Biogeochemical transformation of a nutrient subsidy: salmon, streams, and nitrification

Peter S. Levi · Jennifer L. Tank · Scott D. Tiegs · Dominic T. Chaloner · Gary A. Lamberti

Abstract Migratory animals can alter ecosystem function via the provision of nutrient subsidies. These subsidies are heterogeneous in space and time, which may create hot spots or hot moments in biogeochemical transformations, in turn altering the ecosystem effect of the subsidy by changing the form of the nutrients. Annual migrations of Pacific salmon (*Oncorhynchus* spp.) transport nutrients from the marine environment to their natal freshwater ecosystems. Salmon subsidies provide high quality nutrients (e.g., nitrogen, phosphorus, carbon) that may also be large in quantity where salmon migrations are near historic levels. We hypothesized that the nutrient subsidy provided via the excretion of ammonium (NH_4^+) by live salmon would stimulate microbially mediated nitrification rates in stream sediments and increase streamwater nitrate (NO_3^-) concentrations. We quantified sediment nitrification in seven streams in Southeast Alaska before and during the salmon run in 2007 and 2008. Nitrification rates increased 3-fold from before to during the salmon run (mean $\pm$ SE = 0.07 ± 0.01 to 0.24 ± 0.02 mgN gAFDM^{-1} d^{-1}, respectively). The variation in nitrification was explained by both streamwater and exchangeable NH_4^+ concentrations ($R^2 = 0.50$ and 0.71, respectively), which were low before salmon and increased relative to the size of the salmon run. To experimentally test the effect of salmon subsidies on nitrification rates, we staked senesced salmon carcasses on stream sediments for 3 weeks during the salmon run and then measured nitrification rates directly under the carcasses. Sediment nitrification was 2–5 times higher under the carcasses compared to nearby sediments without the direct carcass influence. Our results confirm that biogeochemical transformations alter the form of salmon-derived nitrogen, representing an overlooked aspect in the dynamics of this subsidy. Therefore, animal-derived nutrient subsidies are not passively retained or exported in recipient ecosystems, but also transformed, thereby influencing the form and incorporation of these nutrient subsidies.

Keywords Nutrient subsidy · Nitrogen cycle · Pacific salmon · Ammonium · Nitrate · Southeast Alaska

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Introduction

The animal-mediated flux of nutrients across ecosystem boundaries can significantly influence recipient ecosystems (Polis et al. 1997; Marcarelli et al. 2011). For instance, organisms that move frequently between ecosystems provide nutrient subsidies that can support primary production and have dramatic consequences for community structure (e.g., Maron et al. 2006). While the effect of animal-derived subsidies on food webs has been well documented, the roles of microbes on and biogeochemical transformations of these nutrients have largely been overlooked (Polis et al. 2004). Subsidies can create "hot spots" or "hot moments" (after McClain et al. 2003) in nutrient transformations, where and when reaction rates are greater than ambient rates, which may alter the influence of the nutrient on the ecosystem (e.g., McIntyre et al. 2008). As a result, biogeochemical transformations may (1) enhance the effect of the subsidy by providing multiple nutrient forms to meet biological demands, or (2) dampen the influence of the subsidy if the demand for the transformed form of the nutrient is less than the initial form. The fate of nutrients from animal-derived subsidies in recipient ecosystems, whether incorporated into the food web or lost from the ecosystem, may ultimately depend on the transformations of those nutrients.

Pacific salmon (*Oncorhynchus* spp.) may create hot spots and hot moments in biogeochemical transformations during their annual spawning runs by transporting a discrete pulse of nutrients from the marine environment to their natal freshwaters (Schindler et al. 2003). Salmon migrations provide a useful model for investigating the effect of biogeochemical transformations on nutrient subsidies, as they deliver inorganic nitrogen (N) to streams as ammonium (NH_4^+) via excretion, often increasing this form of dissolved N by orders of magnitude above background concentrations (see meta-analysis by Janetski et al. 2009). Furthermore, several previous studies have documented concurrent increases in NH_4^+ and NO_3^- during salmon runs, suggesting that microbially mediated nitrification in the sediments transforms salmon-derived NH_4^+ to NO_3^- (Johnston et al. 2004; Levi et al. 2011; Tiegs et al. 2011). While research has demonstrated that resident fish can create hot spots of biogeochemical transformations (McIntyre et al. 2008), no previous study has investigated the potential

hot moment in nutrient transformations associated with anadromous fish migrations.

Nitrogen transformations and cycling have been studied extensively in stream ecosystems (Mulholland et al. 2000), demonstrating that the chemical form of a nutrient (e.g., NH_4^+ vs NO_3^-) influences retention, processing, and export (Peterson et al. 2001; Webster et al. 2003). However, these studies were often conducted in streams with low nitrogen (N) concentrations and not in the context of an animal-derived nutrient subsidy. Nitrification is the oxic transformation of NH_4^+–N to NO_3^-–N by chemoautotrophic bacteria in stream sediments and is controlled primarily by NH_4^+ availability and oxic sediment conditions (Kemp and Dodds 2001; Strauss et al. 2002). Salmon-derived NH_4^+ converted to NO_3^- may produce a less favorable form of inorganic N for biotic assimilation due to the high energy costs of reducing NO_3^- (Dortch 1990; Hall and Tank 2003). Consequently, nitrification may result in a higher loss of salmon-derived NH_4^+ from the recipient stream habitat because NO_3^- often travels a longer distance downstream before being incorporated into the food web (Mulholland et al. 2000; Tank et al. 2008). To date, the transformation of nutrient subsidies and subsequent export of alternative nutrient forms has been overlooked in the mass-balance studies quantifying nutrient subsidies.

To study the role of biogeochemical transformations on salmon subsidies, we quantified sediment nitrification rates in multiple streams before and during an annual salmon migration. We predicted that salmon-derived NH_4^+ during the run would increase sediment nitrification rates, thereby altering the form of the nutrient subsidy and, consequently, increase streamwater NO_3^- concentrations. We also conducted experimental NH_4^+ and carcass amendments to sediments to measure the response of nitrifying bacteria to pulsed N sources. The results of our study further our understanding of the effect and fate of salmon-derived nutrients in stream ecosystems, and the microbial transformation of nutrient subsidies in general.

Methods

Site description and salmon density

We studied seven streams on Prince of Wales Island in Southeast Alaska, which has a coastal temperate

Table 1 Watershed and stream characteristics for the study streams ordered from low to high peak salmon density

Stream	Longitude (N)	Latitude (W)	Watershed size (km^2)	Baseflow discharge (L s^{-1})	Baseflow width (m)	Peak salmon density (fish m^{-2})[a]	Median sediment size (mm)
Trocadero (TRO)	55°22′	132°50′	44.8	478	13.6	0.04	64
Nossuk (NOS)	55°42′	132°17′	19.5	95	10.0	0.07	64
Slide (SLI)	55°44′	132°29′	26.0	279	9.8	0.11	64
Dog Salmon (DOG)	55°19′	132°31′	37.3	411	11.8	0.50	90
Maybeso (MAY)	55°29′	132°40′	38.7	608	18.5	0.63	45
Indian (IND)	55°26′	132°42′	25.9	273	8.8	0.73	45
Twelve Mile (TWE)	55°20′	132°43′	31.1	519	12.3	0.88	23

Stream abbreviations are used throughout the text

[a] Peak salmon density in 2008

climate. As part of a larger project, we selected our study streams across a gradient in watershed timber harvest intensity, and within each stream we selected a representative 300-m study reach located 1–5 km upstream of the estuary (Table 1). The dominant salmon on Prince of Wales Island are pink (*O. gorbuscha*) and chum (*O. keta*) salmon, which migrate and spaw0n during a 6- to 8-week period from August to October and then die after spawning (Quinn 2005). We quantified salmon density by counting all individuals in a 4-m wide belt transect every 10 m in the reach (Tiegs et al. 2008). We scaled the counts to the 300-m reach and calculated density by the average width from measurements taken at every 10 m transect.

Sediment nitrification assay

In each stream, we collected sediment samples for nitrification assays at two time periods in 2007 and three time periods in 2008. In 2007 and 2008, we sampled all streams before the salmon run (*before salmon*) and near the end of the salmon run when <50 % of the peak number of live salmon remained and carcasses were present (*during salmon + carcasses*). Additionally, in 2008, we sampled near the peak of live salmon density when few to no carcasses were present (*during salmon*). During each time period in each stream, we collected sediments from both the thalweg and margin of the stream channel to examine spatial differences in nitrification rates. We collected 4 to 6 sediment cores (30 cm^2 by 2 cm deep; ∼250 mL total) and pooled them to create a composite sediment sample for each of the two stream habitats, replicating this along five transects in each

stream reach, for a total of ten sampling locations in each stream. We sampled to 2 cm depth, and given the unconsolidated nature and low organic matter content of the sediments as well as the high dissolved oxygen concentrations in the overlying streamwater (PS Levi, unpublished data), we are confident that the sediments we sampled were generally oxic. We placed sediment samples in a cooler, returned them to the laboratory, and immediately began the nitrification assays.

We used the nitrapyrin-inhibition method to quantify sediment nitrification rates (Hall 1984; Kemp and Dodds 2001). In the laboratory, we placed 25 mL of sediment from each sample into each of two flasks, an "inhibited" and a "reference", along with 50 mL of unfiltered stream water. In the inhibited flask, we added 20 μL of a 10 % solution of nitrapyrin dissolved in dimethyl sulfoxide (DMSO), which delivers the nitrapyrin across the cell membrane and blocks the conversion of NH_4^+ to NO_3^-, thereby inhibiting nitrification. In the reference flask, we only added 20 μL DMSO, thereby allowing nitrification to occur. We incubated the flasks for 24 to 48 h on a rotary shaker at 175 rpm. After incubation, we sub-sampled 25 mL of the 75-mL sediment slurry, added 25 mL of 2 M KCl, and shook the samples for 1 h to flush NH_4^+ from cation exchange sites. We centrifuged each sample, filtered the supernatant into bottles, and froze the samples for subsequent NH_4^+ analysis (see below). To determine the nitrification rate, we calculated the difference in NH_4^+ between the inhibited and reference flasks and scaled these differences by assay duration and expressed the rates per unit sample area (mgN m^{-2} d^{-1}) and per unit organic matter content (mgN gAFDM^{-1} d^{-1}). Nitrification rates measured

using the nitrapyrin-inhibition method are likely to be higher than in situ rates because redox conditions were optimized with slurries open to the atmosphere (e.g., well-oxygenated) and incubated at room temperature (Strauss et al. 2004). However, we did not amend the sediments with additional NH_4^+ to quantify maximum potential rates (e.g., Arango and Tank 2008). Therefore, the nitrification rates reported here represent an estimate between ambient rates and the maximum potential for nitrification.

To simulate the effect of an NH_4^+ subsidy on sediment nitrification, we conducted an amendment study on sediments in two streams (IND, TWE) prior to the salmon migration. We followed the same method as described above, and for each pooled sediment sample, designated "control" (CTL) flasks that received stream water only and "nitrogen-amended" (+N) flasks that received stream water amended with NH_4Cl to 4 mg NH_4^+–N L^{-1}, a doubling of the highest exchangeable NH_4^+ concentrations observed in 2007 (Bruesewitz et al. 2006). Furthermore, to estimate the small-scale, localized effect that salmon carcasses may have on nitrification, we measured sediment nitrification directly under carcasses in two streams in 2008 (IND, TWE). We staked 8–10 naturally senesced pink salmon carcasses in the stream margin during our second sampling period (*during salmon*). When we returned for the third sampling period 19 to 22 days later (*during salmon + carcasses*), we sampled sediment directly underneath the carcasses ("treatment") and from nearby, non-carcass-amended sediments ("control"). We conducted the nitrification assay on these samples as described above.

Stream sediment characterization

From each sediment sample collected for the nitrification assays, we also measured exchangeable (i.e., porewater) NH_4^+ and NO_3^- and sediment organic matter content. We sub-sampled 30 g of sediment from each pooled sediment sample, added 30 mL of 2 M KCl, and shook these samples to flush all NH_4^+ and NO_3^- from ion exchange sites. After 1 h, we centrifuged the samples, filtered the supernatant, and froze the samples for NH_4^+ and NO_3^- analysis as described below. To determine sediment organic matter content, we sub-sampled 5 mL of sediment from each pooled sample, dried the samples at 60 °C

for 24 h, weighed them for dry mass, ashed them at 550 °C for 4 h, and re-weighed the samples. We calculated percent organic matter (%OM) as one minus the ratio of ash-free dry mass (AFDM) to dry mass multiplied by 100. In addition, we also estimated median sediment size for each stream by measuring the medial diameter of 5 random particles collected along transects every 10 m over our 300-m study reach ($n = 150$) (Bunte and Abt 2001).

Water chemistry and stream discharge

From June to October in 2007 and 2008, we collected 3 replicate water samples for dissolved inorganic nutrient analyses during each sampling visit (range = 6 to 43 samples per stream per year). We filtered the water through a 0.7 μm glass fiber filter and froze the samples for laboratory analysis. We analyzed the samples for NH_4^+–N using the sodium-hypochlorite method (APHA 2005) on a Shimadzu UV-1601 spectrophotometer (Shimadzu, Columbia, MD, USA). To determine NO_3^-–N, we used the cadmium-reduction method on a Lachat QC-8500 Flow Injection Autoanalyzer (Lachat Instruments, Loveland, CO, USA; APHA 2005). To determine stream discharge, we measured stream water velocity at fixed transects using a flow meter on multiple visits to the study reaches ($n = 5$ to 15 per stream per year).

Statistical analyses

To test for differences in inorganic N concentrations in stream water, we used a 2-way randomized-block analysis of variance (2-way RB-ANOVA, $\alpha = 0.05$, Zar 2009) with stream and time period (i.e., before versus during the salmon run) as the factors, and year as the block. We also used a 2-way RB-ANOVA to test for differences among streams and time periods in exchangeable NH_4^+ and NO_3^- concentrations during the nitrification assays (i.e., *before salmon, during salmon, during salmon + carcasses*), using habitat (i.e., channel vs. margin) as the block. Similarly, we used a 2-way RB-ANOVA to test for differences in sediment nitrification rates with stream and time period as the factors and blocked by habitat. Since we measured exchangeable N and nitrification rates for different numbers of time periods during each year (2 in 2007; 3 in 2008), we ran these analyses separately for each year, and used a Bonferroni correction to

determine statistical significance ($\alpha = 0.02$, Zar 2009). To determine whether an N amendment altered nitrification in the two streams *before salmon*, we used a 2-way ANOVA with treatment and stream as the factors. We used a Friedman's non-parametric test in lieu of ANOVA (Zar 2009) to determine whether nitrification rates in sediments beneath carcasses were different than control sediments due to an unbalanced number of samples between the two streams. We used simple linear regression (SLR, $\alpha = 0.05$, Zar 2009) to determine if exchangeable and streamwater NH_4^+ explained significant variation in nitrification rates. Furthermore, we compared our data to previously published nitrification data from reference streams, which we defined as streams with $NH_4^+ \leq 20$ μgN L^{-1} and in watersheds dominated by native vegetation, as well as rates from streams in agricultural and urban landscapes (Kemp and Dodds 2001; Bernhardt et al. 2002; Webster et al. 2003; Arango and Tank 2008). We ran SLRs with our data and these previous studies to determine if streamwater NH_4^+ predicted nitrification

rates and, secondly, if these rates predicted streamwater NO_3^- concentrations. We transformed the data with log or square-root transformations when statistical assumptions were violated. We performed all statistical analyses using SYSTAT 12 (Systat Corp., Chicago, IL, USA).

Results

Salmon nutrients increase stream water N and sediment nitrification

The concentration of both streamwater NH_4^+ and NO_3^- increased during the salmon run (Fig. 1). Prior to salmon, inorganic N concentrations were low in all streams in both years ($NH_4^+ < 15$ μgN L^{-1}; $NO_3^- < 50$ μgN L^{-1}, except for IND in 2007). In contrast, NH_4^+ and NO_3^- were higher during the salmon run (2-way ANOVA, $p < 0.001$). The magnitude of change in N varied by stream ($p < 0.001$), with some
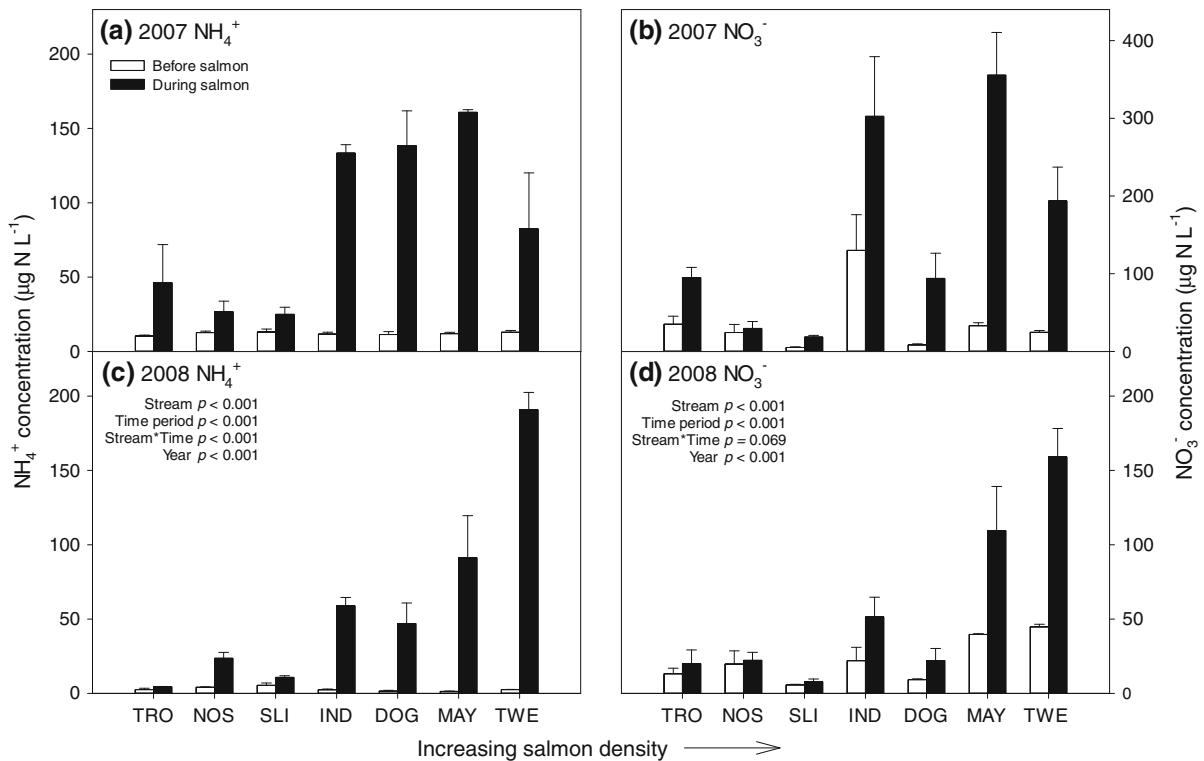
Fig. 1 Stream water concentrations (mean + SE) of dissolved **a**, **c** NH_4^+ and **b**, **d** NO_3^- before salmon and during the peak of the run in 2007 and 2008, respectively. Streams are ordered along the *x*-axis in increasing peak salmon density in 2008. Results from 2-way RB ANOVA listed for NH_4^+ (**c**) and NO_3^- (**d**)

streams changing little between time periods and others increasing orders of magnitude during the salmon run above background concentrations (median magnitude of increase for NH_4^+ = 8.9 times higher; NO_3^- = 2.6). The time period-stream interaction was significant for NH_4^+ ($p < 0.001$), but not for NO_3^- ($p = 0.07$). Similar to dissolved inorganic N, soluble reactive phosphorus (SRP) also significantly increased in all the study streams during the salmon run (see Levi et al. 2011).

Patterns in exchangeable NH_4^+ and NO_3^- from our study reflected differences among streams, time periods, and habitats. In general, exchangeable N was higher in the time periods during the salmon run in 2007 and 2008 (2-way RB-ANOVA, $p < 0.006$; except exchangeable NO_3^- in 2008, $p > 0.05$; Table 2). In both years, exchangeable NO_3^- varied among the study streams (2-way RB-ANOVA, $p < 0.001$), while exchangeable NH_4^+ varied among streams in 2008 only ($p < 0.001$). Finally, exchangeable NH_4^+ was higher in the channel margins relative to the channel thalweg (2-way RB-ANOVA, $p < 0.002$; Table 2). In contrast, exchangeable NO_3^- was higher in the channel thalweg relative to the channel margin in 2008 ($p = 0.004$), but the habitats did not differ in 2007 ($p = 0.5$).

Ambient sediment nitrification rates were low before salmon, but increased during the salmon run (Fig. 2). In both years, the differences in nitrification rate varied significantly by time period (2-way RB-ANOVA, $p < 0.001$) and mean ($\pm$SE) sediment nitrification rates tripled from *before salmon* to *during salmon + carcasses* (0.07 $\pm$ 0.01 to 0.24 $\pm$ 0.02 mgN gAFDM^{-1} d^{-1}, respectively; Fig. 2). In 2008, the mean rate *during salmon* fell between the mean *before salmon* and *during salmon + carcasses* rates (0.14 $\pm$ 0.01 mgN gAF-DM^{-1} d^{-1}). In addition, differences in nitrification rate also varied significantly by stream ($p < 0.001$), but we could not identify pairwise differences between specific time periods or streams (time period-stream interaction $p < 0.01$). Finally, nitrification rates did not differ among the two stream habitats in 2007 or 2008 (2-way RB-ANOVA $p = 0.4$ and 0.5, respectively; Table 2), suggesting that sediment nitrification potential is comparable across the entire wetted width of the stream channel.

The NH_4^+ amendment confirmed that stream sediments have the potential for greater nitrification rates when provided with additional NH_4^+. Prior to the

salmon migration, IND and TWE sediments amended with inorganic NH_4^+ had significantly higher nitrification rates than unamended sediments (2-way ANOVA, $p < 0.001$; Fig. 3a). The response to the N amendment also varied by stream ($p < 0.001$) and the treatment-stream interaction was significant ($p < 0.001$). The carcass addition experiment conducted during the salmon run confirmed the localized effect of salmon-derived NH_4^+; sediment nitrification rates were up to 100-fold higher under salmon carcasses, compared to sediments without carcasses (Friedman's test, $p = 0.014$; Fig. 3b). Interestingly, both amendments in TWE and the carcass amendment in IND increased nitrification similarly (up to ~1.5 mgN gAFDM^{-1} d^{-1}; Fig. 3), which may represent the maximum potential for nitrification in these sediments given the high NH_4^+ conditions of the treatments.

Predictors of stream nitrification rates

Sediment nitrification rates were positively related to exchangeable and streamwater NH_4^+ (SLR, exchangeable NH_4^+ $R^2 = 0.71$, $p = 0.001$; streamwater NH_4^+ $R^2 = 0.50$, $p < 0.001$; Fig. 4a, b), which both increased during the salmon run. Furthermore, nitrification rates weakly predicted streamwater NO_3^- concentrations ($R^2 = 0.19$, $p = 0.008$). Although nitrification rates were higher during the salmon run, the rates were not related to our measure of salmon density (SLR, $p = 0.12$).

We scaled our data to areal rates (mgN m^{-2} d^{-1}) to compare nitrification in Southeast Alaska streams with previously published rates from reference, agricultural, and urban streams. Ambient NH_4^+ concentrations and sediment nitrification rates were similar between our streams *before salmon* and other reference streams (e.g., $NH_4^+ \leq 20$ µgN L^{-1}, nitrification rates <100 mgN m^{-2} d^{-1}; Fig. 5a), although no previous study had been conducted in coastal temperate rainforest streams. In contrast, NH_4^+ concentrations (range = 4–151 µgN L^{-1}) and nitrification rates (range = 26–300 mgN m^{-2} d^{-1}) during the salmon run were up to 10-fold higher than previously reported data in reference streams. Areal nitrification rates were predicted by NH_4^+ concentrations in both our study and studies in other reference streams (SLR; $R^2 = 0.64$ and 0.21, respectively; $p < 0.001$ for both; Fig. 5a). Furthermore, nitrification rates predicted

Table 2 Mean (SE) exchangeable NH_4^+ and NO_3^- concentrations and nitrification rates from the channel and margin habitats with streams ordered in terms of increasing peak salmon density

| | | 2007 | | | | 2008 | | | | | |
| | | Before | | During + Carcasses | | Before | | During | | During + Carcasses | |
		Channel	Margin	Channel	Margin	Channel	Margin	Channel	Margin	Channel	Margin
Porewater NH_4^+ (μg N L^{-1})	TRO	94.1 (47.2)	332.2 (123.1)	85.6 (22.6)	82.3 (12.6)	50.7 (7.9)	58.8 (6.1)	97.0 (n.a.)	85.0 (8.6)	38.9 (3.2)	97.0 (34.8)
	NOS	48.4 (5.3)	283.1 (182.6)	925.5 (243.5)	821.3 (407.1)	63.0 (18.9)	61.1 (10.0)	431.5 (94.3)	319.7 (86.4)	231.1 (62.7)	236.2 (116.6)
	SLI	67.5 (4.0)	157.1 (114.5)	26.1 (5.3)	303.7 (166.2)	75.5 (5.1)	125.6 (48.7)	<0.1 (0.0)	<0.1 (0.0)	22.9 (1.2)	107.2 (55.8)
	DOG	39.1 (9.2)	48.3 (18.9)	206.0 (136.7)	2056.3 (986.2)	47.9 (0.1)	66.4 (17.5)	55.1 (2.5)	244.6 (59.8)	130.4 (16.3)	250.3 (90.3)
	MAY	85.0 (19.0)	326.8 (122.8)	287.7 (34.0)	2722.7 (1255.1)	79.2 (19.0)	246.0 (135.9)	258.8 (24.1)	568.5 (51.9)	178.5 (18.1)	431.1 (78.4)
	IND	101.0 (40.8)	307.2 (158.2)	137.8 (37.6)	429.9 (245.5)	56.2 (8.5)	62.1 (10.3)	126.8 (15.2)	206.0 (96.0)	196.0 (49.4)	122.6 (17.3)
	TWE	145.5 (26.0)	175.9 (26.0)	340.9 (60.1)	989.2 (325.6)	178.7 (56.2)	338.4 (173.9)	185.7 (25.9)	1085.4 (390.1)	295.6 (59.3)	1071.4 (339.9)
Porewater NO_3^- (μgN L^{-1})	TRO	78.0 (59.6)	43.7 (32.0)	150.5 (29.6)	123.6 (31.1)	40.5 (3.0)	43.1 (7.6)	62.8 (0.0)	86.8 (8.7)	23.0 (6.5)	46.1 (5.4)
	NOS	26.2 (14.8)	82.6 (34.1)	350.7 (56.2)	310.5 (86.2)	71.7 (21.4)	67.5 (21.2)	190.4 (42.6)	118.6 (27.2)	116.7 (28.1)	99.1 (17.4)
	SLI	17.8 (8.1)	17.9 (8.0)	98.4 (10.7)	64.8 (11.0)	<0.1 (0.0)	8.1 (8.1)	<0.1 (0.0)	<0.1 (0.0)	9.4 (9.4)	5.7 (2.2)
	DOG	7.3 (4.3)	0.8 (0.8)	157.9 (21.8)	139.9 (34.0)	101.2 (2.5)	77.7 (14.8)	79.9 (12.1)	66.5 (36.8)	135.4 (14.3)	118.5 (33.9)
	MAY	3.4 (3.4)	34.8 (10.1)	459.0 (57.0)	404.6 (169.4)	106.6 (11.9)	112.0 (31.7)	102.6 (10.4)	41.1 (20.4)	140.0 (8.2)	107.0 (33.5)
	IND	118.9 (46.4)	170.3 (66.6)	319.0 (25.8)	312.7 (53.0)	104.1 (9.0)	134.3 (7.2)	252.5 (27.5)	267.4 (110.8)	274.0 (48.6)	153.2 (14.8)
	TWE	51.8 (21.3)	87.3 (11.4)	462.3 (32.1)	294.4 (198.1)	162.8 (15.8)	169.7 (19.7)	187.1 (34.1)	97.3 (51.4)	225.3 (25.3)	78.3 (24.4)
Nitrification rates (mgN gAFDM^{-1} d^{-1})	TRO	0.02 (0.01)	0.02 (0.02)	0.10 (0.05)	0.02 (0.01)	0.03 (0.02)	0.04 (0.01)	<0.01 (n.a.)	0.07 (0.01)	0.04 (0.01)	0.10 (0.02)
	NOS	0.05 (0.02)	0.11 (0.02)	0.24 (0.07)	0.27 (0.11)	0.01 (0.01)	0.03 (0.02)	0.14 (0.02)	0.09 (0.02)	0.11 (0.05)	0.15 (0.02)
	SLI	0.13 (0.01)	0.07 (0.02)	0.04 (0.02)	0.06 (0.01)	0.01 (0.01)	0.04 (0.03)	0.0 (n.a.)	0.0 (n.a.)	0.05 (0.03)	0.07 (0.02)
	DOG	0.13 (0.07)	0.06 (0.03)	0.55 (0.14)	0.44 (0.18)	0.12 (0.05)	0.02 (0.02)	0.19 (0.02)	0.16 (0.04)	0.44 (0.06)	0.33 (0.08)
	MAY	0.04 (0.01)	<0.01 (0.0)	0.24 (0.05)	0.56 (0.14)	0.03 (0.01)	0.04 (0.01)	0.13 (0.01)	0.17 (0.01)	0.10 (0.03)	0.29 (0.12)
	IND	0.04 (0.01)	0.09 (0.01)	0.18 (0.04)	0.20 (0.02)	0.10 (0.06)	0.14 (0.07)	0.27 (0.07)	0.29 (0.06)	0.41 (0.22)	0.15 (0.05)
	TWE	0.08 (0.03)	0.11 (0.05)	0.27 (0.10)	0.29 (0.05)	0.15 (0.04)	0.17 (0.03)	0.19 (0.01)	0.24 (0.04)	0.68 (0.15)	0.27 (0.07)
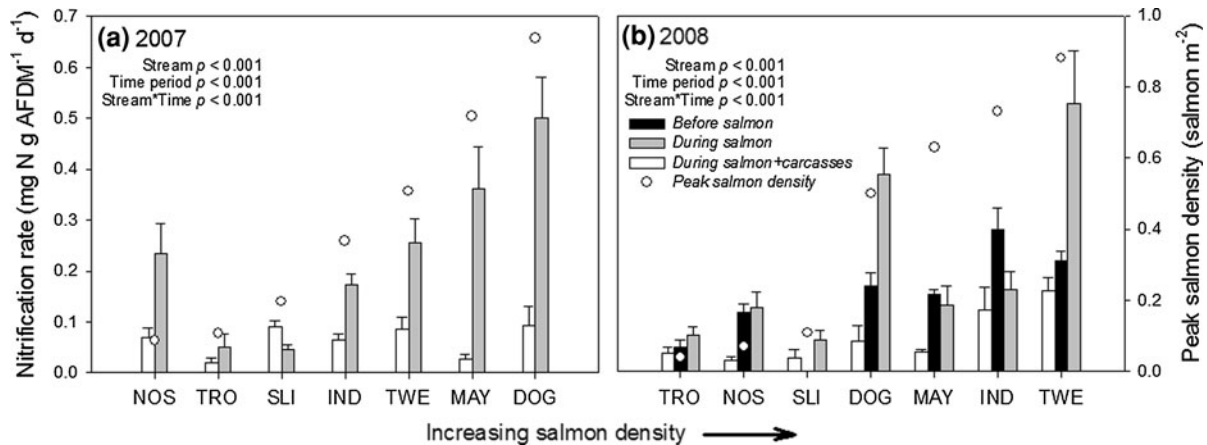
Fig. 2 Stream sediment nitrification rates (mean + SE) during the three time periods, *before salmon*, *during salmon*, and *during salmon + carcasses*, in **a** 2007 and **b** 2008. In 2007, rates were not measured in the *during salmon* period. The *open circles* represent the peak salmon density in each stream, which are arranged along the *x*-axis by increasing density. Note that the stream ordering differs in 2007 and 2008 due to interannual variation in peak salmon density
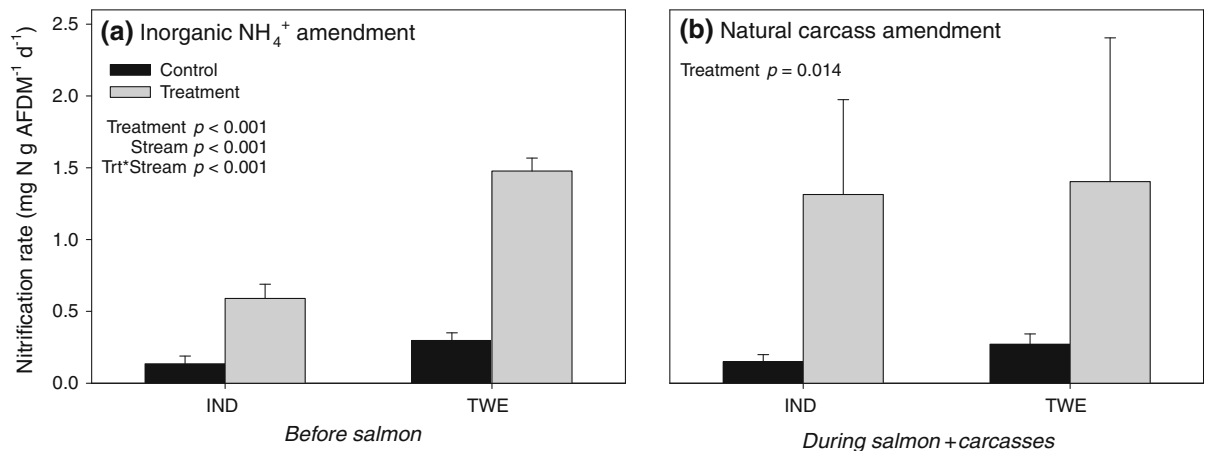
Fig. 3 Stream sediment nitrification rates (mean + SE) resulting from **a** an inorganic NH_4^+ amendment before salmon and **b** a natural carcass amendment *during salmon + carcasses* period in IND and TWE. *Control bars* represent rates of unamended sediments at the same time period (*before salmon* for (**a**) and *during salmon + carcasses* for (**b**))

stream NO_3^- concentrations in both the salmon-bearing streams and reference streams ($R^2 = 0.40$ and 0.35, respectively; $p < 0.001$ for both; Fig. 5b). When pooling all the data from our study and previously published rates from streams draining watersheds of varying land-use (e.g., reference, agriculture, urban), streamwater NH_4^+ predicted nitrification rates (SLR; $R^2 = 0.35$; $p < 0.001$; Fig. 5c) and, consequently, nitrification rates predicted stream NO_3^- ($R^2 = 0.34$; $p < 0.001$; Fig. 5d).

Discussion

Salmon-derived NH_4^+ subsidy increases sediment nitrification in streams

Our research provides multiple lines of evidence that salmon-derived NH_4^+ subsidies can create hot spots and hot moments of sediment nitrification (sensu McClain et al. 2003). First, nitrification during the salmon run was positively related to exchangeable and
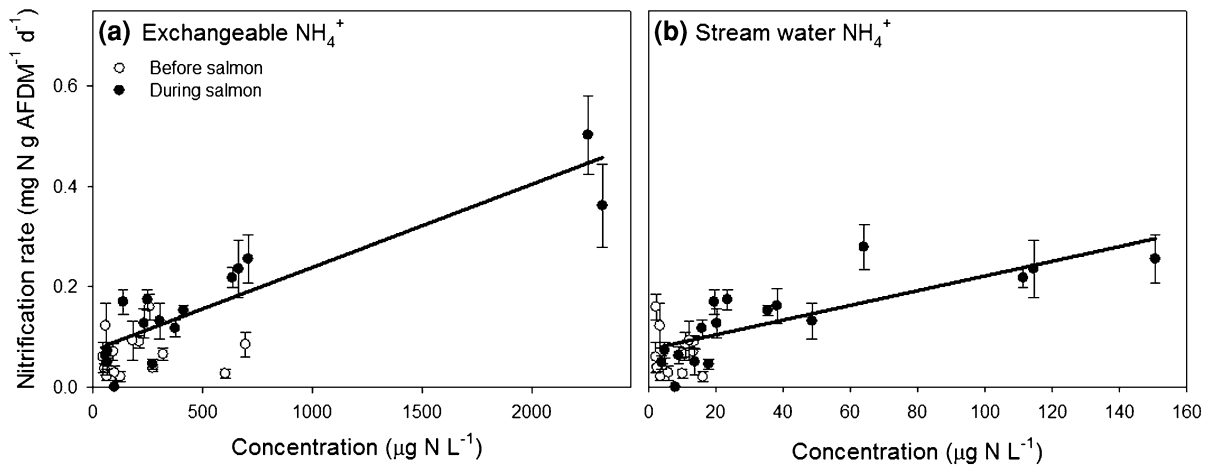
Fig. 4 Linear regressions of sediment nitrification rates (mean $\pm$ SE) and **a** exchangeable NH_4^+ and **b** streamwater NH_4^+. *Bars* on each data point represent standard error
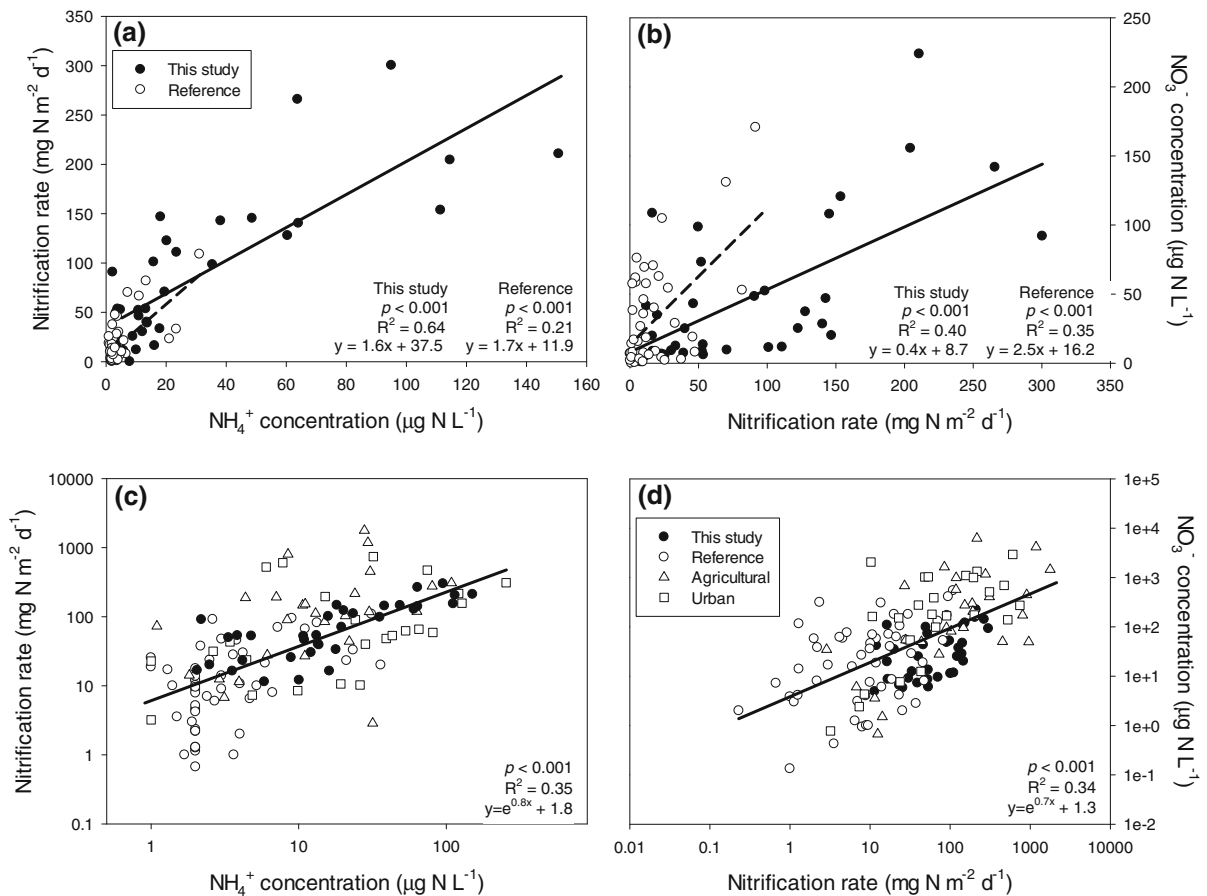
Fig. 5 Linear regressions of **a**, **c** streamwater NH_4^+ and sediment nitrification rates and **b**, **d** sediment nitrification rates and streamwater NO_3^-. *Closed circles* represent data from this study and *open symbols* represent data from previous studies of sediment nitrification from reference, agricultural, and urban streams (*circle*, *triangle*, and *square*, respectively; Bernhardt et al. 2002; Webster et al. 2003; Arango and Tank 2008, LINX, unpublished data)

streamwater NH_4^+, the substrate required for nitrification (Strauss et al. 2002). Second, the experimental amendment of inorganic NH_4^+ *before salmon* consistently increased sediment nitrification rates, evidence a hot moment occurs when NH_4^+ concentrations are elevated, such as during the salmon run. Third, nitrification was higher directly beneath carcasses relative to sediments without carcasses, which suggests that salmon carcasses created a hot spot on a localized scale. Together, these data demonstrate that sediments in salmon-bearing streams have the capacity to rapidly process additional sources of NH_4^+, which may mediate the ecosystem effects of the salmon-derived nutrient subsidy.

While other studies have demonstrated the importance of resident fish on stream nutrient cycling (e.g., McIntyre et al. 2008), no previous study has investigated the role of migratory fish on stream nutrient transformations. Salmon-derived NH_4^+ increased sediment nitrification rates, which in turn altered the form of the subsidized nutrient from NH_4^+–N to NO_3^-–N. This biogeochemical transformation, leading to increased stream water NO_3^-, has potentially important ecological consequences. Often, NO_3^- is less preferred by aquatic autotrophs and heterotrophs than NH_4^+ (e.g., Raven et al. 1992), because the reduction of NO_3^- to NH_4^+ requires additional energy (Dortch 1990). Therefore, as the by-product of nitrification, the temporarily elevated NO_3^- concentrations may alter the community composition of autotrophs in the benthic biofilm (Raven et al. 1992). Furthermore, the transformation may dampen the local enrichment of stream food webs and extend the effects of the subsidy to downstream ecosystems (e.g., Cak et al. 2008), such as estuaries that may be N-limited (Howarth and Marino 2006). Food web responses to the biogeochemical transformations of nutrient subsidies warrants further study in order to better understand the ecological consequences of these transformations.

Our finding that salmon-derived NH_4^+ creates a hot moment in biogeochemical transformation rates is consistent with studies that have reported concurrent increases in NH_4^+ and NO_3^- during salmon runs (Johnston et al. 2004; Levi et al. 2011; Tiegs et al. 2011). However, not all salmon-derived nutrient studies have reported increases in NO_3^- (Janetski et al. 2009). For example, NO_3^- dynamics in other Southeast Alaska streams were similar above and

below salmon barriers before and during the run (Chaloner et al. 2004; Mitchell and Lamberti 2005), suggesting that patterns in NO_3^- were linked to watershed processes. In our study, mean daily discharge did not differ significantly before and during the salmon run across streams, and NO_3^- concentrations did not vary significantly within a stream before the salmon run (Levi et al. 2011); therefore, increases in NO_3^- were likely a result of in-stream nitrification. The contrasting results between studies may be due to localized habitat conditions that could vary from stream to stream, and, as a result, could augment or inhibit nitrification (e.g., sediment organic matter content, pH; Strauss et al. 2002). In addition, variation in watershed and regional characteristics (e.g., riparian vegetation, climate; Compton et al. 2003) may also influence streamwater N concentrations and nitrification rates.

While salmon-derived NH_4^+ increased nitrification rates in temperate rainforest streams, salmon subsidies may not have consistent effects across all salmon-bearing streams. In addition to their role as a nutrient subsidy, salmon act as ecosystem engineers and disturb sediments and benthic biofilm during their migration and spawning (Moore et al. 2004), the magnitude of which depends on physical stream characteristics (e.g., Tiegs et al. 2008). Although sediment size varied among our streams, benthic disturbance by spawning salmon did not appear to negatively influence nitrifying bacteria. However, in sand-bottom or fine-sediment streams, benthic disturbance may disrupt the bacterial assemblage and alter the biogeochemical transformations associated with the salmon nutrient subsidy.

Nitrification of salmon NH_4^+ in context: dissimilatory transformation versus assimilatory uptake

Nitrification rates have been quantified in streams located in eight biomes of North America as part of the Lotic Intersite Nitrogen eXperiment (LINX; Webster et al. 2003), as well as in several other studies (Kemp and Dodds 2001; Bernhardt et al. 2002; Arango and Tank 2008). The areal nitrification rates we quantified before the salmon run (mean $\pm$ SE = 31.9 $\pm$ 7.6 mgN m^{-2} d^{-1}) were similar to rates in other reference streams (19.6 $\pm$ 4.1 mgN m^{-2} d^{-1}), despite differences in geomorphology, geology, and climate. In

contrast, the rates we measured during the salmon run were, on average, 6 times higher (134.4 ± 31.9 mgN m^{-2} d^{-1}) and more similar to rates measured in streams in agricultural and urban watersheds (Webster et al. 2003, LINX II, *unpublished data*; Fig. 5c, d). The higher rates we quantified during the salmon run extend both the range of streamwater NH_4^+ and nitrification rates previously measured in reference streams, defined as streams with low background NH_4^+ and in watersheds dominated by natural vegetation. Furthermore, NH_4^+ concentrations and nitrification rates in our Southeast Alaska study streams, where watershed development is low, were comparable to concentrations and rates in streams draining agricultural or urban watersheds, demonstrating that streams in relatively pristine landscapes have the capacity to transform and process elevated inorganic N loads.

Both assimilatory uptake and dissimilatory processes, such as nitrification, contribute to the removal of dissolved NH_4^+ from the water column. To place our nitrification rates in the context of total NH_4^+ demand, which we did not measure directly, we used NH_4^+ uptake velocities (V_f) from two previously published studies—one conducted in Alaska tundra streams (Webster et al. 2003) and the other in a coastal temperate stream in Oregon (Ashkenas et al. 2004). The V_f estimates from both studies were similar (mean ± SE = 13.3 ± 1.6 mm min^{-1}), suggesting the NH_4^+ V_f in the coastal temperate streams of Southeast Alaska, located midway between those two sites, may be similar. Using this estimate of V_f from the literature, we calculated the NH_4^+ uptake per area in our streams (U; μgNH_4^+–N m^{-2} min^{-1}) using the relationship between V_f and U (U = V_f * [NH_4^+–N]; Stream Solute Workshop 1990). Our calculations suggest that approximately 25 % of the NH_4^+ removed from the water column may be attributed to nitrification, both before and during the salmon run. In salmon-bearing streams, sediment nitrification is likely a significant component of NH_4^+ uptake, especially given the elevated concentrations of NH_4^+ during the salmon run. In the one study of N uptake in a forested stream in the Pacific Northwest, Ashkenas et al. (2004) reported that nitrification accounted for 40–50 % of NH_4^+ uptake, similar to the percentage we estimated for Southeast Alaskan streams. One caveat to our calculations is that we assumed that NH_4^+ demand is not saturated during the

salmon run, such that V_f would remain similar through time. We cannot confirm constant NH_4^+ demand during elevated conditions because no studies have examined NH_4^+ V_f in relation to salmon subsidies. However, we have evidence from our carcass addition experiment that nitrification demand for NH_4^+ was not saturated as the nitrifying bacteria were able to further process even higher concentrations of NH_4^+ (range of exchangeable NH_4^+ = 229–23,900 μgN L^{-1}).

Biogeochemical transformations alter nutrient subsidies

Nitrogen subsidies may undergo diverse, but largely overlooked transformations in their recipient ecosystems. In a study of riparian biogeochemical transformations and salmon nutrient subsidies, Holtgrieve et al. (2009) found that bears foraging on salmon increased NH_4^+ availability and fluxes of nitrous oxide (N_2O) and carbon dioxide (CO_2). Other studies have examined the influence of land use or anthropogenic nutrient inputs (e.g., agricultural run-off) on the N-cycle in streams. For example, Mulholland et al. (2008) reported a strong relationship with NO_3^- concentration and denitrification in streams across multiple biomes and land uses. In our study, nitrifying bacteria responded to a pulsed subsidy of N, both from the natural subsidy during the salmon run and our artificial NH_4^+ amendment (i.e., hot moment) as well as the experimental under-carcass sampling (i.e., hot spot). While natural and anthropogenic nitrogen subsidies have been studied to some extent, less is known about the transformations of other nutrient subsidies, such as carbon and phosphorus.

In summary, biogeochemical transformations can fundamentally alter nutrient subsidies. These transformations may extend or dampen the subsidy's ecosystem effects by diversifying the nutrient form. Such transformations may lead to biogeochemical hot spots or hot moments in the recipient ecosystems (McClain et al. 2003). In our study, we present evidence that an organism's life history can stimulate biogeochemical transformations via the transfer of nutrient subsidies across ecosystem boundaries. A simple mass balance approach to subsidies does not sufficiently explain the dynamics of the subsidized nutrients in recipient ecosystems, because nutrients are not passively retained or exported in their original form. Rather, the nutrients provided by migratory and

highly mobile animals also stimulate biogeochemical transformations, further altering the structure and function of the recipient ecosystem.

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