

Estimation of nutrient contributions from the ocean

K. Nakayama

Estimation of nutrient contributions from the ocean across a river basin using stable isotope analysis

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Abstract

Since marine derived nutrients (MDN) are transported not only in river channels but also across the entire river basin, including via ground water and migratory animals, it is necessary to investigate the contribution of MDN to the forest floor (soils) in order to quantify the true role of MDN at the river ecosystem scale. This study investigated the contribution of pink (*Oncorhynchus gorbuscha*) and chum salmon (*O. keta*) to total oceanic nitrogen (TN) input across a river basin using stable isotope analysis (SIA) of nitrogen ($\delta^{15}\text{N}$). The contribution of TN entering the river basin by salmon was 23.8% relative to the total amount of TN exported from the river basin, providing a first estimate of MDN export for a river basin. The contribution of nitrogen from the ocean to the river basin soils was between 22.9 and 23.8%. Furthermore, SIA showed that the transport of oceanic TN by sea eagles (*Haliaeetus* spp.) was greater than that by bears (*Ursus arctos*), which had previously been that bears are thought to be the major animal transporter of nutrients in the northern part of Japan.

1 Introduction

SIA is increasingly being used to examine connectivity in coastal aquatic-terrestrial ecosystems, such as the input of MDN from the open ocean to coastal and widely river ecosystems (Wyatt et al., 2010a, b, 2012). In the case of river ecosystems, the transportation of nutrients, such as nitrogen and phosphorus, by migrating fish results in enhancement of biofilms and planktonic productivity in river systems (Juday et al., 1932; Cederholm and Peterson, 1985; Bilby et al., 1996; Gresh et al., 2000; Chaloner et al., 2002; Moore and Schindler, 2004; Yanai and Kochi, 2005; Levi and Tank, 2013). Most of those cases, many terrestrial consumers like mammals, birds and insects have been shown to play a large role in terms of providing MDN to watersheds (Donaldson, 1966; Ben-David et al., 1997a; Hinderbrand et al., 1999; Gende et al., 2002; Naiman et al., 2002; Wilkinson et al., 2005; Bartz and Naiman, 2005). Moreover, MDN inputs

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have been shown as important processes controlling the productivity of ecosystem. For example, Merz and Moyle (2006) found that the contribution of MDN to the foliar nitrogen of wine grapes was about 18 to 25 %. Also, Hilderbrand et al. (1999) demonstrated that trees and shrubs near spawning streams receive 24 to 26 % of the foliar nitrogen from MDN, while Helfield and Naiman (2002) suggested that 15.5 to 17.8 % of spruce foliage nitrogen may be provided from MDN. Thus, isotopic methods as intrinsic geospatial tracer provided quantification of cross-ecosystem transfer of nutrients. In particular, migrating fish, such as salmon, have been found to be necessary for a sustainable nutrient-cycle system due to their important role as nutrient transporters (Ben-David et al., 1998; Wipfli et al., 1998; Yanai and Kochi, 2005; Gende et al., 2007; Hocking and Reimchen, 2009; Hocking and Reynolds, 2011). Additionally, MDN has been demonstrated to be important not only for river ecosystems but also potentially for upstream lakes (Kline et al., 1990, 1993; Schindler et al., 2003).

Here we focus on the nutrient budgets at the river basin scale of using stable isotopes. When we consider nutrient flux in a river flowing from the upstream end into the ocean, the flux depends on nutrients supplied not only inside the river itself but from the entire river basin (Dutta and Nakayama, 2010; Alam and Dutta, 2012; Riggsbee et al., 2008). Cederholm et al. (1989) demonstrated that mammals and birds consume migrating fish, which may result in the secondary dispersion of MDN across the river basin associated with the movement of these consumers. Other studies have revealed that mammals incorporate MDN from salmon, which may subsequently lead to re-export to the ocean through river flows (Bilby et al., 1996; Ben-David et al., 1997a, b; Hilderbrand et al., 1999; Szepanski et al., 1999; Reimchen, 2000). However, the contribution of MDN at the river basin scale has not been adequately quantified in natural systems because of difficulty to show those complex food web and accurate biomass.

In this study we present the contribution of MDN to total nutrient inputs across an entire river basin, focusing on the proportion of MDN transported by salmon. Integrated stable isotope researches in the geological, hydrological and biological aspects allowed

us to estimate nutrient budgets in natural river basin and convinced us to conserve the ocean- river connectivity.

2 Geophysical setting

Our target area, the Shiretoko Peninsula, was registered as a World Natural Heritage area in July of 2005. Shiretoko is located at the southernmost extent of drift ice and its ecological systems exhibit high biodiversity and high rates of nutrient circulation, particularly due to runs of pink (*Oncorhynchus gorbuscha*) and chum (*O. keta*) salmon from the Sea of Okhotsk. Potential runs of salmon along the coast of Hokkaido in the Sea of Okhotsk have been estimated at about 29 900 000 individuals a year (Hokkaido National Fisheries Research Institute, Fisheries Research Agency, 2009), equivalent to 2590 t of total nitrogen. The size of the Okhotsk coastal region of Hokkaido is about 24 000 km², which corresponds to that the mean total nitrogen input from the ocean is about 108 kg km⁻² yr⁻¹ if we assume that all salmon run up rivers and the total nitrogen is distributed into the river basins completely. Shiretoko is located on the north-east coast of Hokkaido, Japan (approximately 43°57' N to 44°21' N and 144°58' E to 145°23' E), and has a width, length and maximum altitude of about 15 km, 50 km and 1660 m, respectively (Fig. 1). The Rausu River Basin was selected as a study area because its watershed is the largest in the region and it is considered a representative watershed in the Shiretoko Peninsula. The watershed area, river length, and the mean river slope are 32.5 km², 7 km, 1/7, respectively (Fig. 2). Field experiments were carried out over 5 years from 2008 to 2012.

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3 Methods

3.1 Nitrogen from a river basin to the ocean

TN, total dissolved nitrogen (TDN) and total particulate nitrogen (TPN) were measured at St.0 around the river mouth from 2007 to 2009 (Fig. 2). The nitrogen concentration of filtered and non-filtered water samples were analyzed by the cadmium reduction-colorimetric method. Annual TN and annual TDN export to the ocean were evaluated using the river discharge at St.0 with TDN-discharge and TPN-discharge curves. The TDN-discharge and TPN-discharge curves were produced using ten different peak discharge floods and base flow discharges. As river discharge was not measured during the winter season from January to March, a storage function method was applied to estimate river discharge from 2008 to 2012 (Michael, 1978; Michael et al., 1979). The validity of the storage function method was confirmed through comparison with the observed river discharge from April to December.

3.2 Salmon runs

To evaluate the contribution of salmon to soil organic matter (SOM), the size of salmon runs were investigated in the Rausu River. Salmon were caught at the river mouth for artificial incubation and release, providing an estimate of the number of salmon caught by the apparatus (Hokkaido National Fisheries Research Institute, Fisheries Research Agency, 2009). The apparatus for catching salmon consisted of bamboo, which does not obstruct flood flow or completely block the runs of salmon. Therefore, it was necessary to quantify the capture rate of the apparatus in order to estimate the actual volume of salmon runs. We thus installed infrared cameras (SM-AVIR-602S, Hero Corp., Izumo, Japan) that enabled us to capture images from the 25 to 28 November in 2013 and from the 4 to 7 December in 2013 to count the runs of salmon. Field observation were conducted in the Tokorohoronai River, which is located in the same region

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of Hokkaido and has a width of 3 m and a length of about 6 km, instead of the Rausu River because the latter is too wide (about 15 m) to quantify the total salmon run.

3.3 Stable isotope analysis

MDN, such as nitrogen, are generally supplied from the ocean to surface soils in a river basin as SOM, as feces of mammals, droppings of birds, and the remains of salmon preyed upon by mammals, birds and insects. To focus on the influence of SOM on nitrogen in the river basin soils, soil particles with diameter of less than 500 μm were used in the analysis. In general, some proportion of the nitrogen is reduced into the atmosphere due to denitrification, which indicates the difficulty evaluating total amount of supplied nitrogen. Nevertheless, we made an attempt to estimate the contribution of MDN to SOM by sampling surface soils across the Rausu River Basin (Fig. 2). Surface soil samples were taken at 12, 20 and 21 stations in 2008, 2009 and 2012, respectively. In 2008, fewer samples were taken as we did not have permission to sample surface soils in special protection zones. Surface soils were sampled from three different points at each station in a volume of 5 cm \times 20 cm \times 20 cm (height \times width \times depth). Surface soil sampling stations in 2012 are shown in Fig. 2. Stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) were measured using a Delta Plus Advantage mass spectrometer (Thermo Electron) coupled with an elemental analyzer (Flash EA 1112, Thermo Electron) at the Port and Airport Research Institute, Japan. Stable isotope ratios are expressed in δ notation as the deviation from standards in parts per thousand (‰) according to the following equation:

$$\delta^{13}\text{C}, \delta^{15}\text{N} = [R_{\text{sample}}/R_{\text{standard}} - 1] \cdot 10^3 \quad (1)$$

where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$.

PeeDee Belemnite and atmospheric nitrogen were used as the isotope standards of carbon and nitrogen, respectively. The analytical precision in the mass spectrometer system based on the SD of the internal reference (L-histidine) replicates was < 0.15 for

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both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. The contribution of MDN to SOM in surface soils was evaluated by applying a two source mixing model based on stable isotope analysis (SIA) of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) (Kline et al., 1998; Moore and Semmens, 2008; Hossler and Bauer, 2012). Three soil samples were collected at each sampling station in order to account for small scale variability in SOM (Fig. 2). Salmon tissue isotopes were considered representative of the isotope composition of ocean productivity. To isotopically characterize terrestrial productivity, we considered one terrestrial end-members (sources): Soil Samples exhibiting the Lowest values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (hereafter SSL), and thus assumed to have the highest terrestrial contribution to SOM. Representative soil samples collected in the same river basin were chosen because they have isotopically similar characteristics to the target soil samples in this study.

As bamboo grass (*Sasa senanensis*) is the dominant species in the study area, bamboo grass was collected. Furthermore, to investigate the contribution of the other typical mammals and birds in Shiretoko to SOM, and to roughly understand these animals diets, droppings of sea eagles (*Haliaeetus* spp.) and feces of brown bear (*Ursus arctos*) were collected (Kuwae et al., 2008, 2012). Dropping and feces samples can reduce the uncertainty in terms of SIA fractionation factors when compared to tissue samples (e.g., muscle, liver, and blood). As fractionation occurs during the making or breaking of bonds in small molecules, we might not expect fractionation during food assimilation, i.e., uptake of large molecules, in the absence of the breaking of nitrogen bonds (Fry, 2006). Thus, while tissue samples have variability and uncertainty related to fractionation factors (body conditions such as fasting), we consider that feces and dropping samples do not. However, in the case of multiple food sources, feces and dropping are likely to be enriched in relatively indigestible food sources, when compared with stomach contents or assimilated materials (Sponheimer et al., 2003; Kuwae et al., 2008). A further advantage of using droppings, as opposed to tissues, is that no killing and/or damage to wildlife is involved in collecting samples. Multivariate analysis of variance (MANOVA) and post hoc tests by Tukey–Kramer were used to investigate differences in surface soil samples.

The contribution of MDN was evaluated using a two sources mixing model based on the measured $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. The average contribution in the Rausu River Basin was computed using each sub-basin area obtained from the Thiessen method.

$$f_{\text{C_MDN}} + f_{\text{C_LDN}} = 1 \quad (2)$$

$$f_{\text{C_MDN}}\delta^{13}\text{C}_{\text{salmon}} + f_{\text{C_LDN}}\delta^{13}\text{C}_{\text{SSL}} = \delta^{13}\text{C}_{\text{soil}} \quad (3)$$

$$f_{\text{N_MDN}} + f_{\text{N_LDN}} = 1 \quad (4)$$

$$f_{\text{N_MDN}}\delta^{15}\text{N}_{\text{salmon}} + f_{\text{N_LDN}}\delta^{15}\text{N}_{\text{SSL}} = \delta^{15}\text{N}_{\text{soil}} \quad (5)$$

where $f_{\text{C_MDN}}$ and $f_{\text{C_LDN}}$ are the contributions of MDN and land-derived nutrient (LDN), $\delta^{13}\text{C}_{\text{salmon}}$, $\delta^{13}\text{C}_{\text{SSL}}$ and $\delta^{13}\text{C}_{\text{soil}}$ are the stable isotope ratios of carbon for salmon, SSL and soil samples, respectively, $f_{\text{N_MDN}}$ and $f_{\text{N_LDN}}$ are the contributions of MDN and LDN, $\delta^{15}\text{N}_{\text{salmon}}$, $\delta^{15}\text{N}_{\text{SSL}}$ and $\delta^{15}\text{N}_{\text{soil}}$ are the stable isotope ratios of nitrogen for salmon, SSL and soil samples, respectively.

4 Results and Discussion

4.1 Estimation of nitrogen export to the ocean

During 2007 to 2009 the concentration of TDN was observed to be roughly constant, 0.090 mg L^{-1} (SD 0.022 mg L^{-1}), regardless of the discharge in the Rausu River. In contrast, TPN was revealed to be a function of river discharge ($r^2 = 0.88$; Eq. 5). It should be noted that TPN showed a strong correlation with suspended sediment concentrations (SS), with SS increasing with increasing river discharge. Also, TPN was confirmed to be associated with salmon spawning activities, which has been already revealed by Moore et al. (2007).

$$\text{TPN} = 0.0032 \cdot Q^{1.771} \quad (6)$$

where Q is the river discharge ($\text{m}^3 \text{ s}^{-1}$).

The validity of the storage function method model was confirmed using the observed river discharge from April to September of 2009, which resulted in a Coefficient of Determination (CoD) of 0.61. The reliability of the model has been shown to be high enough for the analysis of river discharge when the CoD is more than 0.6 (Dutta and Nakayama, 2010). Annual mean export of TDN, TPN and TN from 2008 to 2012 were 5210, 14 750 and 19 960 kg yr⁻¹, respectively. Since the size of the Rausu River Basin is 32.5 km², the annual mean export of TDN, TPN and TN per unit catchment area were 160, 454 and 614 kg km⁻² yr⁻¹, respectively (Table 1). The average concentrations of TDN and TPN from 2008 to 2012 were 0.090 and 0.216 mg L⁻¹, which agree with a previous study at the site (Nakayama et al., 2011).

4.2 Contribution of salmon runs to nitrogen input from the ocean

The average number of salmon passing the cameras in the Tokorohoronai River during the 4 days while the apparatus for catching salmon was present was 0.49 h⁻¹. The average numbers for 4 days after the apparatus was removed from the river was 0.61 h⁻¹, so the rate of capture of salmon by the apparatus was estimated as 20 %: (0.61-0.49)/0.61 = 0.20.

In the Rausu River, the annual average numbers of salmon caught by the apparatus at the river mouth were 2075 and 10 580 for chum and pink salmon, respectively, from 2001 to 2009. By assuming that all apparatuses have the same rate of capture, the chum and pink salmon runs can be estimated as 15 375 and 52 900, respectively. The average weight of chum and pink salmon are 3.3 and 2.0 kg, respectively (Makiguchi et al., 2007), with a nitrogen content of about 30.4 g kg⁻¹ (Larkin and Slaney, 1997). Therefore, annual TN transported by chum and pink salmon is estimated to be 1542 and 3216 kg yr⁻¹, respectively. Finally, the annual TN transported by chum and pink salmon per unit catchment area can be estimated as 146 kg km⁻² yr⁻¹ (SD 19 kg km⁻² yr⁻¹), which corresponds to the contribution of TN by salmon, 23.8 % (SD 3.1 %), relative to the annual outflow of TN per unit area (considered to be 100 %) (Table 1).

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4.3 Total input from the ocean to a river basin

The 2012 field experiment suggested that stable isotope ratios, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, were relatively higher close to the ocean (stations 20 and 21) compared to the top of the mountain (station 19), which has been also demonstrated by Kline et al. (1998) (Fig. 2). Tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were highest in the sea eagles ($n = 8$) and lowest in the bamboo grass ($n = 3$). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of SOM ($n = 53$) lay between sea eagles and bamboo grass (Fig. 3). MANOVA suggested that there was no isotopic ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) difference between salmon tissue ($n = 12$) and sea eagle feces. Feces from brown bears ($n = 7$), which were previously thought to be the major terrestrial consumer of spawning salmon, were significantly lower than those of salmon and sea eagles (bear vs. salmon ($\delta^{13}\text{C}$): $P < 0.001$; bear vs. salmon ($\delta^{15}\text{N}$): $P < 0.001$; bear vs. sea eagle ($\delta^{13}\text{C}$): $P < 0.001$; bear vs. sea eagle ($\delta^{15}\text{N}$): $P < 0.001$). The stable isotope ratios in sea eagle droppings were the highest among the animals measured. Also, the contributions of the other predators may impact re-export of nutrient from the ocean across the river basin; their role in marine derived nitrogen input and re-export, such as through release of MDN-rich feces, should be investigated in more detail in future studies.

The isotopic composition of salmon as representative of oceanic $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were 10.99 and -20.54 , respectively. The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of SSL were -3.19 and -29.48 , respectively. Therefore, the three-year average estimate of the contribution of MDN to SOM for $\delta^{15}\text{N}$ depending on the choice of terrestrial isotope values was obtained e.g. 22.9% (SD 3.6%) by using a two sources mixing model. As the reference, the three-year average estimate of the contribution of MDN to SOM for $\delta^{13}\text{C}$ was 17.7% (SD 1.1%) (Fig. 4). Since annual export of TPN per unit area from the Rausu River Basin to the ocean was $454 \text{ kg km}^{-2} \text{ yr}^{-1}$, annual re-export of TPN originally derived from the ocean is estimated to be $104 \text{ kg km}^{-2} \text{ yr}^{-1}$ ($= 454 \text{ kg km}^{-2} \text{ yr}^{-1} * 22.9\%$) (SD $16 \text{ kg km}^{-2} \text{ yr}^{-1} = 454 \text{ kg km}^{-2} \text{ yr}^{-1} \cdot 3.6\%$) based on the contribution of MDN to SOM (Table 1 and Fig. 4). However, it should be noted that this value for MDN re-export is estimated without contribution of marine derived TDN and

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thus should be considered the minimum annual MDN re-export. The actual contribution of MDN to SOM may be more if we assume that TDN ($160 \text{ kg km}^{-2} \text{ yr}^{-1}$) is re-exported with the same contribution as TPN; the contribution increases to 22.9% ($(104 (= 454 \cdot 22.9\%: \text{TPN}) + 37 (= 160 \cdot 22.9\%: \text{TDN})) \text{ kg km}^{-2} \text{ yr}^{-1} / 614 (= \text{TN}) \text{ kg km}^{-2} \text{ yr}^{-1} = 141 \text{ kg km}^{-2} \text{ yr}^{-1} / 614 \text{ kg km}^{-2} \text{ yr}^{-1}$), which is similar to the annual TN transported by chum and pink salmon per unit catchment area, $146 \text{ kg km}^{-2} \text{ yr}^{-1}$ (Table 1).

5 Conclusions

In recent decades, field experiments and stable isotope analyses have been employed to understand the contribution of runs of salmon to river ecosystems. In river ecosystems, runs of salmon are thought to play a large role in the sustainability of nutrient circulation due to their contribution to mammals that incorporate MDN and disperse it across the entire river basin, with the MDN potentially re-exported to the ocean through river flows. In previous studies, the input of total nitrogen (TN) from the ocean to river basin ecosystems has been actively investigated, since it can control ecosystems in which salmon run upstream for spawning, but the contribution of TN from the ocean across an entire river basin has not been examined in detail. This is despite the fact that waterfalls and the other obstacles, which inhibit runs of salmon are known to reduce the transport of MDN upstream. Therefore, this study quantifies the role of salmon in transporting MDN across an entire river basin of the Shiretoko World Natural Heritage area using stable isotope analysis.

Soil organic matter on the forest floor was investigated to understand the contribution of TN, which led to new understanding regarding the transportation potential of MDN by mammals, birds and insects. Contributions of MDN to SOM obtained from a two source mixing model based on $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ were almost the same using two different proxies for terrestrial production, providing a high degree of confidence in the estimated contribution of MDN in the river basin. Annual TN transport estimated for pink salmon

was twice that for chum salmon, which suggests that pink salmon play a greater role in the input of TN across the Rausu River Basin.

The contribution of nitrogen from the ocean across the river basin was 22.9% (= $141 \text{ kg km}^{-2} \text{ yr}^{-1} / 614 \text{ kg km}^{-2} \text{ yr}^{-1}$), while the contribution of TN by salmon was 23.8% (SD 3.1%). Therefore, the annual contribution of MDN to nitrogen may be between 22.9% (= $141 \text{ kg km}^{-2} \text{ yr}^{-1}$) and 23.8% (= $146 \text{ kg km}^{-2} \text{ yr}^{-1}$), which provides valuable support for a strong influence of MDN on the ecological systems across this river basin (Table 1 and Fig. 5).

Author contributions. K. Nakayama designed the field experiments and wrote most of the paper. Also, K. Nakayama performed mixing model analysis. Also, Y. Maruya produced the figures using the GIS technical input and carried out runoff analysis. K. Komai helped the river discharge and nitrogen concentration analysis. M. Komata, and K. Komai measured total nitrogen, dissolved total nitrogen and particulate total nitrogen. K. Matsumoto carried out the field experiments of salmon runs and conducted statistical analysis of stable isotopes. T. Kuwae designed the field experiment regarding stable isotopes and carried out stable isotope measurements. All authors read and commented on drafts of this paper.

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Table 1. Summary of annual export and re-export of nitrogen per unit area.

	N export		N re-export	
	N kg y ⁻¹	N kg km ⁻² y ⁻¹	Salmon run (% ^a) N kg km ⁻² y ⁻¹	MDN input (% ^b) N kg km ⁻² y ⁻¹
TDN	5210	160	–	37 (22.9)
TPN	14 750	454	–	104 (22.9)
TN	19 960	614	146 (23.8)	141 (22.9)

^a = (Salmon run)/(N export).

^b = (N export) (MDN contribution = 22.9).

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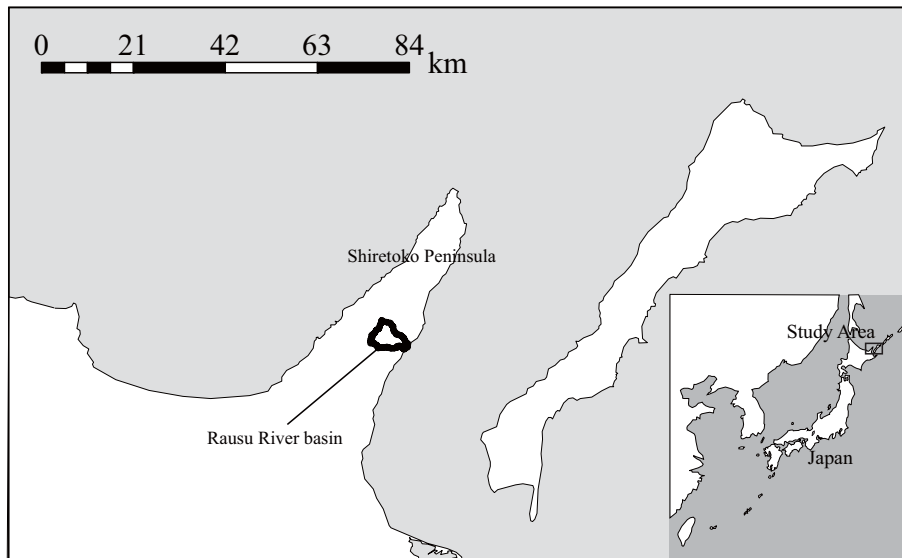


Figure 1. Coastline around the Shiretoko Peninsula and the Rausu River Basin.

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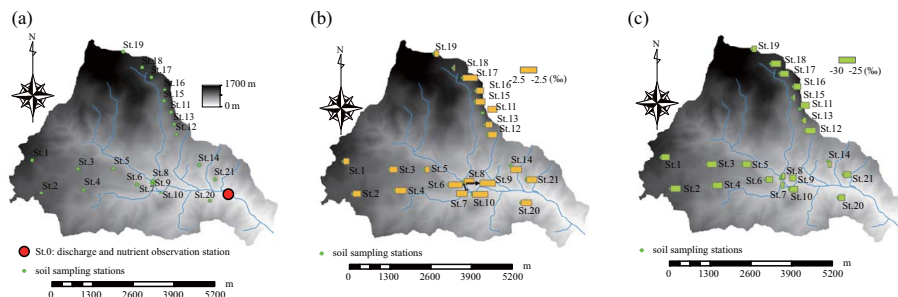


Figure 2. (a) Elevation of the Rausu River Basin. Green circles indicate surface soil sampling stations in September of 2012. Red circles indicates a field observation station for discharge, TDN and TPN. (b) $\delta^{15}\text{N}$ and sampling stations in 2012. (c) $\delta^{13}\text{C}$ and sampling stations in 2012.

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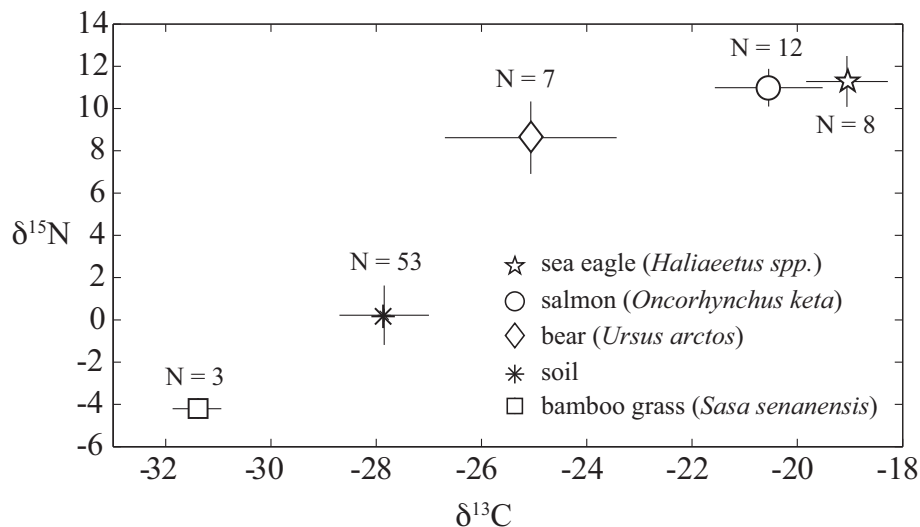


Figure 3. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of bamboo grass (*Sasa senamensis*), SSL, soil samples, bear feces (*Ursus arctos*), salmon (*Oncorhynchus keta*), and sea eagles feces (*Haliaeetus spp.*).

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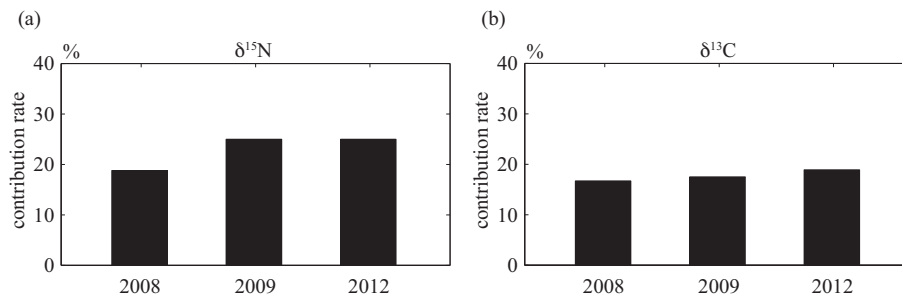


Figure 4. Contribution of MDN from the ocean to the Rausu River basin in 2008, 2009 and 2012 using the two sources mixing model. **(a)** Average contributions of MDN based on SSL for $\delta^{15}\text{N}$ were 22.9%. **(b)** Average contributions of MDN based on SSL for $\delta^{13}\text{C}$ were 17.7%.

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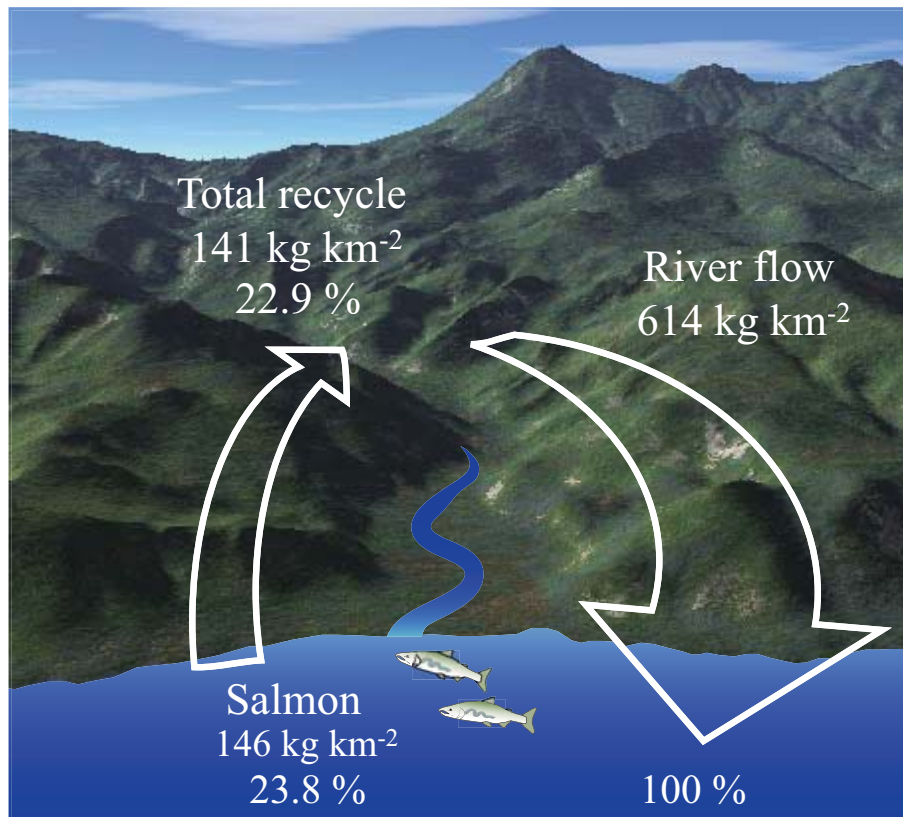


Figure 5. Annual input of TN per unit area from the ocean to the Rausu River Basin, and annual TN transported by salmon per unit area, relative to the annual outflow of TN per unit area (considered to be 100%).

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