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

K. Nakayama<sup>1</sup>, Y. Maruya<sup>2</sup>, K. Matsumoto<sup>3</sup>, M. Komata<sup>4</sup>, K. Komai<sup>3</sup>, and T. Kuwae<sup>5</sup>

<sup>1</sup>Department of Civil Engineering, Kobe University, Kobe, Japan

<sup>2</sup>Department of Civil and Earth Resources Engineering, Kyoto University, Kyoto, Japan

<sup>3</sup>Department of Civil and Environmental Engineering, Kitami Institute of Technology, Kitami, Japan

<sup>4</sup>Department of Biotechnology and Environmental Chemistry, Kitami Institute of Technology, Kitami, Japan

<sup>5</sup>Coastal and Estuarine Environment Research Group, Port and Airport Research Institute, Yokosuka, Japan

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Correspondence to: K. Nakayama (nakayama@phoenix.kobe-u.ac.jp)

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## Abstract

Total nitrogen (TN), which consists of total particulate nitrogen (TPN) and total dissolved nitrogen (TDN), is transported with not only in river channels but also across the entire river basin, including via ground water and migratory animals. In general, TPN export from an entire river basin to the ocean is larger than TDN in a mountainous region. Since marine derived nutrients (MDN) are hypothesized to be mainly transported as suspended matters from the ground surface, 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 TN export from an entire river basin, and also we 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 by salmon was found to be 23.8% relative to the total amount of TN exported from the river basin. The contribution of particulate nitrogen based on suspended sediment from the ocean to the river basin soils was 22.9% with SD of 3.6% by using stable isotope analysis (SIA) of nitrogen ( $\delta^{15}\text{N}$ ).

## 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, fishes and insects have been shown to play a large role in terms of providing MDN to watersheds

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(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). Moreover, MDN inputs 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 is 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).

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 also from the entire river basin (Dutta and Nakayama, 2010; Alam and Dutta, 2012; Riggsbee et al., 2008). Also, particulate nutrient flux, which is given from surface soils dominantly, is revealed to be larger than dissolved nutrient generally in a mountainous region (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, b; Hilderbrand et al., 1999; Szepanski et al., 1999; Reimchen, 2000). 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





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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 and has a custom to remove its apparatus before and after salmon run seasons, allowing us to monitor the salmon escapement from the apparatus and the salmon run under the open condition at the same place. The capture rate of the apparatus was calculated with numbers of salmon passing the observation point which has a channel section of 3 m in width and 0.2 m in depth, instead of the Rausu River because its river width (about 15 m) is too wide to cover the entire width. We used two infrared cameras (SM-AVIR-602S, Hero Corp., Izumo, Japan) placed 2 m above the river surface and recorded videos in all day to monitor the individual salmon passing this 3 m section. Videos were taken from the 25 to 28 November (before removal of the apparatus) and from the 4 to 7 December (after removal of the apparatus) in 2013. The number of salmon runs was calculated as the differences of the numbers of individuals running to upstream and those to downstream 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

MDN, such as nitrogen, are generally supplied from the ocean to surface soils in a river basin as SOM, which includes 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 particulate nitrogen in the river basin soils, soil particles with diameter of less than 500  $\mu\text{m}$  after rinsing in 1N-HCL solution were used in the analysis. Therefore, it cannot be allowed to evaluate how much TN is exported from the river basin to the ocean. However, TPN export from an entire river basin is revealed to be larger than TDN in the Rausu River basin due to the steep slope (Nakayama et al., 2011). In general, some proportion of the nitrogen is reduced due to denitrification, which indicates the increase in  $\delta^{15}\text{N}$  of soil (Yamada et al., 1996). Wada et al. (1984) demonstrated that denitrification seems to have a small effect on the variation of  $\delta^{15}\text{N}$  of SOM under



equation:

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

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

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  $< 0.15\text{‰}$  for 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 and Table 1). 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. 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  $\delta^{13}\text{C}$  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.

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

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

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

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

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where  $f_{C\_MDN}$  and  $f_{C\_LDN}$  are the contributions of MDN and land-derived nutrient (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.

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 are typical migratory mammals and birds 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. no isotopic fractionation 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 chloroform-methanol solution (2:1) prior to SIA, to remove isotopically fractionated metabolites, such as Metabolites in the samples were removed by urea and ammonium (Kuwae et al., 2008, 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,  $0.090 \text{ mg L}^{-1}$  (SD  $0.022 \text{ mg L}^{-1}$ ), 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

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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 are 3.3 and 2.0 kg, respectively (Makiguchi et al., 2007), with a nitrogen content of about  $30.4 \text{ g kg}^{-1}$  (Larkin and Slaney, 1997). Therefore, annual TN potentially transported by chum and pink salmon is estimated to be 1542 and 3216  $\text{kg yr}^{-1}$  (CS 20%), 617 and 1287  $\text{kg yr}^{-1}$  (CS 50%), and 386 and 804  $\text{kg yr}^{-1}$  (CS 80%), respectively. Finally, the annual TN transported by chum and pink salmon per unit catchment area can be estimated as  $146 \text{ kg km}^{-2} \text{ yr}^{-1}$  (CS 20%),  $59 \text{ kg km}^{-2} \text{ yr}^{-1}$  (CS 50%), and  $37 \text{ kg km}^{-2} \text{ yr}^{-1}$  (CS 80%), (SD  $19 \text{ kg km}^{-2} \text{ yr}^{-1}$ ), 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  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of SOM were lower than those of salmon (Fig. 4). Interestingly, SSL has almost the same value of the mean  $\delta^{15}\text{N}$  of bamboo grass, which may suggest that bamboo grass can be considered to be as LDN. The stable isotope ratios in sea eagle droppings and brown bear feces were higher than LDN, indicating that sea eagles and bears are also one of the transporter of MDN to SOM. In the case of multiple food sources, feces and droppings 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). 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 previously 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  $\delta^{15}\text{N}$  is associated with soil

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organic matter, which is given as leaf litters, droppings from birds, feces from animals and so on. Also, Wada et al. (1984) revealed that  $\delta^{15}\text{N}$  is almost identical with organic nitrogen in natural forests. Therefore, it is important to understand the influence of sea eagles and bears on nutrient-cycle system. However, from Fig. 4, we cannot quantify the relative contribution of sea eagles and bears to total MDN transport.

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 (Fig. 5). 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. 5). 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  $104 \text{ kg km}^{-2} \text{ yr}^{-1}$  ( $= 454 \text{ kg km}^{-2} \text{ yr}^{-1} \times 22.9\%$ ) (SD  $16 \text{ kg km}^{-2} \text{ yr}^{-1} = 454 \text{ kg km}^{-2} \text{ yr}^{-1} \times 3.6\%$ ) based on the contribution of MDN to SOM (Fig. 5 and Table 2).

Wada et al. (1984) demonstrated that  $\delta^{15}\text{N}$  of SOM in the forest has significant variation in the surface soil, such as about  $-3$  to  $-2\%$  at Jumonji in Chichibu and at Mt. Shigayama, and about 1 to 5% at Memuro in the eastern Hokkaido.  $\delta^{15}\text{N}$  of about 1 to 5% 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 values of about 1 to 5% at Memuro are expected to include the influence of emission of anthropogenic nitrogen.  $\delta^{15}\text{N}$  of about  $-3$  to  $-2\%$  in surface soil at Jumonji and at Mt. Shigayama may support our assumption that the larger the  $\delta^{15}\text{N}$  is, the higher the contribution of MDN becomes. In order to confirm our assumption, we carried out the similar field observation at 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 area of the Rusa River is covered by a special protection zone. The Rusa River basin is thus considered as more protected and natural area as

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the Natural World Heritage compared to the Rausu River. Therefore, the contribution of MDN is expected to be larger in the Rusa River basin compared to the Rausu River basin (Fig. 2). As a result, the spatial average of  $\delta^{15}\text{N}$  in the Rusa River basin was obtained as 1.1‰, which is 1.0‰ larger than the Rausu River basin. It may be thus suggested that the higher value of  $\delta^{15}\text{N}$  of SOM in surface soil is associated with the contribution of MDN. However, it should be noted that this value for MDN re-export is estimated without contribution of marine derived TDN and thus should be considered the minimum annual MDN re-export from the viewpoint of TN.

## 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 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.

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 potential contribution of TN by salmon was 23.8% (CS 20%), 9.5% (CS 50%), and 6.0% (CS 80%), while the contribution of MDN to SOM was 22.9% (SD 3.6%). Therefore, the annual potential contribution of salmon to TN may be

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146 kg km<sup>-2</sup> yr<sup>-1</sup> (CS 20 %), 59 kg km<sup>-2</sup> yr<sup>-1</sup> (CS 50 %), and 37 kg km<sup>-2</sup> yr<sup>-1</sup> (CS 80 %), which provides valuable support for an influence of MDN on the ecological systems across this river basin (Table 2).

*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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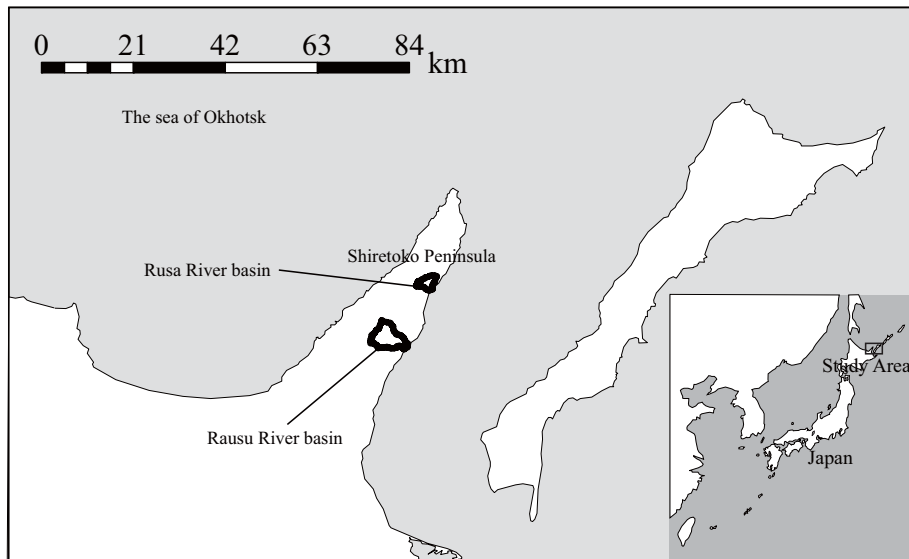
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**Figure 1.** Coastline around the Shiretoko Peninsula and the Rausu River basin.

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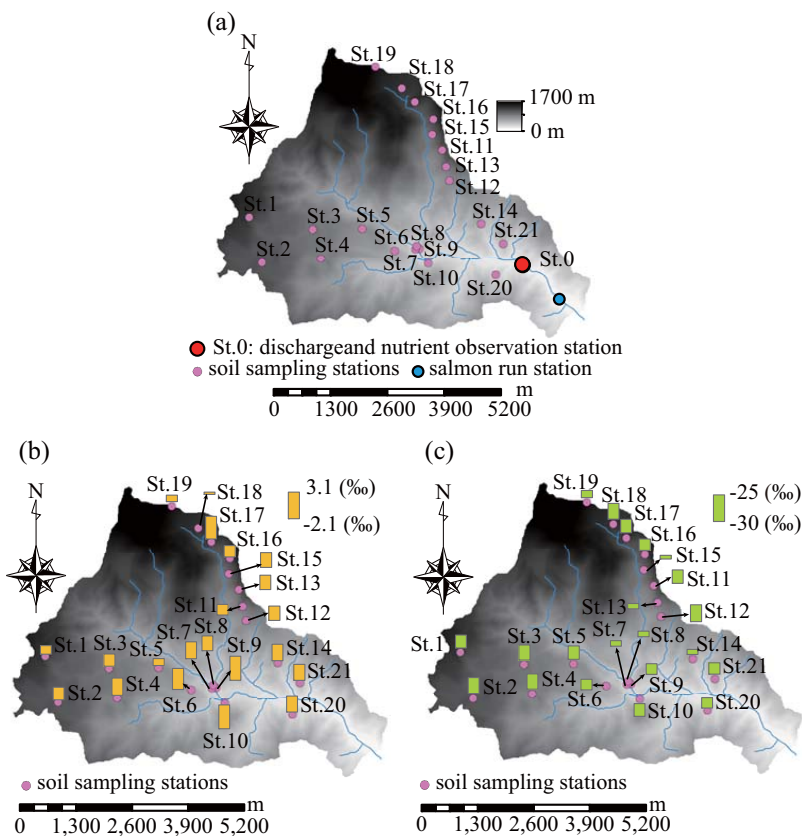
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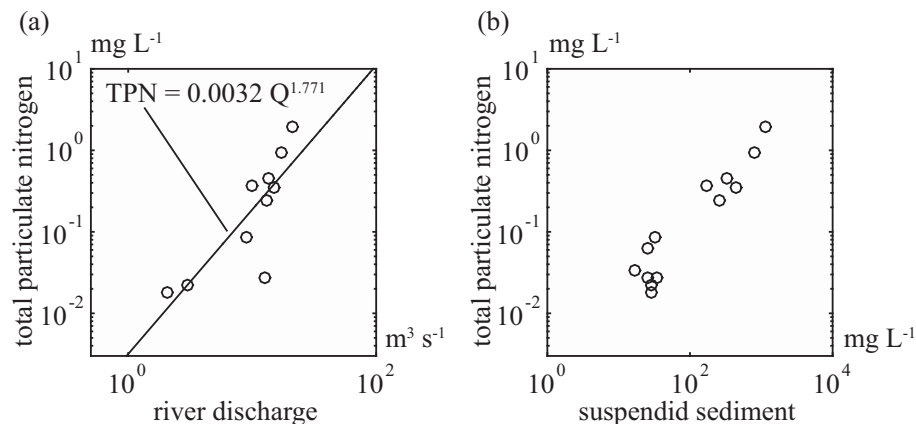
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**Figure 2.** (a) Elevation of the Rausu River basin. Green circles indicate surface soil sampling stations in September of 2012. Red circles indicates a field observation station for discharge, TDN (total dissolved nitrogen) and TPN (total particulate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

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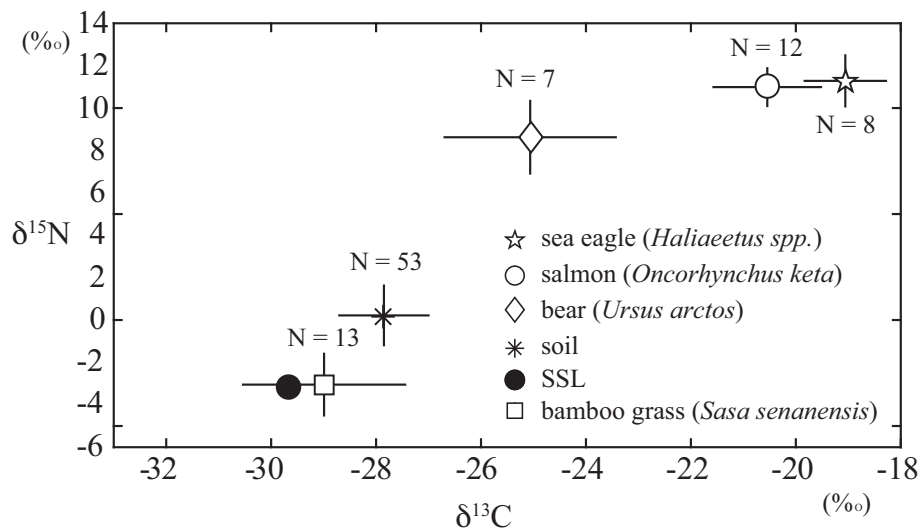
**Figure 3.** River discharge, total particulate nitrogen and suspended sediment at the river mouth of Rausu River. **(a)** River discharge and concentration of total particulate nitrogen. **(b)** Concentration of suspended sediment and concentration of total particulate nitrogen.

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

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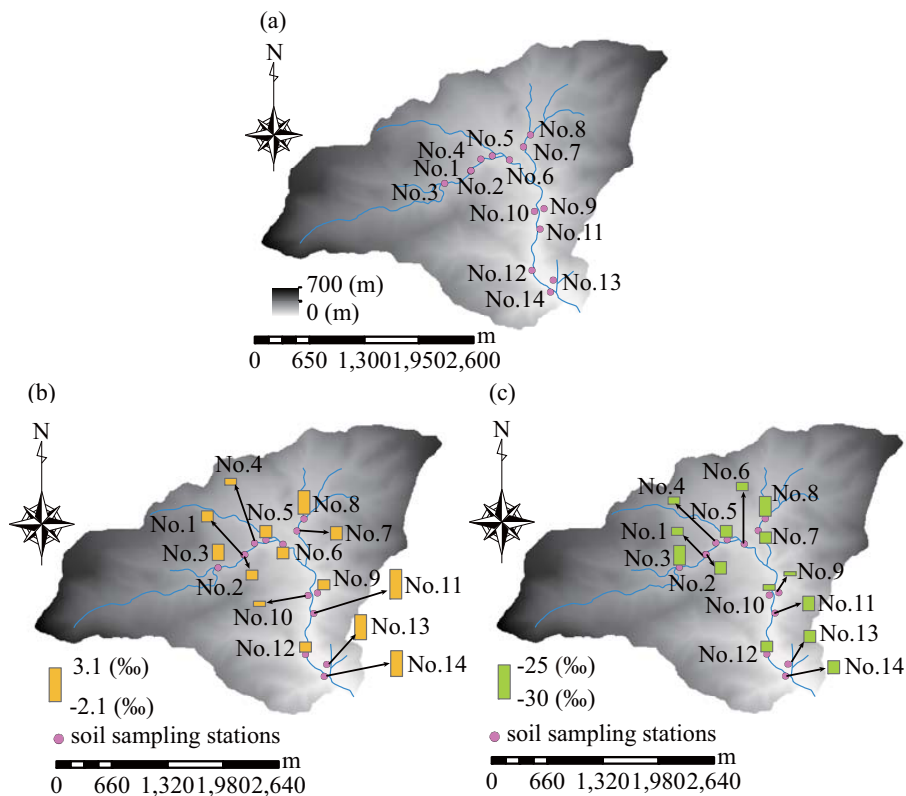
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**Figure 6.** (a) Elevation of the Rusa River basin. Green circles indicate surface soil sampling stations in September of 2012. (b)  $\delta^{15}\text{N}$  and sampling stations in 2014. (c)  $\delta^{13}\text{C}$  and sampling stations in 2014.

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