Nitrogen fluxes between the ocean and a river basin using stable isotope analysis

Nakayama, Keisuke
Maruya, Yasuyuki
Matsumoto, Kei
Komai, Katsuaki
Kuwae, Tomohiro

(Citation)
Estuarine Coastal and Shelf Science,212:286-293

(Issue Date)
2018-11-15

(Resource Type)
journal article

(Version)
Accepted Manuscript

Nitrogen fluxes between the ocean and a river basin using stable isotope analysis

Keisuke Nakayama [a], Yasuyuki Maruya [b], Kei Matsumoto [c], Katsuaki Komai [c] and Tomohiro Kuwae [d]

[a] Graduate School of Engineering, Kobe University, Rokkodai-cho 1-1, Nada-ku, Kobe city, 657-8501, Japan

[b] River Basin Research Center, Gifu University, 1-1 Yanagido, Gifu city, 501-1193, Japan

[c] Civil and Environmental Engineering department, Kitami Institute of Technology, Kohencho 165, Kitami city, 090-8507, Japan

[d] Coastal and Estuarine Environment Research Group, Port and Airport Research Institute, 3-1-1, Yokosuka city, 239-0826, Japan

[d] Corresponding author. E-mail: nakayama@phoenix.kobe-u.ac.jp

Tel: +81 78 803 6056

Postal address: Rokkodai-cho 1-1, Nada-ku, Kobe city, 657-8501, Japan

Abstract

Total nitrogen (TN), consisting of total particulate nitrogen (TPN) and total dissolved nitrogen (TDN) pools, may be transported to the ocean not only via river channels but also from across the entire river basin via groundwater and migratory animals. Since marine derived nitrogen (MDN) is hypothesized to largely be transported in particulate form, it is necessary to investigate the contribution of particulate MDN in ground surface soils to the total MDN at the river ecosystem scale. In this study, we investigated TN export from an entire river basin to the ocean, and also estimated the contribution of pink (*Oncorhynchus gorbuscha*) and chum salmon (*O. keta*) to total oceanic nitrogen input across a river basin. The maximum potential contribution of MDN entering the river basin from the ocean as salmon was 23.8 % relative to the total amount of TN exported from the river basin. The contribution of MDN from the ocean to particulate nitrogen in river basin soils was estimated to be 22.9 % with standard deviation (SD) of 3.6 % using stable isotope analysis (SIA) of nitrogen ($\delta^{15}N$).

Keywords: marine derived nutrient; nutrient flux; particulate nitrogen; particulate organic matter; salmon

1. Introduction

In river ecosystems, marine derived nitrogen (MDN) inputs have been shown to be important processes controlling the productivity of the ecosystem between the ocean and watersheds. For example, Merz and Moyle (2006) found that the contribution of MDN to the foliar nitrogen of wine grapes was about 18 to 25 % from the ocean. Also, Hilderbrand et al. (1999) demonstrated that trees and shrubs near spawning streams received 24 to 26 % of their foliar nitrogen from MDN, while Helfield and Naiman (2002) suggested that 15.5 to 17.8 % of spruce foliage nitrogen may be provided by MDN. About the transport of MDN, Terrestrial consumers like mammals, birds, fishes and insects have been shown to play a large role in terms of providing MDN from the ocean to watersheds (Donaldson, 1966; Ben-David et al., 1997a; Hilderbrand et al., 1999; Gende et al., 2002; Naiman et al., 2002; Wilkinson et al., 2005; Bartz and Naiman, 2005, Koshino et al. 2013). In the other studies in natural rivers in cold regions, as a particularly important transfer mechanism, migrating fish such as salmon have been shown to be necessary for sustainable nutrient-cycles (nutrient fluxes) 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, the transportation of nitrogen 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, Marcarelli et al. 2014). For example, Cederholm et al. (1989) demonstrated that mammals and birds consume migrating fish, which may result in the secondary dispersion of MDN from the ocean 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; Ben-David et al., 1997b; Hilderbrand et al., 1999; Szepanski et al., 1999; Reimchen, 2000, Holtgrieve et al. 2009). Therefore, it is needed to clarify the nutrient fluxes from a river basin to the ocean and from the ocean to a river basin.

When we consider nitrogen flux in a river flowing from its upstream end into the ocean, the flux depends on nitrogen supplied from the entire river basin (Dutta and Nakayama, 2010; Alam and Dutta, 2012; Riggsbee et al., 2008). In mountainous regions, the total nitrogen flux is comprised mainly of particulate nitrogen, which is derived from surface soils. For example, in the mountainous regions of the Shiretoko Peninsula (site of this study), total particulate nitrogen comprised 75% to 95% of total nitrogen during a flood (Aynur et al., 2012). It is thus necessary to consider surface soils including particulate organic matter in order to analyze nitrogen transport from the river basin to the ocean. However, as mentioned in previous studies, surface soils including particulate organic matter consist of not only land derived materials but also marine derived materials, which means that surface soils may include more marine derived materials close to the ocean.

As one of the methods for analyzing the contribution of MDN, stable isotope analysis (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 river ecosystems (Wyatt et al., 2010a; Wyatt et al., 2010b; Wyatt et al., 2012, Havik et al. 2014, Adame et al. 2015). Isotopic methods as an intrinsic geospatial tracer may thus provide a means to quantify cross-ecosystem transfer of nutrients from the ocean to watersheds. Therefore, this study aims to estimate the contribution of MDN to the particulate organic matter of surface soils by considering salmon as an oceanic source (end-member) in stable isotope analysis. In this study, we present total nitrogen (TN) transport across an entire river basin to the ocean, the potential contribution of MDN to the river basin by salmon, and the contribution of MDN to surface soils in the river basin. Integrated stable isotope analysis of geological, hydrological and biological compartments of the ecosystem allowed us to estimate the nitrogen budget between a natural river basin and the ocean, suggesting it may be important to conserve ocean-river connectivity in such systems.

2. Methods

2.1 Geophysical setting

Our target area, coastal land region and the ocean around the Shiretoko Peninsula, was registered as a World Natural Heritage area in July of 2005 (Fig. 1). Shiretoko is located at the southernmost extent of drift ice and its ecological systems exhibit high biodiversity and high rates of nitrogen circulation, particularly due to runs of pink (*Oncorhynchus gorbuscha*) and chum (*O. keta*) salmon from the Sea of Okhotsk (Aynur et al., 2012). 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). The average weight of chum and pink salmon in this region are 3.3 kg and 2.0 kg, respectively (Makiguchi et al., 2007), which include nitrogen of about 0.100 kg and 0.0608 kg, respectively (Larkin and Slaney, 1997). Therefore, the potential runs of salmon equivalents to 2590 tons of total nitrogen. The size of the Okhotsk coastal region of Hokkaido is about 24,000 km^2, which corresponds to that mean total nitrogen input from the ocean of about 108 kg km^{-2} yr^{-1} if we assume that all salmon run up rivers and the total nitrogen is completely distributed into river basins. Shiretoko is located on the northeast 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). Therefore, the Shiretoko Peninsula is suitable for investigating nitrogen fluxes between coastal land region and the ocean. The Rausu River basin was selected as the main 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 km^2, 7 km, 1/7 (m/m), respectively. Because of the steep slope, nitrogen flux during a flood due to suspended particulate organic matters is larger than due to dissolved nitrogen (Aynur et al., 2012). Field experiments were carried out over 5 years from 2008 to 2012. For comparison with the Rausu River basin, stable isotope analyses were also carried out in 2014 in the Rusa River basin, which provided us equivalent plant and soil environments based on its old growth and conserved forests similar to those of Rausu River basin. Here, the watershed area, river length, and the mean river slope are 9.2 km^2, 5.5 km and 1/7, respectively (Fig. 1).

2.2 Nitrogen from a river basin to the ocean

MDN supplied from the ocean to surface soils in a river basin generally includes feces of mammals, droppings of birds, and the remains of salmon preyed upon by mammals, birds and insects. These MDN are recycled within the terrestrial ecosystems and mainly stored as soil organic matter (SOM). Thus, to focus on the influence of SOM on total particulate nitrogen (TPN) export, soil particles with diameter of less than 500 μm after rinsing in 1N-HCL solution were used in this analysis. The 500 μm cut-off was necessary because soil samples tended to include relatively large, low SOM particles because of the steep slope. The analysis does not allow evaluation of TN (TPN+TDN, TDN: total dissolved nitrogen) export from the river basin to the ocean. However, TPN export from an entire river basin has been revealed to be larger than TDN in the Rausu River basin due to its steep slope (Aynur et al., 2012).

We made an attempt to estimate the contribution of MDN to SOM resulting from the accumulation of particulate organic matter by sampling surface soils across the Rausu River basin (Fig. 2). It should be directly related to the potential riverine transport of MDN back to the ocean as suspended sediments. TDN was measured based on TN and TPN from glass microfiber filters 0.7 μm at St.0 around the river mouth from 2007 to 2009 in the Rausu River basin (TDN = TN – TPN; Fig. 2). The nitrogen concentration of filtered and non-filtered water samples were analyzed by the cadmium reduction-colorimetric method. Annual TN, TPN and TDN exports 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, river discharge was estimated using a storage function method 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.

Surface soil samples were taken at 12, 20 and 21 stations in 2008, 2009 and 2012, respectively. Three soil samples (15 cm height × 15 cm width × 5 cm depth) were collected at each sampling station in order to account for small-scale variability in SOM (Fig. 2 and Table 1). In 2008, fewer samples were taken as we did not have permission to sample surface soils in special protection zones. Stable isotope analysis was conducted for fine particles whose diameter is less than 500 μm. After sieving through a 500 μm sieve with distilled water, the soil was dried for 3 days at 60 to 70 degree Celsius. The dried soil was ground using an alumina mortar. Surface soil sampling stations in 2012 are shown in Fig. 2. Since previous studies have revealed that surface soil transport is related to the spatial distribution of surface soil type, land-use type and vegetation (Ishida et al., 2010), the location of each sampling station was selected by dividing the river basin into 21 domains (sub-basin areas) that vary in soil type and vegetation (Figure 1). The spatial distribution of surface soil types was divided into 6 categories. Although the spatial pattern in vegetation is complicated, the vegetation can generally be categorized in terms of altitude. Since Shiretoko is protected as a natural World Heritage area, all areas studied are classified as forest and have high vegetation cover by bamboo grass.

2.3 Salmon runs

To evaluate the contribution of salmon to SOM, 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 a lattice fence, which does not obstruct flood flows 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. Field observations were conducted in the Tokorohoronai River, which is located in the same region of Hokkaido but where it is customary to remove the catching apparatus before and after the salmon run season, allowing us to monitor salmon escape rates from the apparatus and the salmon run under open conditions at the same place. The capture rate of the apparatus was calculated using the number of salmons passing the observation point in a channel section of 3 m width and 0.2 m depth; the Rausu River width (about 15 m) is too wide for this type of observation. We used two infrared cameras (SM-AVIR-602S, Hero Corp., Izumo, Japan) placed 2 m above the river surface and recorded continuous videos to monitor the individual salmon passing this 3 m section. Videos were taken from the 25th to 28th of November (before removal of the apparatus) and from the 4th to 7th of December (after removal of the apparatus) in 2013. The number of salmon was calculated as the net number of salmon running upstream by identifying individual salmons at the observation point. No salmon were captured and tagged for individual identification. There was no influence of rainfall during the observation period.

2.4 Stable isotope analysis

Stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) 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 (Table 1 for $\delta^{13}C$ and $\delta^{15}N$ of SOM in 2012). Stable isotope ratios are expressed in δ notation as the deviation from standards in parts per thousand (‰) according to the following equation:

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

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

Vienna Pee Dee 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 standard deviation (SD) of the internal reference (L-histidine) replicates was <0.15‰ for both $\delta^{13}C$ and $\delta^{15}N$. The C/N values ranged from 10 to 20 for surface soil samples. The contribution of MDN to SOM was evaluated by applying a two source mixing model based on stable isotope analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) (Kline et al., 1998; Moore and Semmens, 2008; Hossler and Bauer, 2012). Salmon tissue isotopes were considered representative of the isotope composition of ocean productivity. To isotopically characterize terrestrial productivity, we considered one terrestrial end-member (source): Soil Samples exhibiting the Lowest $\delta^{13}C$ and $\delta^{15}N$ at St.18 in 2009 (hereafter SSL), and thus assumed to have the highest terrestrial contribution to SOM. To validate the assumption that $\delta^{15}N$ is lowest close to the top region of the mountain, the mean of $\delta^{15}N$ for each elevation was plotted against the representative elevation (Fig. 2d), which demonstrates that at higher elevations $\delta^{15}N$ is lower. It should be noted that representative soil samples collected in the same river basin were chosen because they have isotopically similar characteristics to the other soil samples in this study.

The contribution of MDN to SOM was evaluated using a two sources mixing model based on the measured $\delta^{13}C$ and $\delta^{15}N$. The average contribution in the Rausu River basin was computed using each sub-basin area obtained from the Thiessen method (Thiessen, 1911).

$$f_{C\_MDN} + f_{C\_LDN} = 1 \quad (2)$$

$$f_{C\_MDN}\delta^{13}C_{salmon} + f_{C\_LDN}\delta^{13}C_{SSL} = \delta^{13}C_{soil} \quad (3)$$

$$f_{N\_MDN} + f_{N\_LDN} = 1 \quad (4)$$

$$f_{N\_MDN}\delta^{15}N_{salmon} + f_{N\_LDN}\delta^{15}N_{SSL} = \delta^{15}N_{soil} \quad (5)$$

where f_{C_MDN} and f_{C_LDN} are the contributions of MDN and land-derived nitrogen (LDN) by carbon, $\delta^{13}C_{salmon}$, $\delta^{13}C_{SSL}$ and $\delta^{13}C_{soil}$ are the stable isotope ratios of carbon for salmon, SSL and soil samples, respectively, f_{N_MDN} and f_{N_LDN} are the contributions of MDN and LDN by nitrogen, $\delta^{15}N_{salmon}$, $\delta^{15}N_{SSL}$ and $\delta^{15}N_{soil}$ are the stable isotope ratios of nitrogen for salmon, SSL and soil samples, respectively. Salmon tissues were sampled at the mouth of the Rausu River from October to December of 2007, 2008 and 2012.

As bamboo grass (*Sasa senanensis*) is the dominant species in the study area, bamboo grass was collected at 13 soil sampling points (St.1, St.2, St.3, St.4, St.7, St.8, St.10, St.11, St.12, St.13, St.14, St.17, and St.21). Furthermore, droppings of sea eagles (*Haliaeetus* spp.) around the mouth of the Rausu River in winter season from 2013 to 2014 and feces of brown bear (*Ursus arctos*) around

St. 14 in August of 2009, which represent a typical migratory bird and mammal in Shiretoko, were collected to investigate whether or not they include MDN and thus contribute to SOM. Samples of feces and droppings for SIA analysis offer a major advantage, i.e. little isotopic fractionation expected and thus ideal to use the stable isotope values as a MDN tracer (Fry, 2006). The samples were pre-treated by rinsing with a chloroform-methanol solution (2:1) prior to SIA to remove isotopically fractionated metabolites, such metabolites in the samples were removed as urea and ammonium (Kuwae et al., 2008; Kuwae et al., 2012). It should be noted that there may be the possibility that a substantial difference in plant community in the Rausu River basin influences $\delta^{15}N$, which has not been explored in our study.

3. Results and Discussions

3.1 Estimation of nitrogen export to the ocean

During 2007 to 2009 the concentration of TDN was observed to be constant, 0.090 mg L^{-1} (SD 0.022 mg L^{-1}), regardless of the discharge in the Rausu River, and TPN was revealed to be a function of river discharge (r^2=0.88; Eq. 6) (Fig. 3). TPN showed a strong correlation with suspended sediment (SS) concentrations, with SS concentration increasing with increasing river discharge (Fig. 3). TPN was modeled using our field observation results, discharge and TPN as in (6).

$$\mathrm{TPN} = 0.0032 \times Q^{1.771} \tag{6}$$

where Q is the river discharge (m^3 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 (Draper and Smith, 1998).

$$CoD = \frac{\left(\sum_{i=1}^{N}\left(Cal_i - \overline{Cal}\right)\cdot\left(Obs_i - \overline{Obs}\right)\right)^2}{\sum_{i=1}^{N}\left(Cal_i - \overline{Cal}\right)^2 \cdot \sum_{i=1}^{N}\left(Obs_i - \overline{Obs}\right)^2} \quad (7)$$

where Cal is the calculated river discharge, $\overline{Cal}$ is the mean calculated river discharge, Obs is the observed river discharge, $\overline{Obs}$ is the mean observed river discharge, respectively.

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 exports of TDN, TPN and TN from 2008 to 2012 were 5210 kg yr^{-1}, 14750 kg yr^{-1} and 19960 kg yr^{-1}, respectively. Since the size of the Rausu River basin is 32.5 km^2, the annual mean exports of TDN, TPN and TN per unit catchment area equate to 160 kg km^{-2} yr^{-1}, 454 kg km^{-2} yr^{-1} and 614 kg km^{-2} yr^{-1}, respectively (Table 2). The average concentrations of TDN and TPN from 2008 to 2012 were 0.090 mg L^{-1} and 0.216 mg L^{-1}, which agrees with a previous study at the site (Aynur et al., 2012).

3.2 Contribution of salmon runs to nitrogen input from the ocean

To evaluate annual MDN, we made an attempt to estimate the potential number of salmon running up a river. 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 hr^{-1}. The average numbers for 4 days after the apparatus was removed from the river was 0.61 hr^{-1}, so the rate of capture of salmon by the apparatus (CS) was estimated as 20 %: (0.61-0.49) / 0.61 = 0.20. Since the field observations were conducted at the end of November and the beginning of December after the peak of salmon runs, floods may damage the apparatus for catching salmon and the 20 % capture rate may be an underestimate. Therefore, we attempted to apply two larger capture rates, 50 % and 80 %, in order to demonstrate the influence of this estimate on our calculations of the possible nitrogen input from the ocean due to salmon runs.

In the Rausu River, the annual average numbers of salmon caught by the apparatus at the river mouth were 3075 and 10580 for chum and pink salmon, respectively, from 2001 to 2009. By assuming that all apparatuses have the same rate of capture, the potential for chum and pink salmon runs can be estimated as 15375 and 52900 (CS 20 %), 6150 and 21160 (CS 50 %), and 3844 and 13225 (CS 80 %), respectively. Therefore, annual TN potentially transported by chum and pink salmon is estimated to be 1542 kg yr^{-1} and 3216 kg yr^{-1} (CS 20 %), 617 kg yr^{-1} and 1287 kg yr^{-1} (CS 50 %), and 386 kg yr^{-1} and 804 kg yr^{-1} (CS 80 %), 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} (CS 20 %), 59 kg km^{-2} yr^{-1} (CS 50 %), and 37 kg km^{-2} yr^{-1} (CS 80 %), (SD 19 kg km^{-2} yr^{-1}), which corresponds to the contribution of TN by salmon, 23.8 % (CS 20 %), 9.5 % (CS 50 %), and 6.0 % (CS 80 %), relative to the annual outflow of TN per unit area (considered to be 100 %) (Table 2). It is clear that the contribution of salmons to MDN is significant because $\delta^{13}C$ and $\delta^{15}N$ of salmon are much higher than SOM (Fig. 4).

3.3 Contribution of MDN to SOM in the Rausu River basin

The $\delta^{15}N$ and $\delta^{13}C$ of SSL were -3.2 ‰ and -29.5 ‰, respectively. Interestingly, SSL had almost the same value for mean $\delta^{15}N$ as bamboo grass ($\delta^{15}N$ = -3.0 ‰ with SD 1.5 ‰, $\delta^{13}C$ = -29.0 ‰ with SD 1.5 ‰), which confirms that bamboo grass can be considered a major source of LDN. The isotopic composition of salmon as representative of oceanic $\delta^{15}N$ and $\delta^{13}C$ were 11.0 ‰ (SD 0.9 ‰) and -20.5 ‰ (SD 1.0 ‰), respectively. Therefore, the three-year average estimate of the contribution of MDN to SOM based on $\delta^{15}N$, depending on the choice of terrestrial isotope values, was 22.9 % (SD 3.6 %) using a two sources mixing model, which is similar to Hilderbrand et al. (1999) (Fig. 5). The 22.9% estimate also appears similar to Merz and Moyle (2006), 18-25% contribution of MDN to foliar Nitrogen. The yearly contribution of MDN to SOM was 18.8 %, 25.0 % and 25.0 % for 2008, 2009 and 2012, respectively. It should be noted that the contribution in 2008 is different from 2009 and 2012 because the sampling area was smaller in 2008. Therefore, it appears that a two sources mixing model can enable us to evaluate the contribution of MDN to SOM precisely though there is variation (0.9 ‰ SD) in the $\delta^{15}N$ of salmon. For reference, the three-year average estimate of the contribution of marine derived carbon (MDC) to SOM based on $\delta^{13}C$ was 17.7 % (SD 1.1 %) (Fig. 5). Since the annual export of TPN per unit area from the Rausu River basin to the ocean was 454 kg km^{-2} yr^{-1}, annual input of TPN originally derived from the ocean is estimated to be 104 kg km^{-2} yr^{-1} (= 454 kg km^{-2} yr^{-1} * 22.9 %) (SD 16 kg km^{-2} yr^{-1} = 454 kg km^{-2} yr^{-1} * 3.6 %) based on the contribution of MDN to SOM (Fig. 5 and Table 2).

We assume that the larger $\delta^{15}N$ is, the higher the contribution of MDN from the ocean. In order to confirm our assumption, we carried out similar field observations in the Rusa River basin (Fig. 6). In the Rausu River, only a part of the area is registered as a special protection zone of the Natural World Heritage region, but the whole of the Rusa River basin is covered by a special protection zone and is closer to the ocean compared to the Rausu River basin. The Rusa River basin is thus considered a more protected and natural area as defined by the natural World Heritage conditions compared to the Rausu River. Therefore, the contribution of MDN could be expected to be larger in the Rusa River basin compared to the Rausu River basin (Fig.2). The spatial average of $\delta^{15}N$ was computed using 14 sub-basin areas in the Rusa River basin obtained from the Thiessen method (Thiessen, 1911). The spatial average of $\delta^{15}N$ in the Rusa River basin was 1.1 ‰, which is 1.0 ‰ larger than in the Rausu River basin. It could thus be suggested that the higher $\delta^{15}N$ of SOM in surface soils around the Rusa River is associated with higher contributions from MDN from the ocean.

It should be noted that the effect of denitrification on the $\delta^{15}N$ is negligible in our case. In general, some proportion of the nitrogen is reduced due to denitrification, which results in an increase in $\delta^{15}N$ of the soil. However, Rennie et al. (1976) revealed that the isotope ratio of nitrogen in ground surface soils is identical to that in organic nitrogen in the natural forest, which suggests that denitrification does not involve any isotope fractionation. Mckinley et al. (2013) also demonstrated that surface soil is aerobic in forests when the water table is not close to the ground surface. Since our sampling was carried out within the top 5 cm of the soil and the surface soil is not saturated due to the steep slope, the SOM sampled was considered to be under aerobic conditions. Furthermore, Hilton et al. (2013) demonstrated that erosional processes decrease $\delta^{15}N$ of the soil, and Craine et al. (2015) found that $\delta^{15}N$ of the soil tends to increase due to microbial processing. In the Rausu River basin, since the slope is very steep and there is no correlation between C/N and $\delta^{15}N$ of the soil, erosional loss may be dominant compared to microbial processing. Microbial processing in addition to MDN might both be enriching $\delta^{15}N$ of the soil, suggesting the SIA-based estimate of 22.9% MDN contribution to SOM is an upperbound. Additionally, when CS is 20 %, the contribution of TN by salmon is estimated 23.8 % which is similar to the SIA-based estimate of 22.9% MDN contribution, which means that TN transportation is expected of a system at steady state with inputs = outputs between the Rausu River basin and the ocean. Therefore, salmon runs and CS management may play a great role in a nitrogen input from the ocean compared to the other nitrogen inputs given from the outside of the Rausu River basin. Also, we may have identified that salmon runs should be conserved in order to protect sound nature due to nitrogen cycle in the World Natural Heritage, “Shiretoko Peninsula”.

The stable isotope ratios in sea eagle droppings ($\delta^{15}N$ = 11.3 ‰ with SD 1.2 ‰, $\delta^{13}C$ = -19.1 ‰ with SD 0.8 ‰) and brown bear feces ($\delta^{15}N$ = 8.6 ‰ with SD 1.7 ‰, $\delta^{13}C$ = -25.1 ‰ with SD 1.6 ‰) were higher than LDN, indicating that sea eagles and bears likely transport MDN to the SOM (Fig. 4). In the case of multiple food sources, feces and droppings are likely to be enriched in relatively indigestible food sources, when compared with assimilated materials (Sponheimer et al. 2003; Kuwae et al.2008). Therefore, in the present study, feces and droppings are likely to be enriched in LDN (e.g., plants) because LDN would be more indigestible than MDN (e.g., fishes). However, such an enrichment does not affect the qualitative investigation, i.e., whether or not feces and droppings include MDN and thus contribute to SOM. Since brown bears are thought to be the major terrestrial consumer of spawning salmon, they may impact input of nitrogen from the ocean across the river basin, such as through release of MDN-rich urine and feces (Hilderbrand et al. 1999). Rennie et al. (1976) demonstrated that the $\delta^{15}N$ of surface organic matter is associated with the total organic matter, which includes among other components leaf litter, droppings from birds, and feces from animals. Therefore, it is important to quantify the influence of sea eagles and bears on the nitrogen flux (nitrogen cycle) in these systems. However, based on Fig. 4, we cannot as yet quantify the relative contribution of sea eagles and bears to total MDN transport. Also, it is necessary to clarify CS for evaluating the contribution of sea eagles and bears to total MDN transport.

4. Conclusions

In recent decades, field experiments and stable isotope analyses have been employed to understand the contribution of salmon runs to river ecosystems. Runs of salmon are thought to play a large role in the sustainability of nitrogen cycling in coastal systems because of their contribution to mammals that incorporate MDN and disperse it across the entire river basin. The input of MDN to river basin ecosystems has been actively investigated in previous studies, since it can exert great control on ecosystems in which salmon run upstream for spawning. However, the contribution of MDN across an entire river basin has not previously been examined in detail. This study provides an important quantification of the role of salmon in transporting MDN from the ocean across an entire river basin of the Shiretoko World Natural Heritage area using stable isotope analysis, and indicates that this is likely an important nitrogen pathway that should be preserved in these ecosystems between coastal land region and the ocean.

5. Author contribution

K. Nakayama designed the field experiments and wrote most of the paper and performed mixing model analysis. Y. Maruya produced the figures using the GIS technical input and carried out runoff analysis. K. Komai helped with 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 on 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.

6. Acknowledgment

We wish to thank Tetsunori Inoue, and anonymous reviewers, for their constructive comments, which have contributed to a significant improvement in the manuscript. We also wish to thank Professor Pierre Regnier for the comments for a significant improvement in the manuscript. This work was supported by Mitsui & Co., Ltd. Environment fund, the Sumitomo foundation, River fund by the River Foundation, and a Grant-in-Aid for Scientific Research from the Japan Society for the

Promotion of Science (JSPS). The data for this paper are available, please contact the corresponding author, Keisuke Nakayama, keisuke_n@mui.biglobe.ne.jp.

References

Adame, M. F., Fry, B., Gambora, J. N. & Herrera-Silveira, J. A. 2015. Nutrient subsidies delivered by seabirds to mangrove islands, *Marine Ecology Progress Series* **525**, 15-24, doi10.3354/meps11197.

Alam, M. J. & Dutta, D. 2012. A process-based and distributed model for nutrient dynamics in river basin: development, testing and applications, *Ecological Modelling* **247**, 112-124, doi:10.1016/j.ecolmodel.2012.07.031.

Aynur, A., Nakayama, K., Maruya, Y., Inoue, T., Kuwae, T., Tateyama, K., Okada, T., Kato, J. & Ishida, T. 2012. Clarification of mechanisms of nutrient circulation between ocean and inland in Shiretoko, *Annual Journal of Hydraulic Engineering, JSCE* **68(4)**, 721-726 (English abstract in Japanese).

Bartz, K. K. & Naiman, R. J. 2005. Effects of salmon-borne nutrients on riparian soils and vegetation in Southwest Alaska, *Ecosystems* **8**, 529-545, DOI:10.1007/s10021-005-0064-z.

Ben-David, M., Flynn, R. W. & Schell, D. M. 1997a. Annual and seasonal changes in diets of martens: evidence from stable isotope analysis, *Oecologia* **111**, 280-291, doi:10.1007/s004420050236.

Ben-David, M., Hanley, T. A., Klein, D. R. & Schell, D. M. 1997b. Seasonal changes in diets of coastal and riverine mink: the role of spawning Pacific salmon, *Canadian Journal of Zoology* **75**, 803-811, doi:10.1139/z97-102.

Ben-David, M., Hanley, T. A. & Schell, D. M. 1998. Fertilization of terrestrial vegetation by spawning Pacific salmon: the role of flooding and predator activity, *Oikos* **83**, 47-55.

Bilby, R. E., Fransen, B. R. & Bisson, P. A. 1996. Incorporation of nitrogen and carbon from spawning coho salmon into the trophic system of small streams : evidence from stable isotopes, *Canadian Journal of Fisheries and Aquatic Sciences* **53**, 164-173, doi:10.1139/f95-159.

Biodiversity Center of Japan, Ministry of the Environment. 2010. Vegetation survey, http://gis.biodic.go.jp/webgis/sc-025.html?kind=vg67.

Cederholm, C. J. & Peterson, N. P. 1985. The retention of coho salmon (Oncorhynchus kisutch) carcasses by organic debris in small streams, *Canadian Journal of Fisheries and Aquatic Science* **42**, 1222-1225, doi:10.1139/f85-150.

Cederholm, C. J., Houston, D. B., Cole, D. I. & Scarlett, W. J. 1989. Fate of coho salmon (Oncorhynchus kisutch) carcasses in spawning streams, *Canadian Journal of Fisheries and Aquatic Sciences* **46**, 1347-1355, doi:10.1139/f89-173.

Chaloner, D. T., Wipfli, M. S. & Caouette, J. P. 2002. Mass loss and macroinvertebrate colonization of Pacific salmon carcasses in southeastern Alaskan streams, *Frechwater Biology* **47**, 263-273, doi:10.1046/j.1365-2427.2002.00804.x.

Craine, J. M., Brookshire, E. N. J., Cramer, M. D., Hasselquist, N. J., Koba, K., Spiotta, E. M. & Wang, L. 2015. Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils, *Plant and Soil* **396(1)**, 1–26, doi:10.1007/s11104-015-2542-1.

Donaldson, J. R. 1966. The phosphorus budget of Iliamna Lake, Alaska, as related to the cyclic abundance of sockeye salmon, Dissertation, University of Washington, Seattle.

Dutta D. & Nakayama, K. 2010. Impacts of Spatial data resolution in River flow Simulation by Physically Based Distributed Modeling Approach: A Case Study in Tsurumi River Basin, Japan, *Hydrological Processes* **23**, 534-545, doi: 10.1002/hyp.7183.

Draper, N. R. & Smith, H. 1998. Applied Regression Analysis, Wiley, New York.

Fry, B. J. 2006. Stable isotope ecology, Springer Verlag, New York.

Gende, S. M., Edwards, R. T., Willson, M. F. & Wipfli, M. S. 2002. Pacific Salmon in Aquatic and Terrestrial Ecosystems, *BioScience* **52(10)**, 917-928, doi:10.1641/0006-3568(2002)052[0917:PSIAAT]2.0.CO;2.

Gende, S. M., Miller, A. E. & Hood, E. 2007. The effects of salmon carcasses on soil nitrogen pools in a riparian forest of southeastern Alaska, *Canadian Journal of Forest Research* **37**, 1194-1202, doi:10.1139/X06-318.

Gresh, T., Lichatowich, J. & Schoonmaker, P. 2000. An estimation of historic and current levels of salmon production in the Northeast Pacific ecosystem: evidence of a nutrient deficit in the freshwater systems of the Pacific Northwest, *Fisheries* **25**, 15–21, doi:10.1577/1548-8446(2000)0252.0.CO;2.

Havik, G., Catenazzi, A. & Holmgren M. 2014. Seabird nutrient subsidies benefit non-nitrogen fixing tree and alder species composition in South American coastal dry forests, *PLoS One* **9(1)**, e86381, doi:10.1371/journal.pone.0086381.

Helfield, J. M. & Naiman, R. J. 2002. Salmon and alder as nitrogen sources to riparian forests in a boreal Alaskan watershed, *Oecologia* **133**, 573-582, doi:10.1007/s00442-002-1070-x.

Hilderbrand, G. V., Hanley, T. A., Robbins, C. T. & Schwartz, C. C. 1999. Role of brown bears (Ursus arctos) in the flow of marine nitrogen into a terrestrial ecosystem, *Oecologia* **121**, 546-550, doi:10.1007/s004420050961.

Hilton, R. G., Galy, A., West, A. J., Hovius, N. & Roberts, G.G. 2013. Geomorphic control on the $\delta^{15}N$ of mountain forests, *Biogeosciences* **10**, 1693-1705, doi:10.5194/bg-10-1693-2013

Hocking, M. D. & Reimchen, T. E. 2009. Salmon species, density and watershed size predict magnitude of marine enrichment in riparian food webs, *Oikos* **118**, 1307-1318.

Hocking, M. D. & Reynolds, J. D. 2011. Impacts of salmon on riparian plant diversity, *Science* **331**, 1609-1612, DOI:10.1126/science.1201079.

Hokkaido National Fisheries Research Institute, Fisheries Research Agency, 2009. http://salmon.fra.affrc.go.jp/zousyoku/result/result.htm.

Holtgrieve, G. W., Schindler, D. E. & Jewett, P. K. 2009. Large predators and biogeochemical hotspots: brown bear (*Ursus arctos*) predation on salmon alters nitrogen cycling in riparian soils, *Ecological Research* **24**, 1125-1135, doi:10.1007/s11284-009-591-8.

Hossler, K. & Bauer, J. E. 2012. Estimation of riverine carbon and organic matter source contributions using time-based isotope mixing models, *Journal of Geophysical Research* **117**, G03035, doi:10.1029/2012JG001988.

Ishida, T., Nakayama, K., Okada, T., Maruya, Y., Onishi, K. & Omori, M. 2010. Suspended sediment transport in a river basin estimated by chemical composition analysis, *Hydrological Research. Letters* **4**, 55-59, doi:10.3178/hrl.4.55.

Juday, C. W., Rich, H., Kemmerer, G. I. & Mann, A. 1932. Limnological studies of Karluk Lake, Alaska, 1926-1930, *Bulletin of the U.S. Fish Commission* **47**, 407-436.

Kline, T. C. Jr., Wilson, W. J. & Goering, J. J. 1998. Natural isotope indicators of fish migration at Prudhoe Bay, Alaska, *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 1494-1502, doi:10.1139/f98-012.

Koshino, Y., Kudo, H. & Kaeriyama, H. 2013. Stable isotope evidence indicates the incorporation into Japanese catchments of marine-derived nutrients transported by spawning Pacific salmon, *Freshwater Biology* **58**, 1864-1877, doi:10.1111/fwb.12175.

Kuwae, T., Beninger, P. G., Decottignies, P., Mathot, K. J., Lund, D. R. & Elner, R. W. 2008. Biofilm grazing in a higher vertebrate: the Western Sandpiper, *Calidris mauri*, *Ecology* **89(3)**, 599-606, http://dx.doi.org/10.1890/07-1442.1.

Kuwae, T., Miyoshi, E., Hosokawa, S., Ichimi, K., Hosoya, J., Amano, T., Moriya, T., Kondoh, M., Ydenberg, R. C. & Elner, R. W. 2012. Variable and complex food web structures revealed by exploring missing trophic links between birds and biofilm, *Ecology Letters* **15**, 347-356, doi:10.1111/j.1461-0248.2012.01744.x.

Larkin, G. A. & Slaney, P. A. 1997. Implications of trends in marine-derived nutrient influx to south coastal British Columbia salmonid production, *Canadian Journal of Forest Research* **22(11)**, 16-24, doi:10.1577/1548-8446(1997)022<0016:IOTIMN>2.0.CO;2.

Levi, P. S. & Tank, J. L. 2013. Nonnative Pacific salmon alter hot spots of sediment nitrification in Great Lakes tributaries, *Journal of Geophysical Research* **118**, 436-444, doi:10.1002/jgrg.20044.

Makiguchi, Y., Nii, H., Nakao, K. & Ueda, H. 2007. Upstream migration of adult chum and pink salmon in the Shibetsu River, *Hydrobiologia* **582**, 43-54, doi:10.1007/s10750-006-0555-8.

Marcarelli, A. M., Baxter, C. V. & Wipfli, M. S. 2014. Nutrient additions to mitigate for loss of Pacific salmon: consequences for stream biofilm and nutrient dynamics, *Ecosphere* **5(6)**, A69, doi:10.1890/ES13-00366.1.

Mckinley, R., Radcliffe, D. & Mukundan, R. 2013. A streamlined approache for sediment source fingerprinting in a Southern Piedmont watershed, USA, *Journal of Soils and Sediments* **13**, 1754-1769, doi:10.1007/s11368-013-0723-1.

Merz, J. E. & Moyle, P. B. 2006. Salmon, wildlife, and wine: marine-derived nutrients in human-dominated ecosystems of central California, *Ecological Applications* **16(3)**, 999-1009, doi:10.1890/1051-0761.

Michael, J. B. 1978. A storage-routing model relating drainage basin hydrology and geomorphology, *Water Resources Research* **14**, 921-928, doi:10.1029/WR014i005p00921.

Michael, J. B., Pilgrim, D. H. & Cordery, I. 1979. A storage routing model based on catchment geomorphology, *Journal of Hydrology* **42**, 209-230, http://dx.doi.org/10.1016/0022-1694(79)90048-9.

Moore, J. W. & Schindler, D. E. 2004. Nutrient export from freshwater ecosystems by anadromous sockeye salmon (Oncorhynchus nerka), *Canadian Journal of Fisheries and Aquatic Sciences* **61**, 1582-1589, doi:10.1139/F04-103.

Moore, J. W. & Semmens, B. X. 2008. Incorporating uncertainty and prior information into stable isotope mixing models, *Ecology Letters* **11(5)**, 470-480, doi: 10.1111/j.1461-0248.2008.01163.x.

Naiman, R. J., Bilby, R. R., Schindler, D. E. & Helfield, J. M. 2002. Pacific salmon, nutrients, and the dynamics of freshwater and riparian ecosystems, *Ecosystems* **5**, 399-417, doi:10.1007/s10021-001-0083-3.

Reimchen, T. E. 2000. Some ecological and evolutionary aspects of bear - salmon interactions in coastal British Columbia, *Canadian Journal of Zoology* **78**, 448-457, doi:10.1139/z99-232.

Rennie, D. A., Paul, E. A. & Johns, L. E. 1976. Natural nitrogen-15 abundance of soil and plant samples, *Canadian Journal of Soil Science* **56(1)**, 43-50, doi: 10.4141/cjss76-006.

Riggsbee, J. A., Orr, C. H., Leech, D. M., Doyle, M. W. & Wetzel, R. G. 2008. Suspended sediments in river ecosystems: Photochemical sources of dissolved organic carbon, dissolved organic nitrogen, and adsorptive removal of dissolved iron, *Journal of Geophysical Research* **113**, G03019, doi:10.1029/2007JG000654.

Sponheimer, M., Robinson, T., Ayliffe, L., Passey, B., Roeder, B., Shipley, L., Lopez, E., Cerling, T., Dearing, D. & Ehleringer, J. 2003. An experimental study of carbon-isotope fractionation between diet, hair, and feces of mammalian herbivores, *Canadian Journal of Zoology* **81**, 871–876, doi: 10.1139/Z03-066.

Szepanski, M. M., Ben-David, M. & Ballenberghe, V. V. 1999. Assessment of anadromous salmon resources in the diet of the Alexander Archipelago wolf using stable isotope analysis, *Oecologia* **120**, 327-335, doi:10.1007/s004420050866.

Thiessen, A. H. 1911. Precipitation averages for large areas, *Monthly Weather Review* **39**, 1082-1084, doi:http://dx.doi.org/10.1175/1520-0493(1911)39<1082b:PAFLA>2.0.CO;2.

Wilkinson, C. E., Hocking, M. D. & Reimchen, T. E. 2005. Uptake of salmon-derived nitrogen by mosses and liverworts in Coastal British Columbia, *Oikos* **108**, 85-98.

Wipfli, M. S., Hudson, J. & Caouette, J. 1998. Influence of salmon carcasses on stream productivity: response of biofilm and benthic macroinvertebrates in southeastern Alaska, USA, *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 1503-1511, doi:10.1139/cjfas-55-6-1503.

Wyatt, A. S. J., Waite, A. M. & Humphries, S. 2010a. Variability in isotope discrimination factors in coral reef fishes: Implications for diet and food web reconstruction, *PLoS ONE* **5(10)**, e13682, doi: 13610.11371/journal.pone.0013682.

Wyatt, A. S. J., Lowe, R. J., Humphries, S. & Waite, A. M. 2010b. Particulate nutrient fluxes over a fringing coral reef: relevant scales of phytoplankton production and mechanisms of supply, *Marine Ecology Progress Series* **405**, 113-130, doi:10.3354/meps08508.

Wyatt, A. S. J., Falter, J. L., Lowe, R. J., Humphries, S. & Waite, A. M. 2012. Oceanographic forcing of nutrient uptake and release over a fringing coral reef, *Limnology and Oceanography* **57(2)**, 401-419, doi:10.4319/lo.2012.57.2.0401.

Yanai, S. & Kochi, K. 2005. Effects of salmon carcasses on experimental stream ecosystems in Hokkaido, Japan, *Ecological Research* **20**, 471-480, doi:10.1007/s11284-005-0056-7.

Figure captions

Fig. 1. (a) Coastline around the Shiretoko Peninsula and the Rausu River basin. (b) Surface soil type. (c) Vegetation. Pink circles indic ate surface soil sampling stations in September of 2012 (Biodiversity Center of Japan, Ministry of the Environment. 2010).

Fig. 2. (a) Elevation of the Rausu River basin. Pink circles indicate surface soil sampling stations in September of 2012. Red circles indicate field observation stations for discharge, TDN (total dissolved nitrogen) and TPN (total particulate nitrogen). (b) $\delta^{15}N$ of soil samples and sampling stations in 2012. (c) $\delta^{13}C$ of soil samples and sampling stations in 2012. (d) Mean $\delta^{15}N$ at an interval of 250 m in 2012. Error bars indicate standard deviation.

Fig. 3. River discharge, total particulate nitrogen and suspended sediment at the river mouth of Rausu River. (a) River discharge and concentration of total particulate nitrogen. (b) Concentration of suspended sediment and concentration of total particulate nitrogen.

Fig. 4. $\delta^{13}C$ and $\delta^{15}N$ of bamboo grass (*Sasa senanensis*), SSL (Soil Samples exhibiting the Lowest values of $\delta^{13}C$ and $\delta^{15}N$), soil samples, bear feces (*Ursus arctos*), salmon (*Oncorhynchus keta*), and sea eagles droppings (*Haliaeetus spp.*). The bars indicate standard deviation.

Fig. 5. Contributions of MDN (marine derived nitrogen) and MDC (marine derived carbon) 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 (Soil Samples exhibiting the Lowest values of $\delta^{13}C$ and $\delta^{15}N$) for $\delta^{15}N$ are 22.9 %. (b) Average contributions of MDC based on SSL for $\delta^{13}C$ are 17.7 %.

Fig. 6. (a) Elevation of the Rusa River basin. Pink circles indicate surface soil sampling stations in September of 2014. (b) $\delta^{15}N$ and sampling stations in 2014. (c) $\delta^{13}C$ and sampling stations in 2014.

Table captions

Table 1. $\delta^{15}N$ and $\delta^{13}C$ of SOM in 2012.

Table 2. Summary of annual export and input of nitrogen per unit area.

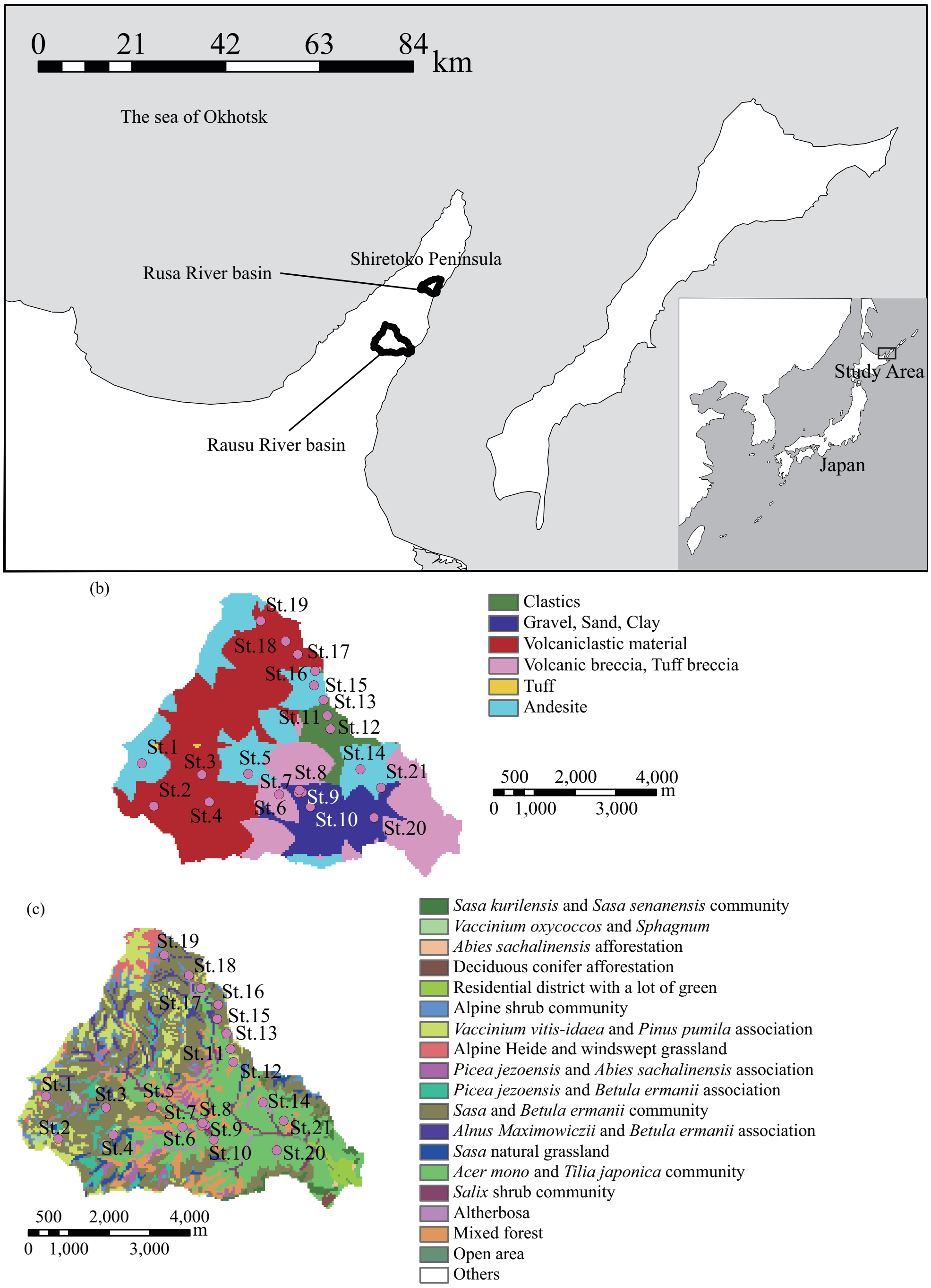

Fig. 1. (a) Coastline around the Shiretoko Peninsula and the Rausu River basin. (b) Surface soil type. (c) Vegetation. Pink circles indic ate surface soil sampling stations in September of 2012 (Biodiversity Center of Japan, Ministry of the Environment. 2010).

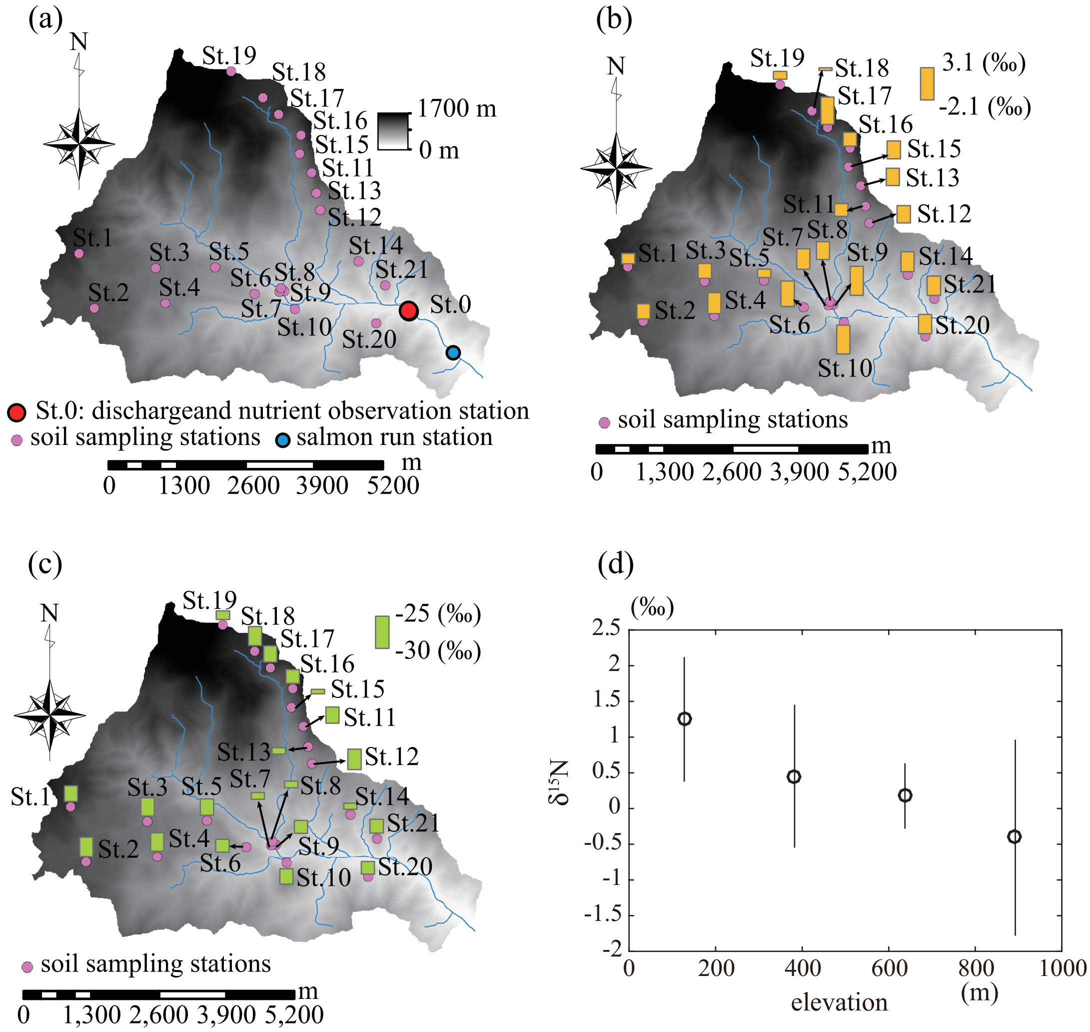

Fig. 2. (a) Elevation of the Rausu River basin. Pink circles indicate surface soil sampling stations in September of 2012. Red circles indicate a field observation station for discharge, TDN (total dissolved nitrogen) and TPN (total particulate nitrogen). (b) $\delta^{15}N$ of soil samples and sampling stations in 2012. (c) $\delta^{13}C$ of soil samples and sampling stations in 2012. (d) Mean $\delta^{15}N$ at an interval of 250 m and elevation in 2012. Error bars indicate standard deviation.

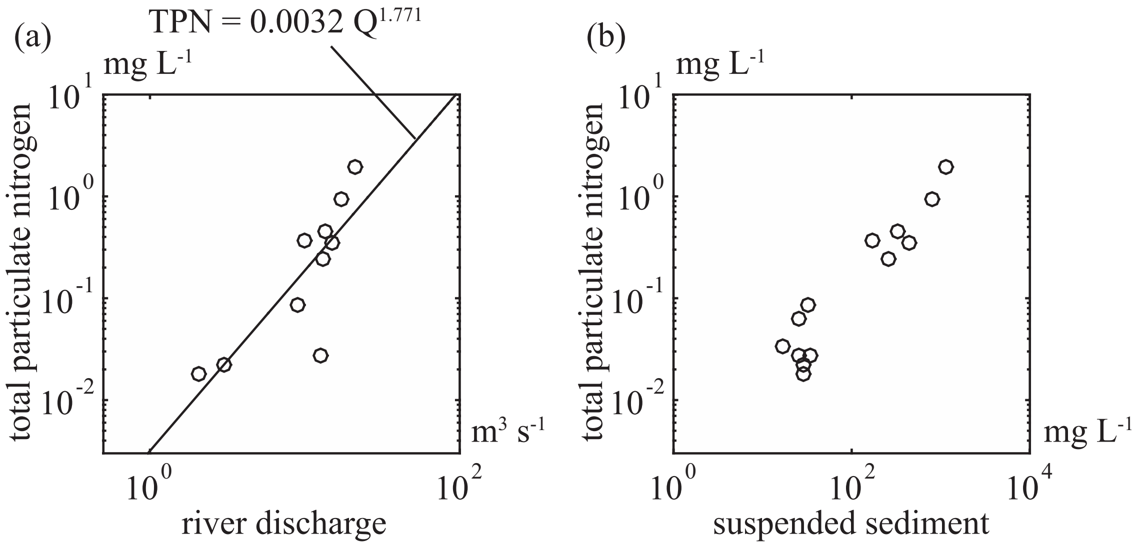

Fig. 3. River discharge, total particulate nitrogen and suspended sediment at the river mouth of Rausu River. (a) River discharge and concentration of total particulate nitrogen. (b) Concentration of suspended sediment and concentration of total particulate nitrogen.

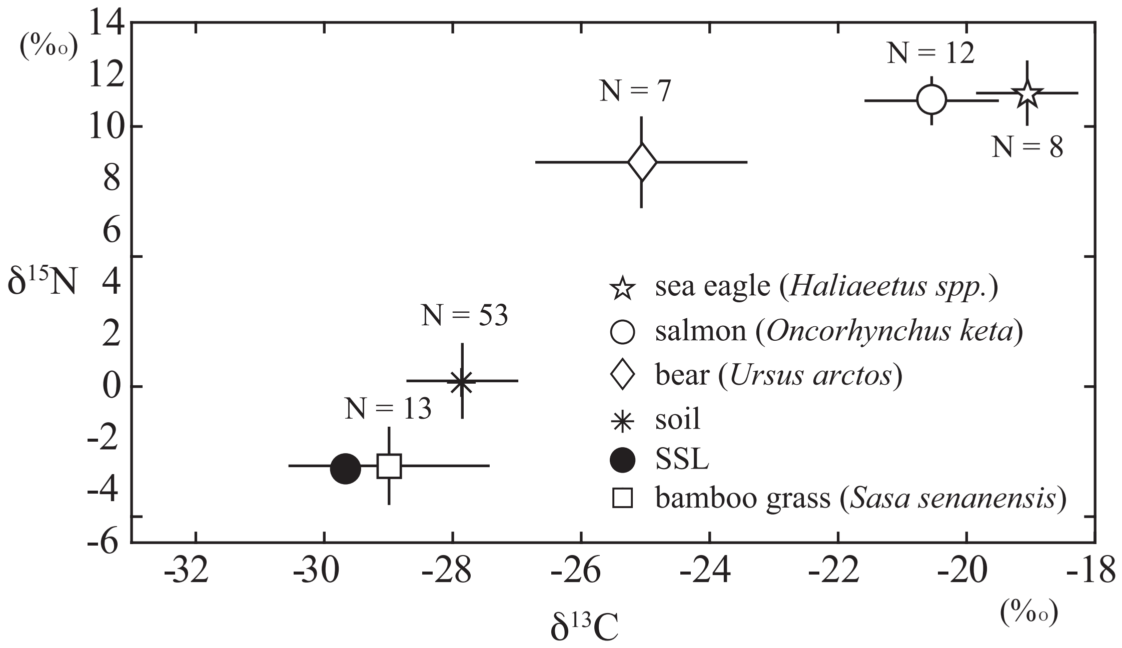

Fig. 4. $\delta^{13}C$ and $\delta^{15}N$ of bamboo grass (*Sasa senanensis*), SSL (Soil Samples exhibiting the Lowest values of $\delta^{13}C$ and $\delta^{15}N$), soil samples, bear feces (*Ursus arctos*), salmon (*Oncorhynchus keta*), and sea eagles droppings (*Haliaeetus spp.*). The bars indicate the standard deviation.

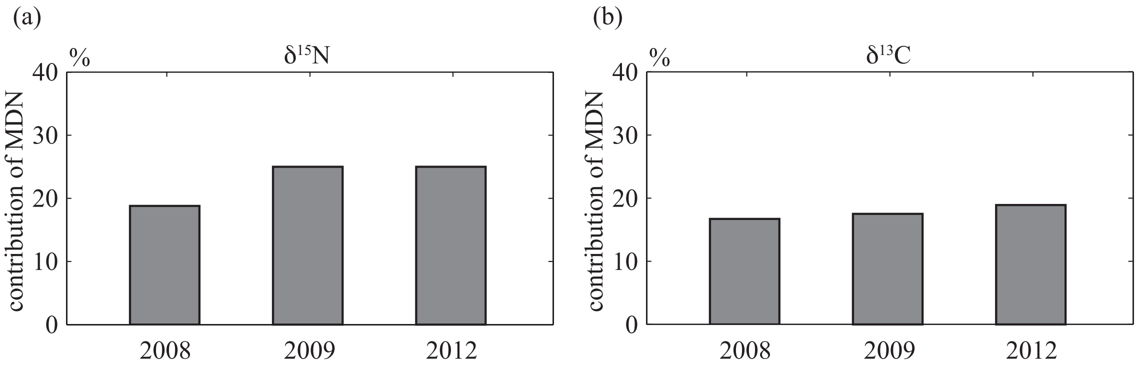

Fig. 5. Contributions of MDN (marine derived nitrogen) and MDC (marine derived carbon) from the ocean to the Rausu River basin in 2008, 2009 and 2012 using the two sources mixing model. (a) Average contributions of MDN based on SSL (Soil Samples exhibiting the Lowest values of $\delta^{13}C$ and $\delta^{15}N$) for $\delta^{15}N$ are 22.9 %. (b) Average contributions of MDC based on SSL for $\delta^{13}C$ are 17.7 %.

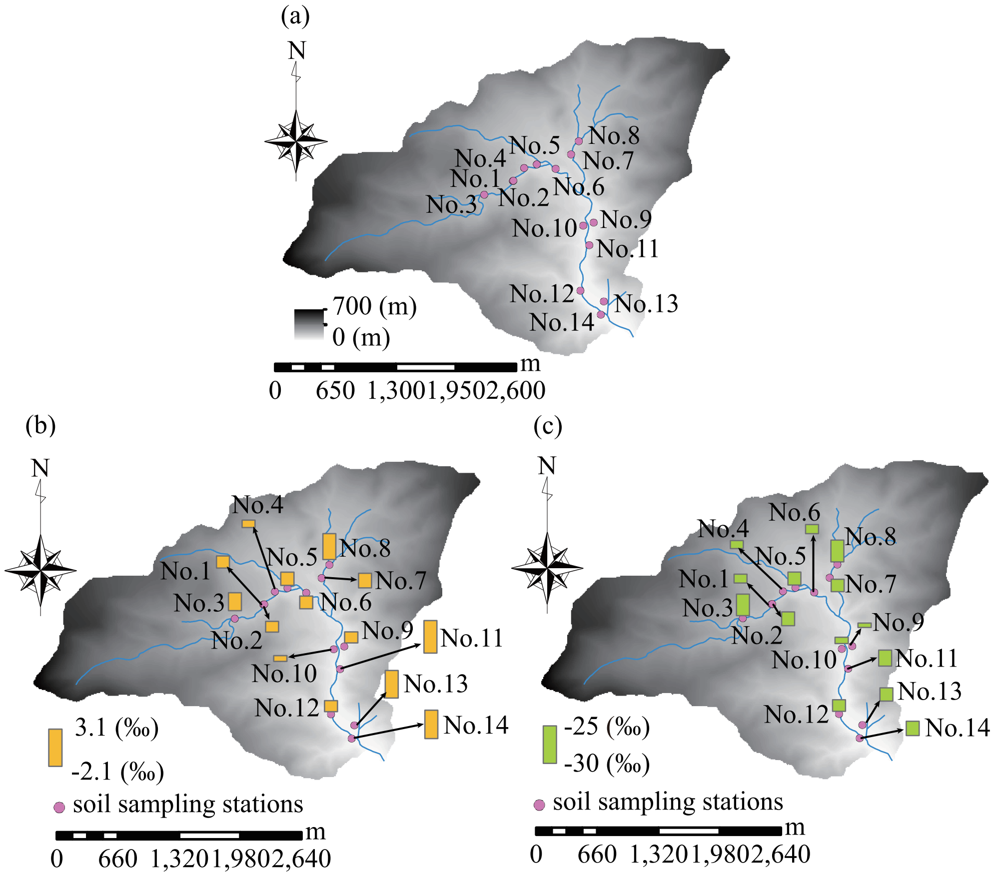

Fig. 6. (a) Elevation of the Rusa River basin. Pink circles indicate surface soil sampling stations in September of 2014. (b) $\delta^{15}N$ and sampling stations in 2014. (c) $\delta^{13}C$ and sampling stations in 2014.

Table 1. $\delta^{15}N$ and $\delta^{13}C$ of SOM in the Rausu River basin in 2012.

Station number	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
St.1	-0.8	-27.6
St.2	-0.1	-27.0
St.3	-0.1	-27.1
St.4	0.9	-27.1
St.5	-1.1	-27.5
St.6	1.7	-28.0
St.7	0.8	-29.0
St.8	0.4	-29.0
St.9	2.2	-28.0
St.10	2.2	-27.6
St.11	0.3	-27.5
St.12	0.3	-26.8
St.13	-0.4	-29.0
St.14	0.7	-29.0
St.15	0.4	-29.3
St.16	-0.3	-27.8
St.17	2.0	-27.5
St.18	-2.1	-27.1
St.19	-1.3	-28.7
St.20	0.6	-28.0
St.21	0.7	-27.8

Table 2. Summary of annual export and input of nitrogen per unit area.

	N export		N input	
			Salmon run (%*)	MDN input (%)**
	N kg· y^{-1}	N kg· km^{-2}· y^{-1}	N kg· km^{-2}· y^{-1}	N kg· km^{-2}· y^{-1}
TDN	5210	160	-	-
TPN	14750	454	-	104 (22.9)
TN	19960	614	CS 20 %, 146 (23.8) CS 50 %, 59 (9.5) CS 80 %, 37 (6.0)	-

* = (Salmon run)/(N export)

** = (N export)×(MDN contribution = 22.9)