $F_{20,60} = 1.8$ ,  $p = 0.035$ ), with the largest differences driven by high nitrate-N concentrations in one control stream. In that stream, nitrate-N made up 47% of the TDN load, compared to 7% on average in the other two control streams and 19% on average across all streams, treatments and dates. Ammonium-N loads were only measured in 2010 (data not shown), and increased in all streams post-

treatment (period,  $F_{2,12} = 9.75$ ,  $p = 0.003$ ), but showed no significant responses to treatment. Ammonium-N loads averaged 13% of TDN load; the fact that nitrate + ammonium-N together accounted for only 34% of the TDN load suggests DON as an important form of N export in these streams.

Both TP and TDP significantly increased post-treatment in most years, with particularly large increases in analog-treated streams in years 1 and 2 and carcass-treated streams in year 2 (Fig. 6). RMANOVA indicated significant effects of treatment and time in combination for both TP and TDP (Fig. 6b, c; treatment  $\times$  period,  $F_{20,57} = 3.9$ ,  $p < 0.0001$ ; TDP treatment  $\times$  period,  $F_{20,59} = 4.6$ ,  $p < 0.001$ ). SRP concentrations also showed significant changes through time (Fig. 6a; treatment  $\times$  period,  $F_{20,60} = 3.7$ ,  $p < 0.0001$ ). Similar to nitrogen, TDP comprised 89% of the TP load on average, but in contrast SRP comprised 80% of the TDP load, suggesting a less important role for both particulate and DOP export in these streams.

We also observed spatial patterns in nitrate-N and ammonium-N concentrations along a subset of the study reaches 3–4 weeks post-treatment. In particular, ammonium-N concentrations increased from upstream to downstream 5.3-fold in carcass and 3.4-fold in analog-treated reaches, but remained flat in control reaches three weeks post-treatment in 2009 (Fig. 7a; ANCOVA treatment,  $F_{2,21} = 35.0$ ,  $p < 0.001$ ; period,  $F_{6,21} = 3.7$ ,  $p = 0.001$ , treatment  $\times$  period interaction,  $p > 0.05$ ). Similar reach-scale patterns were not observed for nitrate-N (Fig. 7b;  $p > 0.05$  for all effects in ANCOVA), again possibly because of the high and variable concentrations of nitrate-N in one control stream relative to all of the other study streams.

When integrated over the month following treatment in 2008, TDN load increased 50% in the carcass and 70% in the analog treated streams relative to the control streams (Fig. 8), although not significantly (one-way ANOVA,  $F_{2,6} = 1.3$ ,  $p = 0.3$ ). TN loads were only slightly higher than TDN load, and similarly increased 25% in the carcass and 51% in the analog treated streams (Fig. 8), but these increases were also not significant (one-way ANOVA,  $F_{2,6} = 0.6$ ,  $p = 0.6$ ). In contrast, TP loads were 70% and TDP loads were 79% higher from analog streams than



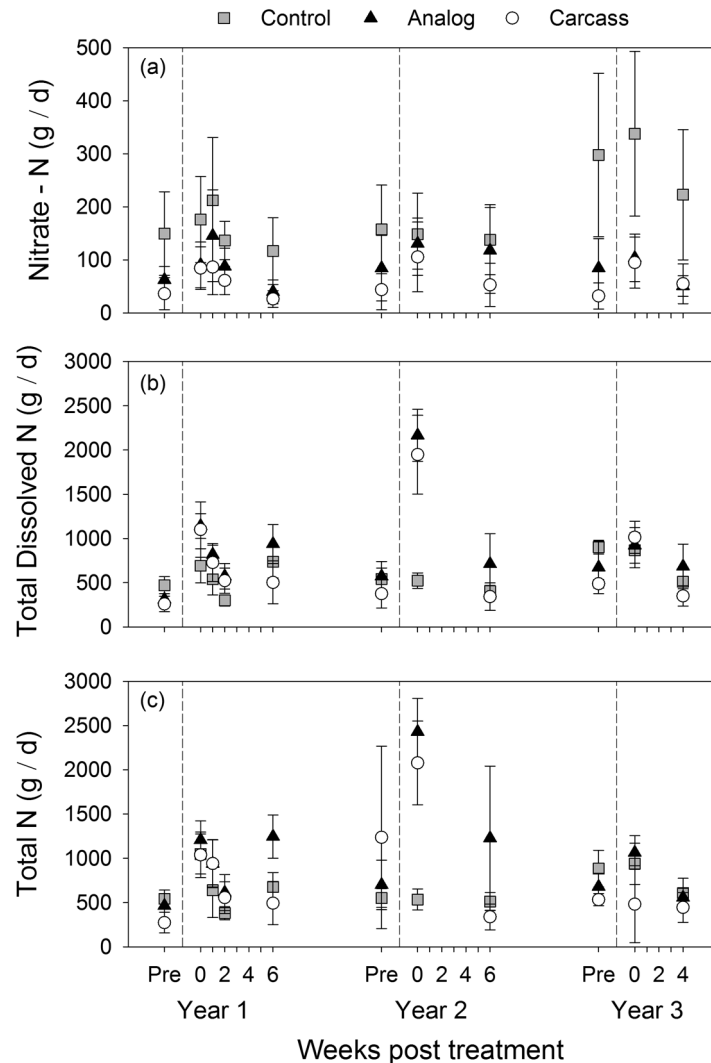


Fig. 5. (a) Nitrate-N was significantly elevated in control vs. treatment streams due to one control stream with consistently high concentrations, while (b) total dissolved N, and (c) total N both increased following treatment with carcass and analog in years 1 and 2 but not year 3. Vertical dashed lines represent treatment application in each year. Error bars are  $\pm 1$  SE,  $n = 3$  for each point.

from carcass and control streams combined, but neither of these increases were significant (Fig. 8; one-way ANOVA for TP,  $F_{2,6} = 1.2$ ,  $p = 0.4$ ; for TDP,  $F_{2,6} = 1.5$ ,  $p = 0.3$ ).

*Whole-stream nutrient uptake.*—The predominant trend in nutrient uptake was a tendency towards short uptake lengths of ammonium-N and nitrate-N in control streams on all sampling dates and in all streams prior to treatment (Table 2). Both uptake velocities and uptake rates of nitrate-N in control streams and pre-treatment in

all streams ranged an order of magnitude, but averaged  $0.033 \pm 0.011$  mm/s for  $V_f$  and  $2.19 \pm 0.72$  mg·m<sup>-2</sup>·h<sup>-1</sup> for  $U$  (Table 2). Uptake velocities and rates of ammonium-N in the two control streams were faster and lower than for nitrate-N ( $V_f = 0.062 \pm 0.003$  mm/s;  $U = 0.79 \pm 0.13$  mg·m<sup>-2</sup>·h<sup>-1</sup>). Measurements of nitrate-N uptake before treatment in July 2009 and 2010 suggest there were no inter-annual changes, but this could not be tested statistically due to lack of replication (Table 2). We were unable to measure

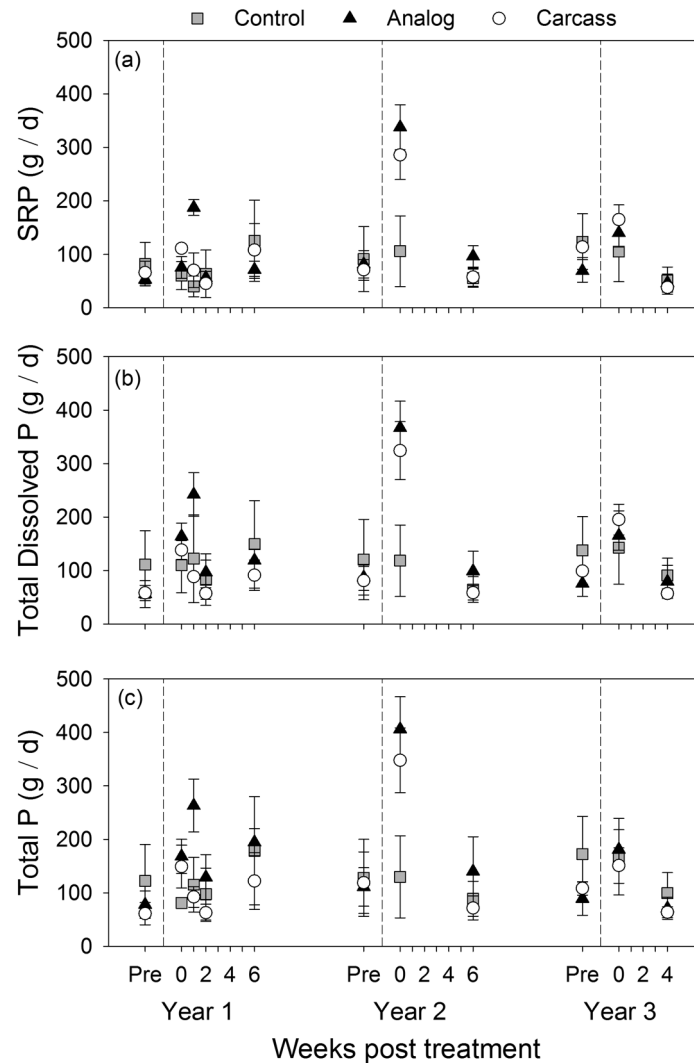


Fig. 6. (a) Soluble reactive P (SRP) increased significantly in analog but not carcass treatments in years 1 and 2, while (b) total dissolved P, and (c) total P increased following treatment applications but were not significantly different among treatments. Vertical dashed lines represent treatment application in each year. Error bars are  $\pm 1$  SE,  $n = 3$  for each point.

ammonium-N uptake post-treatment in the carcass-treated streams, and only able to measure nitrate-N uptake in one of these streams (Table 2), because of increasing background nutrient concentrations along the study reaches (Fig. 7). Across all treated streams where uptake was detectable, uptake velocities of both nitrate-N and ammonium-N were similar to those measured in control streams and pre-treatment (nitrate-N,  $V_f = 0.030 \pm 0.021$  mm/s; ammonium-N,  $V_f = 0.047 \pm 0.036$  mm/s), but uptake rates

were lower for nitrate-N but not ammonium-N (nitrate-N,  $U = 0.31 \pm 0.16$  mg·m<sup>-2</sup>·h<sup>-1</sup>; ammonium-N,  $U = 2.93 \pm 2.14$  mg·m<sup>-2</sup>·h<sup>-1</sup>) although this could not be tested statistically (Table 1).

## DISCUSSION

Nutrient addition to mitigate for the loss of marine-derived nutrients delivered by salmon in the Pacific Northwest is a widely applied management strategy (Compton et al. 2006) with

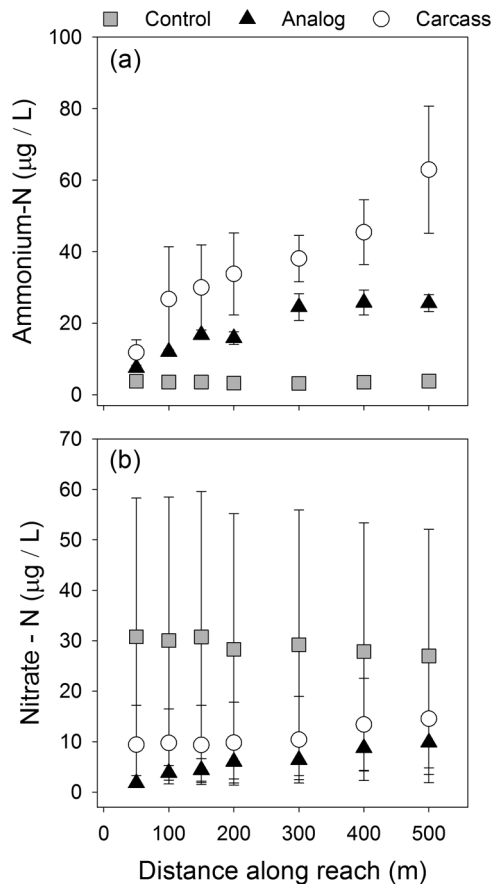


Fig. 7. (a) Ammonium-N concentrations significantly increased along reaches treated with carcass or analog, but not control reaches approximately three weeks post-treatment in August 2009. (b) No significant patterns vs. distance or treatment were observed for nitrate-N. Error bars are  $\pm 1$  SE,  $n = 2$  for each point.

the ultimate goal of increasing production of resident and/or migratory fish via both direct (consumption) and indirect (nutrient stimulation of biofilm) pathways. Stimulation of the indirect pathway is predicated on the notion that added nutrients are incorporated by microbes, particularly those comprising stream biofilms, which then serve as a link to pass those nutrients up the food web. Similar to other, but not all, studies that have measured biofilm responses to both naturally spawning salmon and to nutrient additions for mitigation, we observed that biofilms in study streams increased their biomass and incorporated nutrients from salmon carcasses and analog in the short-term, post-treatment

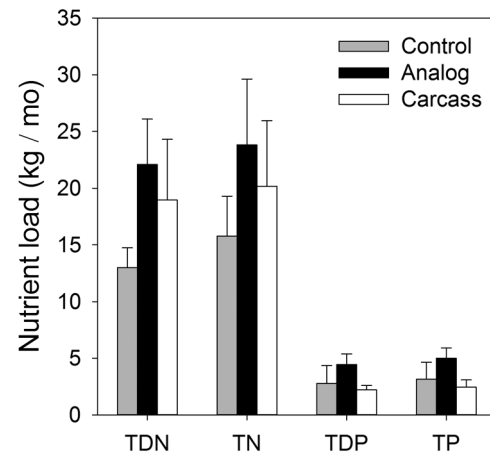


Fig. 8. Total dissolved N (TDN) and total N (TN) loads were elevated in analog and carcass treated streams when interpolated across the month following treatment application, but were not significantly higher than loads in control streams. In contrast, total dissolved P (TDP) and total P (TP) loads were elevated only in analog streams, but not significantly compared to loads in control and carcass-treated streams. Bars show mean and 1 SE,  $n = 3$  for each point.

period. We also observed that biofilm nutrient limitation changed following treatment with salmon carcasses, but not analog. If we had only measured these biofilm-specific responses, we might have concluded that treatment with these materials could result in incorporation of large amounts of nutrients by biofilms in the treatment reaches. However, our measurements of nutrient loads and uptake suggest that the ability of biofilms and other stream microbes to respond to added nutrients is limited, such that much of the nutrient added may be stored or exported downstream. These results suggest that better understanding of microbially driven nutrient uptake and demand could provide insight into the efficacy of nutrient mitigation for stimulating fish production via the bottom-up pathway, and that the effects of nutrient additions should be viewed at larger segment or watershed scales rather than at the scale of treated reaches.

Streams in the central Idaho area we studied may be considered ideal candidates for nutrient additions for mitigation; they are located in a region of very low geologic nutrient availability, have little to no anthropogenic nutrient loading or watershed habitat degradation (Compton et

al. 2006, Budy and Schaller 2007), and have stream biofilms that are strongly nutrient limited (Marcarelli and Wurtsbaugh 2007, Sanderson et al. 2009). The results of our current study confirmed that at least the autotrophic portion of biofilms in these streams are primarily N limited and that additions of some salmon materials may alleviate nutrient limitation of biofilms; we observed no nutrient limitation of chlorophyll *a* following treatment in carcass-treated streams, but biofilms remained primarily N-limited in analog-treated streams. These observed differences between biofilm responses in carcass and analog-treated streams could be due to the different N application rates associated with each material (50 vs. 27 g N/m<sup>2</sup>, respectively). Others have found that naturally spawning salmon in Alaska may alleviate nutrient limitation of both autotrophs and heterotrophs (Rüegg et al. 2011), but that analog additions may not alleviate biofilm nutrient limitation in other central Idaho streams (Kohler et al. 2008, Ebel 2012). Suppression of biofilm standing crops by P additions that we observed here has been observed in many studies, and has been hypothesized to be due to competition between autotrophs and heterotrophs for nutrients (e.g., Marcarelli et al. 2009, Sanderson et al. 2009).

Although demonstration of nutrient limitation in any study suggests that biofilms have the capacity to respond to nutrient additions such as those proposed via nutrient mitigation, it does not indicate the degree or extent to which biofilms may respond nor the potential of the stream microbial community to retain or process nutrients. Biofilm growth on NDS can best be thought of as an indicator of whether portions of biofilms could respond to nutrients added to a stream ecosystem, but their applicability to natural stream biofilms may be limited for a variety of reasons. First, NDS characterize biofilms that grow on specific substrates in streams like wood and rock, but exclude microbes living on other substrates and those living in hyporheic zones, all of which influence stream nutrient uptake and demand (Davis and Minshall 1999). Moreover, NDS may inadequately represent biofilms growing on substrates that they are designed to mimic. For example, inorganic substrates, like the fritted glass disks in our experiment, tend to favor growth of autotrophs

over heterotrophs (Johnson et al. 2009), whereas natural stream biofilms are composed of complex communities of algae, cyanobacteria, bacteria and fungi.

Responses on natural rock substrates in our study suggest that biofilms have the capacity to increase standing crop and incorporate nutrients from treatment materials. Similar to other studies of carcass and analog additions to streams (Kiernan et al. 2010, Kohler et al. 2012, but see Ambrose et al. 2004), we observed short-term increases in chlorophyll *a* and AFDM in the six weeks following treatment. Moreover, biofilm isotope composition became enriched by approximately 4‰ for  $\delta^{15}\text{N}$  and 4.5‰ for  $\delta^{13}\text{C}$ ; these increases were larger than those observed in response to natural spawning runs (Bilby et al. 1996, Reisinger et al. 2013), but still resulted in biofilm isotope composition that was distinct from those of the treatment materials (Fig. 3). This suggests that though biofilms did incorporate nutrients from treatments, they either did not turn over all of their stored nitrogen during the time period of observation (4–6 weeks post-treatment), and/or they continued to obtain nitrogen from other sources. Nutrients enter streams from a variety of sources including atmospheric and geologic input, biological fixation, lateral riparian inputs, and groundwater (Triska et al. 1984); nutrients introduced for mitigation should be considered as an additional source to these others (Compton et al. 2006). In the case of N, biological inputs via N fixation by in-stream cyanobacteria (Marcarelli and Wurtsbaugh 2007, 2009) and riparian alder (Compton et al. 2003) may be particularly important in mountain, headwater streams. Previous work in streams draining the Idaho Batholith has demonstrated that fluxes from in-stream N fixation may exceed hydrologic N fluxes during the late summer (Marcarelli and Wurtsbaugh 2009), which is when we (and others employing such methods) apply mitigation treatments to coincide with historic peaks in spawning activities. Although we did not monitor biofilm assemblages in the current study, shifts among different taxa might explain the year-to-year or stream-to-stream differences in standing crop and nutrient uptake that we observed.

Biofilm isotope composition in the treatment streams returned to values similar to those in the



control streams by 1-year post-treatment, suggesting that although these nutrients may be important for fueling short-term biofilm growth, they did not translate into effects that could be detected year-to-year during the 3-year duration of this study. This loss of isotope from the biofilms could be due to natural biofilm turnover; in a regional stream, Hall et al. (2009) found a net residence time of 117 d for N in biofilms on rocks. Another likely loss mechanism is mobilization of streambeds and biofilm scour during the spring snowmelt flood (Davis et al. 2013). Overwinter monitoring of biofilm isotopes following mitigation treatment could help determine which mechanism of biofilm turnover is most important. In streams with natural spawning runs and long-term delivery of marine-derived nutrients, legacy effects on  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of biofilms have been reported (Reisinger et al. 2013), and could possibly manifest in response to nutrient additions over longer timescales if those nutrients were moved into long-term storage pools like riparian soils and vegetation (Naiman et al. 2002, Koshino et al. 2013).

Despite the responses of stream biofilms observed, our measurements of whole-stream nutrient uptake and load provide little evidence that nutrient additions altered the capacity of microbes to take up and retain ammonium-N or nitrate-N. All of the uptake rates and velocities measured in the study streams before and after treatment were comparable to those measured in other, non-nutrient enriched central Idaho streams (Davis and Minshall 1999, Arp and Baker 2007). If we use the maximum uptake rates of ammonium and nitrate measured in our study and add them together to estimate uptake of dissolved inorganic N (DIN), then extrapolate to estimate how much DIN could be taken up by stream microbes over the month-long period during which we also monitored nutrient loads, we estimate that uptake could account for a maximum of  $6 \text{ g N}\cdot\text{m}^{-2}\cdot\text{mo}^{-1}$ ; if we use the median uptake rates measured then we would estimate a median uptake of  $0.9 \text{ g N}\cdot\text{m}^{-2}\cdot\text{mo}^{-1}$ . In comparison, we added 50 and  $27 \text{ N/m}^2$  in the carcass and analog treatments, respectively. Therefore, nutrient uptake in the month following treatment could account for a maximum of 12–20% and a median of 2–3% of nutrients added during the treatment applications. In compari-

son, a  $^{15}\text{N}$  tracer experiment in nearby Spring Creek, Idaho found that 58% of nitrate-N was retained in August under baseflow conditions (Hall et al. 2009). It is important to note that even our median uptake rates may overestimate actual uptake because we assumed that nutrient uptake rates were constant over this month-long period despite evidence that they can vary with daily and seasonal patterns of biological activity (Roberts and Mulholland 2007). Our rates were measured during the middle of the day when primary production and nutrient uptake tend to be highest.

The contradictory observations that biofilms in the streams clearly demonstrated N limitation using NDS, yet only took up a small portion of the N added in treatment reaches can be resolved by the fact that biofilm production and nutrient uptake in streams is limited and constrained by multiple interacting factors. First, the ability of microbes to take up nutrients in any stream is not limitless, but rather follows either Michaelis-Menten (Earl et al. 2006) or Efficiency Loss models (O'Brien et al. 2007). Either model predicts that microbes will take up a smaller proportion of the total nutrients in streams as the supply increases, as the maximum amount taken up will be based on the ability of microbes to increase their growth and transport nutrients from the water column into their cells. These abilities, in turn, are constrained by types of microbes present and other environmental factors (Borchardt 1996). For example, Ambrose et al. (2004) found that primary production in California streams did not respond to nutrient enrichment because of the overriding effects of light limitation. We have some evidence that grazing by macroinvertebrates controlled biofilm standing crops in years 2–4 post-treatment in the study streams, but are not certain how this might be related to biofilm nutrient uptake (Collins 2014). Regardless of which factors constrained responses, if stream microbial communities cannot increase their uptake rates or storage of available nutrients in response to mitigation treatments they will be exported via hydrologic transport, as we observed; this is an important contrast to nutrient fertilization in lakes (Hyatt et al. 2004) where nutrients may be retained in place by slow rates of lateral and vertical transport and long residence times.

Finally, we observed increases in nutrient loads at the downstream end of the study reaches in terms of TDN, TDP, TN, and TP in the initial week following treatment applications, although these increases did not translate into significantly higher nutrient loads when scaled over the month period following treatment. Similar increases in nutrient concentrations in response to nutrient additions for mitigation have been observed in some studies (Claeson et al. 2006, Kiernan et al. 2010), but not others (Kohler et al. 2008, Kohler et al. 2012). However, these studies all varied in terms of form of material added (inorganic nutrients, analog or carcasses), treatment application rate, timing of nutrient sampling vs. treatment application, location of experiment (natural vs. artificial streams or mesocosms), and sensitivity of the analytical chemistry methods employed. Overall, we observed that approximately 20 kg N/month could be exported from these streams in the month following treatment, mostly in dissolved form; in contrast, we added 42.5 and 61.5 kg N to these streams in the analog and carcass treatments, respectively. We likely underestimated loads as our first sampling time was two days post-addition, missing a likely pulse of nutrient export immediately following treatment. In addition, N that is taken up by biofilms as described in our study may be temporarily retained as standing crop, but some portion will be remineralized to dissolved form, then exported downstream on a time scales of days to weeks (O'Brien et al. 2012) or exported in particulate form during storm-flow and snowmelt (Hall et al. 2009).

That a potentially large portion of the nutrients added to the treatment reaches were exported may actually mimic the fate of nutrients delivered via naturally spawning salmon. Others have observed short-term increases in stream nutrient concentrations following salmon spawning, although these studies suggest that excretion by live salmon may be a more important source of nutrients than carcass material (Janetski et al. 2009, Tiegs et al. 2011). Studies of biofilm responses to naturally spawning salmon have demonstrated that the building of redds in the streambed can be a strong direct disturbance that leads to reduction of benthic standing crops of primary producers in the locations where salmon spawn (Janetski et al. 2009, Verspoor et al. 2010).

However, export of nutrients and organic matter driven by this disturbance may lead to increased delivery of salmon nutrients to downstream river reaches (Albers and Petticrew 2012), rearing lakes (Moore et al. 2007), and estuaries (Cak et al. 2008), and in some cases, these exported nutrients have been linked to increased biofilm standing crop in these downstream ecosystems (Albers and Petticrew 2012). Therefore, the effects of salmon spawning may be more appropriately viewed at the scale of stream segments or watersheds, rather than reaches. Similarly, the observed export of nutrients from the treatment reaches suggests that responses may be more appropriately measured at these larger scales (Fausch et al. 2002). Although nutrient export may be a vital process distributing salmon nutrients among distant spawning and natal rearing habitats, export of nutrients from targeted reaches for nutrient mitigation may contribute to downstream eutrophication depending on the amount and form of nutrients added, particularly in areas of high nutrient availability (Compton et al. 2006) and/or where oligotrophic headwaters are in close proximity to nutrient-rich mainstem rivers and reservoirs such as those along the Snake River in Idaho.

After accounting for export and uptake by biofilms, a large amount of added nutrients are processed or retained in the reaches in the month following treatment, and it is important to consider the range of possible pathways and fates for these retained nutrients. Direct consumption of materials used for nutrient mitigation by both invertebrates and fish is certainly an important fate with direct consequences for success of mitigation strategies. In a parallel investigation we found that resident fish in our study streams derived 17% and 6% of their annual production via the direct consumption of carcass (3–5% of treatment applied) and analog (4–11% of treatment applied) material, respectively (Collins 2014). We also observed that salmon carcasses were removed from our study streams by bears, attracting terrestrial diptera and potentially delivering nutrients to riparian plants and consumers (Collins 2014). It is likely that treatment material that was not taken up by biofilms, consumed by fishes or invertebrates, or exported in the fall was stored in the stream bed and hyporheic zone, then exported during

snowmelt when discharge and nutrient export is greatest from streams in this region (Hall et al. 2009). Finally, denitrification rates appear to be low under natural conditions in other regional streams (Hall et al. 2009) but can be quite high when supplemented with nitrate-N and organic C (Washbourne et al. 2011); both N and organic C are supplied via the treatment materials used in this study, suggesting that denitrification losses may be stimulated by these treatments and should be carefully quantified to understand the possible nutrient loss via this dissimilatory pathway.

Application of ecosystem techniques focused on microbially driven nutrient dynamics can help address important questions facing fisheries managers, including when nutrient additions to mitigate for the loss of salmon should be considered, at what scale we can expect to detect responses to nutrient additions, what application rates should be applied to ensure efficient and cost-effective management, and how to optimize or limit downstream consequences of nutrient additions. Our study suggests that effective applications that maximize in-situ responses while minimizing nutrient export to downstream ecosystems might be achieved at much lower treatment rates than those used in the current study, and should be evaluated further in a dose-response framework to determine the treatment level at which effective responses can be expected. Estimation of spiraling lengths and nutrient loads in streams and rivers that are targets for nutrient additions for mitigation may help managers design plans that maximize the spatial arrangement of treatment reaches while minimizing impacts far downstream from treatment areas (Thomas et al. 2003). All of these considerations will be important when scaling nutrient mitigation up from the scale of stream reaches (10s of meters) to that of river segments or watersheds (10s to 100s of km), where the cost and logistics of acquiring and applying nutrient mitigation materials like salmon carcasses and analog may become prohibitive.

Restoration activities like the nutrient mitigation we tested here may be best thought of as attempts to allow streams to re-express their capacity for food web and ecosystem productivity (Ebersole et al. 1997). As ecologists and managers, we can only expect mitigation efforts

to elicit responses within the capacity of the ecosystem. It is possible that these central Idaho stream ecosystems have entered an alternative stable state (Folke et al. 2004), and that increasing the capacity of microbes to incorporate added nutrients may require large-scale, long-term additions of nutrients or restoration of naturally spawning runs of salmon. Determining whether microbially driven nutrient uptake and subsequent movement of nutrients into stream food webs may eventually respond to nutrient additions as the ecosystem regains historical capacity would require longer time periods of observation than the three years encompassed by this study. Other long-term nutrient addition studies in streams have revealed that non-linear responses emerge after five or more years of treatment (Slavik et al. 2004, Davis et al. 2010), whereas whole-watershed studies suggest that responses from historical disturbances can persist after as long as 50 years of observation (Bernal et al. 2012). Evaluating the potential capacity of stream and lake ecosystems to respond to nutrient additions through long-term studies should be prioritized as a research goal as implementation of salmon restoration and mitigation plans continue in the Pacific Northwest.

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