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

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Abstract

Total nitrogen (TN), consisting of total particulate nitrogen (TPN) and total dissolved nitrogen (TDN) pools, may be transported in not only river channels but across entire river basins such as via ground water and migratory animals. In general, the amount of TPN exported from mountainous river basins to the ocean is expected to be larger than TDN. Since marine derived nutrients (MDN) are hypothesized to largely be transported in particulate form, it is necessary to investigate the contribution of particulate MDN in forest ground surface soils to the total MDN at the river ecosystem scale. In this study we investigated TN export from an entire river basin, 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 TN entering the river basin as salmon was 23.8 % relative to the total amount of TN exported from the river basin. The contribution of MDN to particulate nitrogen in river basin soils was estimated to be 22.9 % with SD of 3.6 % using stable isotope analysis (SIA) of nitrogen ($\delta^{15}N$).

1. Introduction

Stable Isotope Analysis (SIA) is increasingly being used to examine connectivity in coastal aquatic-terrestrial ecosystems, such as the input of marine derived nutrients (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). 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, Marcarelli et al. 2014). In most of those cases, terrestrial consumers like mammals, birds, fishes and insects have been shown to play a large role in terms of providing MDN 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). Moreover, MDN inputs have been shown to be an important processes controlling the productivity of the 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 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. Thus, isotopic methods as an intrinsic geospatial tracer can provide a means to quantify cross-ecosystem transfer of nutrients. As a particularly important transfer mechanism, migrating fish such as salmon have been shown to be necessary for sustainable nutrient-cycles 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; Kline et al., 1993; Schindler et al., 2003).

When we consider nutrient flux in a river flowing from its upstream end into the ocean, the flux depends on nutrients supplied not only inside the river itself but also from the entire river basin (Dutta and Nakayama, 2010; Alam and Dutta, 2012; Riggsbee et al., 2008). Particulate nutrient flux from a basin, which is derived mainly from surface soils, is generally larger than the flux from dissolved nutrients in mountainous regions (Nakayama et al., 2011). 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; Ben-David et al., 1997b; Hilderbrand et al., 1999; Szepanski et al., 1999; Reimchen, 2000, Holtgrieve et al. 2009). However, the contribution of MDN to surface soils, which may be transported from a river basin to the ocean as suspended sediments, at the river basin scale has not been adequately quantified in natural systems due to the difficulty in quantifying complex food webs and making accurate biomass estimates.

In this study we present TN transport across an entire river basin to the ocean, the potential contribution of TN from the ocean 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 nutrient budget for a natural river basin, suggesting it may be important to conserve ocean-river connectivity in such systems.

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 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). 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, respectively. Because of the steep slope, nutrient flux due to suspended sediments is larger than due to dissolved nutrients (Nakayama et al., 2011). 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. Here, the watershed area, river length, and the mean river slope are 9.2 km^2, 5.5 km, 1/7, respectively (Fig. 1).

3. Methods

3.1 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 TPN export, soil particles with diameter of less than 500 μm after rinsing in 1N-HCL solution were used in this analysis. The analysis does not allow evaluation of TN (TPN+TDN) 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 (Nakayama et al., 2011).

We believe 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 (Yamada et al., 1996). However, Wada et al. (1984) demonstrated that denitrification seems to have a small effect on the variation of $\delta^{15}N$ in SOM under aerobic conditions close to the ground surface in a natural forest. Moreover, 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 the δ^{15}N of 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.

We made an attempt to estimate the contribution of MDN to SOM resulting from the accumulation of particulate organic matter, which should be directly related to the potential riverine transport of MDN back to the ocean as suspended sediments, by sampling surface soils across the Rausu River basin (Fig. 2). TN, TDN and TPN were measured at St.0 around the river mouth from 2007 to 2009 in the Rausu River basin (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 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, 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.

Surface soil samples were taken at 12, 20 and 21 stations in 2008, 2009 and 2012, respectively. Three soil samples 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. Surface soils were sampled from three different points at each station in a volume of 15 cm × 15 cm × 5 cm (height × width × depth). 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.

3.2 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 running upstream salmon 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.

3.3 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:

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

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 of the internal reference (L-histidine) replicates was <0.15‰ for both δ^{13}C and δ^{15}N. The contribution of MDN to SOM was evaluated by applying a two source mixing model based on stable isotope analysis (SIA) of carbon (δ^{13}C) and nitrogen (δ^{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 δ^{13}C at St.14 and δ^{15}N at St.18 in 2009 (hereafter SSL), and thus assumed to have the highest terrestrial contribution to SOM. SSL was collected close to the top of the mountain, where MDN is not expected to influence isotope values. 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.

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

$$f_{C\_MDN} + f_{C\_LDN} = 1 \tag{2}$$

$$f_{C\_MDN}\delta^{13}\mathrm{C}_{salmon} + f_{C\_LDN}\delta^{13}\mathrm{C}_{SSL} = \delta^{13}\mathrm{C}_{soil} \tag{3}$$

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

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

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

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.) and feces of brown bear (*Ursus arctos*), 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). Chum salmon tissues and droppings of sea eagles were collected at the river mouth and feces of brown bear were collected at St.14. 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).

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 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. 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. 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 (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 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 nutrient re-export 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. 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 a nitrogen content of about 0.100 kg and 0.0608 kg, respectively (Larkin and Slaney, 1997). 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).

4.3 Contribution of MDN to SOM in the Rausu River basin

Both δ^{13}C and δ^{15}N of SOM were lower than those of the salmons (Fig. 4). Interestingly, SSL had almost the same value for mean δ^{15}N as bamboo grass, which confirms that bamboo grass can be considered as a major source of LDN. The isotopic composition of salmon as representative of oceanic δ^{15}N and δ^{13}C were 11.0 and -20.5, respectively. The δ^{15}N and δ^{13}C of SSL were -3.2 and -29.5, respectively. Therefore, the three-year average estimate of the contribution of MDN to SOM based on δ^{15}N, depending on the choice of terrestrial isotope values, was 22.9 % (SD 3.6 %) using a two sources mixing model (Fig. 5). For reference, the three-year average estimate of the contribution of MDN to SOM based on δ^{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 re-export 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 believe that the higher δ^{15}N of SOM in surface soils around the Rusa River is not associated with human impacts but with higher contributions from MDN. Wada et al. (1984) demonstrated that the δ^{15}N of SOM in forests may show significant variation in the surface soil, for example by as much as about -3 ‰ to -2 ‰ at Jumonji in Chichibu and at Mt. Shigayama, and about 1 ‰ to 5 ‰ at Memuro in the eastern Hokkaido. The $\delta^{15}N$ variation at Memuro was obtained in the Hokkaido Agricultural Experimental Station, which is located 10 km from the center of Obihiro city where 150,000 people live. Therefore, the 1 ‰ to 5 ‰ variation there likely reflects the influence of anthropogenic nitrogen emissions. The $\delta^{15}N$ variation of about -3 ‰ to -2 ‰ in surface soils at Jumonji and at Mt. Shigayama may support our assumption that the larger $\delta^{15}N$ is, the higher the contribution of MDN. 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. 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 $\delta^{15}N$ 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. However, it should be noted that MDN re-export as TN was estimated without the contribution of marine derived TDN, for example, in dissolved form such as urine from bears, and thus should be considered the minimum annual MDN re-export.

The stable isotope ratios in sea eagle droppings and brown bear feces were higher than LDN, indicating that sea eagles and bears likely transport MDN to the SOM. 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 re-export of nutrient 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 δ^{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. Wada et al. (1984) also revealed that δ^{15}N of surface soils is almost identical within different organic nitrogen pools in natural forests. Therefore, it is important to quantify the influence of sea eagles and bears on the nutrient-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.

5. 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 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. The input of TN from the ocean 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 TN from the ocean across an entire river basin has not previously been examined in detail. This is despite the fact that waterfalls and other obstacles, which inhibit salmon runs, are known to reduce the transport of MDN upstream. This study provides an important quantification of the role of salmon in transporting MDN across an entire river basin of the Shiretoko World Natural Heritage area using stable isotope analysis, and indicates that this is likely an important nutrient pathway that should be preserved in these ecosystems.

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.

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Fig. captions:

Fig. 1. (a) Coastline around the Shiretoko Peninsula and the Rausu River basin. (b) Surface soil type. (c) Vegetation.

Fig. 2. (a) Elevation of the Rausu River basin. Green 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) $\delta^{15}N$ and sampling stations in 2012. (c) $\delta^{13}C$ and sampling stations in 2012.

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

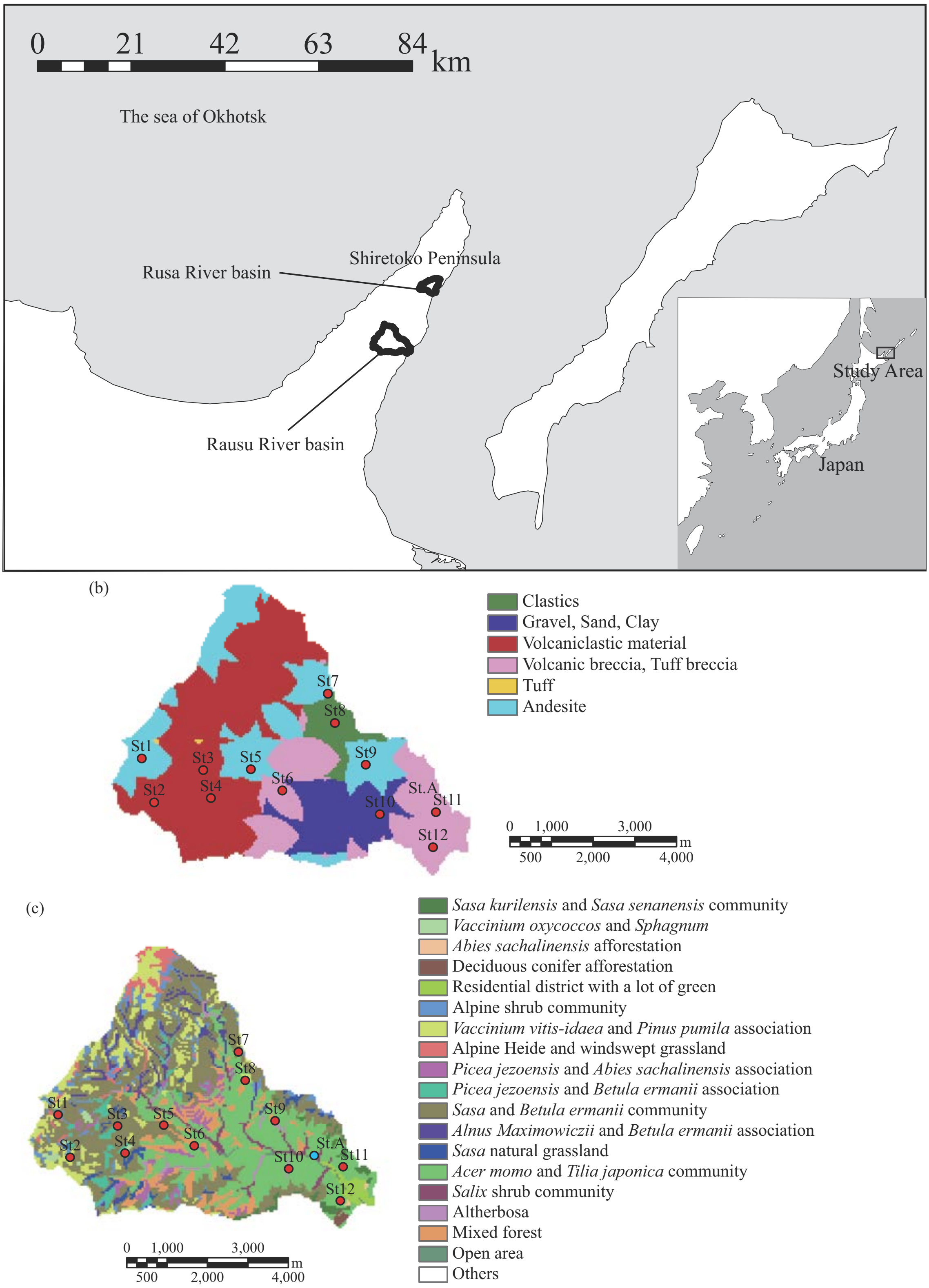
Fig. 5. Contribution of MDN (marine derived nitrogen) from the ocean to the Rausu River basin in 2008, 2009 and 2012 using the two sources mixing model. (a) Average contributions of MDN based on SSL (Soil Samples exhibiting the Lowest values of $\delta^{13}C$ and $\delta^{15}N$) for $\delta^{15}N$ were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

Fig. 6. (a) Elevation of the Rusa River basin. Green circles indicate surface soil sampling stations in September of 2012. (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 re-export of nitrogen per unit area.



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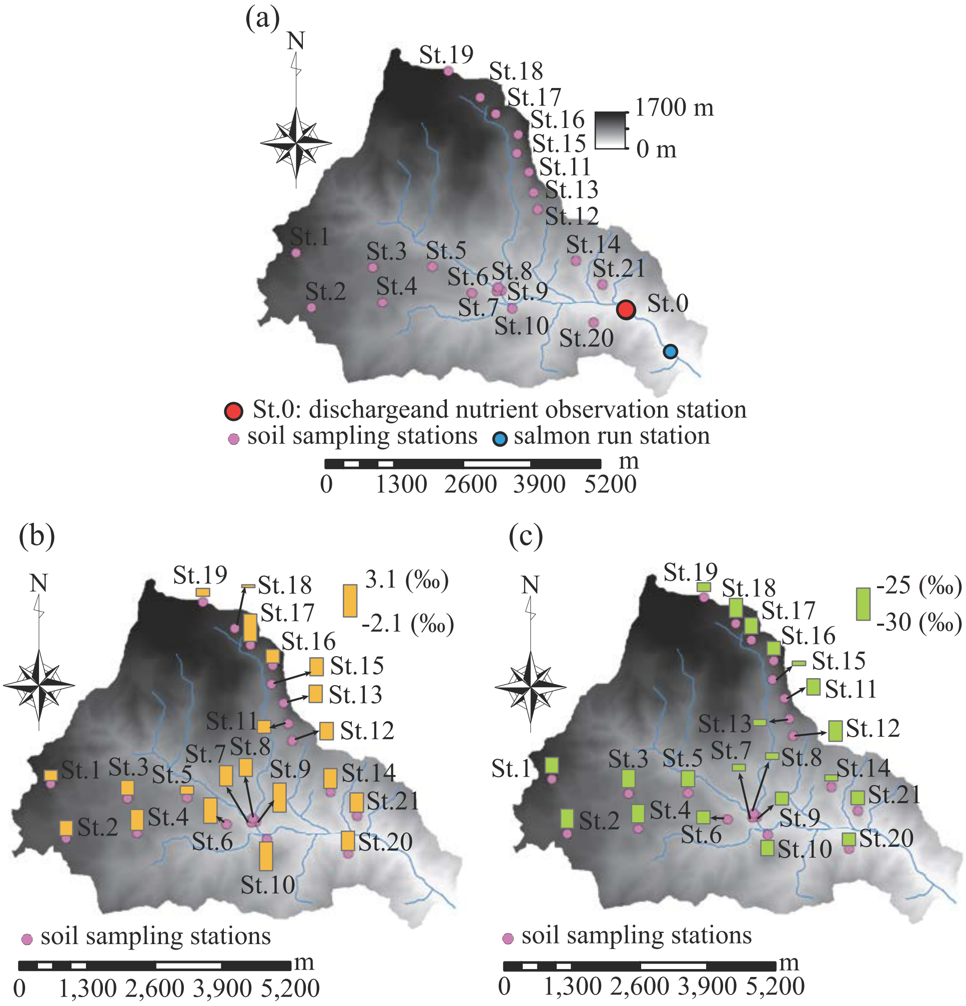

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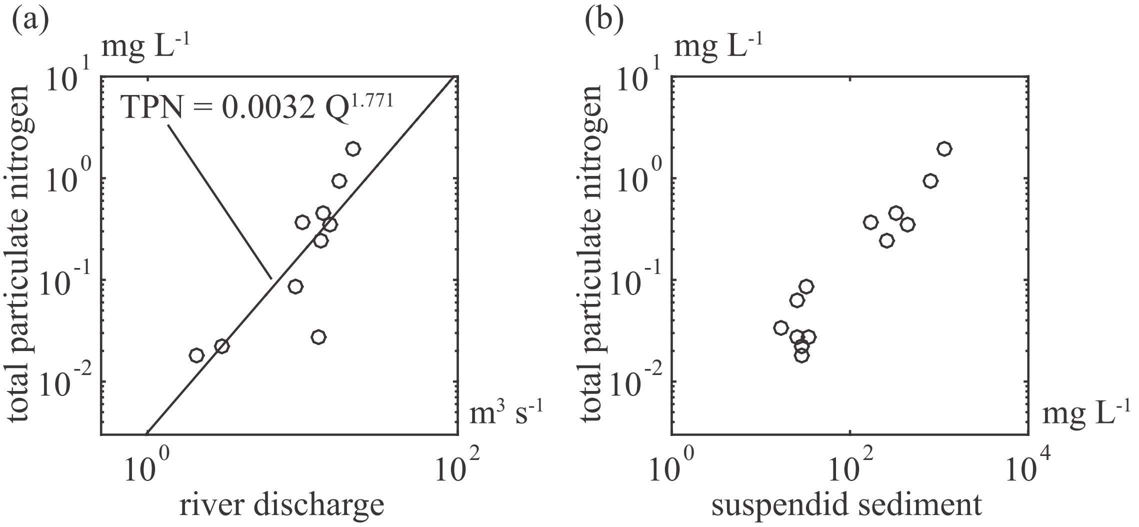

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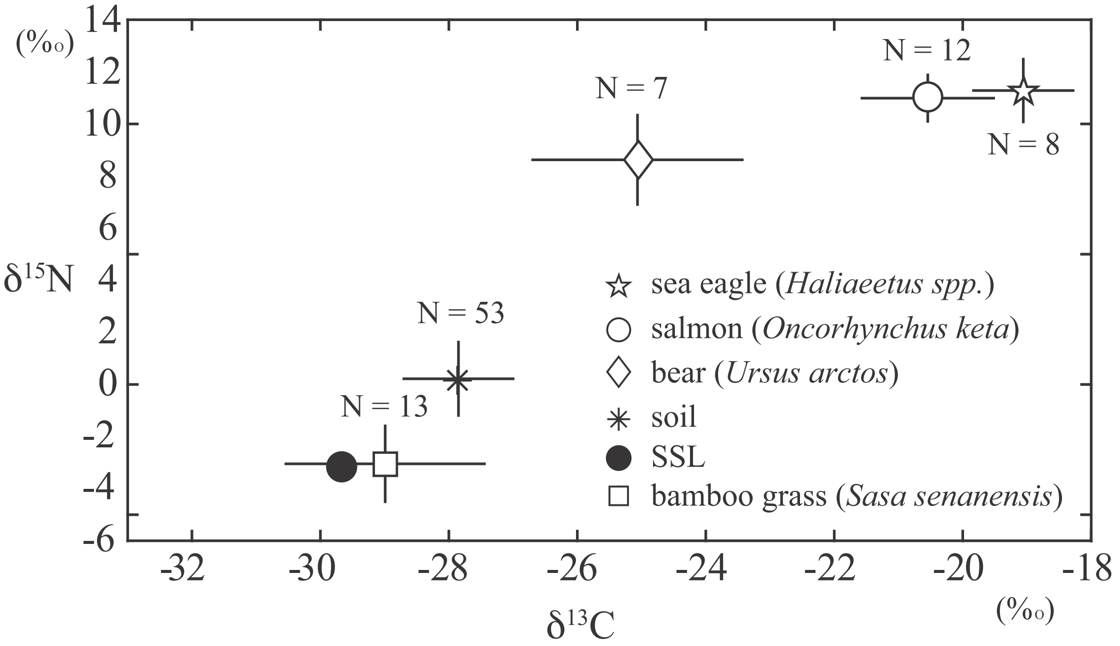

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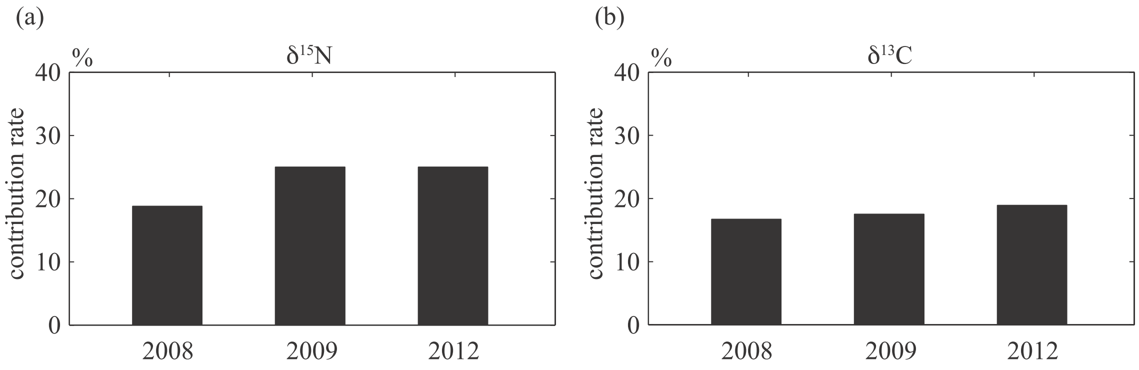

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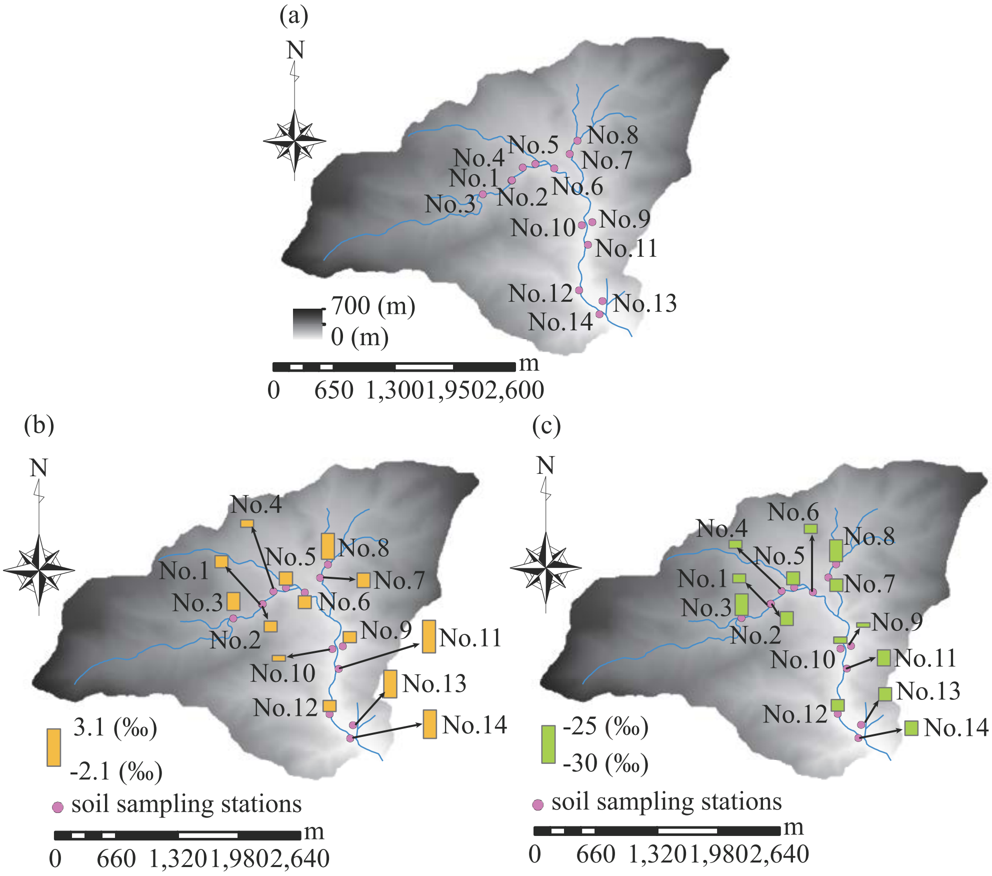

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Table 1. $\delta^{15}N$ and $\delta^{13}C$ of SOM in the Rausu River basin in 2012.

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 re-export of nitrogen per unit area.

			N re-export	
	N export		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)