

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

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A-28
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12 **Abstract**

13 Total nitrogen (TN), which consists of total particulate nitrogen (TPN) and total
14 dissolved nitrogen (TDN), is transported with not only in river channels but also across
15 the entire river basin, including via ground water and migratory animals. In general,
16 TPN export from an entire river basin to the ocean is larger than TDN in a mountainous
17 region. Since marine derived nutrients (MDN) are hypothesized to be mainly
18 transported as suspended matters from the ground surface, it is necessary to investigate
19 the contribution of MDN to the forest floor (soils) in order to quantify the true role of
20 MDN at the river ecosystem scale. This study investigated TN export from an entire
21 river basin, and also we estimated the contribution of pink (*Oncorhynchus gorbuscha*)
22 and chum salmon (*O. keta*) to total oceanic nitrogen input across a river basin. The
23 potential contribution of TN entering the river basin by salmon was found to be 23.8 %
24 relative to the total amount of TN exported from the river basin. The contribution of
25 particulate nitrogen based on suspended sediment from the ocean to the river basin soils
26 was 22.9 % with SD of 3.6 % by using stable isotope analysis (SIA) of nitrogen ($\delta^{15}\text{N}$).

A-3

27 Furthermore, SIA showed that the transport of oceanic TN by sea eagles (*Haliaeetus*
28 spp.) was greater than that by bears (*Ursus arctos*), which had previously been that
29 bears are thought to be the major animal transporter of nutrients in the northern part of
30 Japan.

31

32 **1. Introduction**

33 SIA is increasingly being used to examine connectivity in coastal aquatic-terrestrial
34 ecosystems, such as the input of MDN from the open ocean to coastal and widely river
35 ecosystems (Wyatt et al., 2010a; Wyatt et al., 2010b; Wyatt et al., 2012). In the case of
36 river ecosystems, the transportation of nutrients, such as nitrogen and phosphorus, by
37 migrating fish results in enhancement of biofilms and planktonic productivity in river
38 systems (Juday et al., 1932; Cederholm and Peterson, 1985; Bilby et al., 1996; Gresh et
39 al., 2000; Chaloner et al., 2002; Moore and Schindler, 2004; Yanai and Kochi, 2005;
40 Levi and Tank, 2013). Most of those cases, many terrestrial consumers like mammals,
41 birds, fishes and insects have been shown to play a large role in terms of providing
42 MDN to watersheds (Donaldson, 1966; Ben-David et al., 1997a; Hilderbrand et al.,
43 1999; Gende et al., 2002; Naiman et al., 2002; Wilkinson et al., 2005; Bartz and Naiman,
44 2005). Moreover, MDN inputs have been shown as important processes controlling the
45 productivity of ecosystem. For example, Merz and Moyle (2006) found that the
46 contribution of MDN to the foliar nitrogen of wine grapes was about 18 to 25 %. Also,
47 Hilderbrand et al. (1999) demonstrated that trees and shrubs near spawning streams
48 receive 24 to 26 % of the foliar nitrogen from MDN, while Helfield and Naiman (2002)
49 suggested that 15.5 to 17.8 % of spruce foliage nitrogen is provided from MDN. Thus,
50 isotopic methods as intrinsic geospatial tracer provided quantification of

B-4

A-5

51 cross-ecosystem transfer of nutrients. In particular, migrating fish, such as salmon, have
52 been found to be necessary for a sustainable nutrient-cycle system due to their important
53 role as nutrient transporters (Ben-David et al., 1998; Wipfli et al., 1998; Yanai and
54 Kochi, 2005; Gende et al., 2007; Hocking and Reimchen, 2009; Hocking and Reynolds,
55 2011). Additionally, MDN has been demonstrated to be important not only for river
56 ecosystems but also potentially for upstream lakes (Kline et al., 1990; Kline et al., 1993;
57 Schindler et al., 2003).

58

59 ~~Here we focus on the particulate nutrient budgets due to suspended sediment at the river~~
60 ~~basin scale of using stable isotopes.~~ When we consider nutrient flux in a river flowing
61 from the upstream end into the ocean, the flux depends on nutrients supplied not only
62 inside the river itself but also from the entire river basin (Dutta and Nakayama, 2010;
63 Alam and Dutta, 2012; Riggsbee et al., 2008). Also, particulate nutrient flux, which is
64 given from surface soils dominantly, is revealed to be larger than dissolved nutrient
65 generally in a mountainous region (Nakayama et al., 2011). Cederholm et al. (1989)
66 demonstrated that mammals and birds consume migrating fish, which may result in the
67 secondary dispersion of MDN across the river basin associated with the movement of
68 these consumers. Other studies have revealed that mammals incorporate MDN from
69 salmon, which may subsequently lead to re-export to the ocean through river flows

A-6

70 (Bilby et al., 1996; Ben-David et al., 1997a; Ben-David et al., 1997b; Hilderbrand et al.,
71 1999; Szepanski et al., 1999; Reimchen, 2000). However, the contribution of MDN to
72 surface soils, which may be transported from a river basin to the ocean as suspended
73 sediments, at the river basin scale has not been adequately quantified in natural systems
74 because of difficulty to show those complex food web and accurate biomass.

A-7

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76 In this study we present the TN transport across an entire river basin to the ocean, the
77 potential contribution of TN from the ocean to a river basin by salmon, and the
78 contribution of MDN to surface soils in a river basin. Integrated stable isotope
79 researches in the geological, hydrological and biological aspects allowed us to estimate
80 nutrient budgets in natural river basin and convinced us to conserve the ocean river
81 connectivity.

A-8

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83 **2. Geophysical setting**

84 Our target area, the Shiretoko Peninsula, was registered as a World Natural Heritage
85 area in July of 2005. Shiretoko is located at the southernmost extent of drift ice and its
86 ecological systems exhibit high biodiversity and high rates of nutrient circulation,
87 particularly due to runs of pink (*Oncorhynchus gorbuscha*) and chum (*O. keta*) salmon
88 from the Sea of Okhotsk. Potential runs of salmon along the coast of Hokkaido in the

89 Sea of Okhotsk have been estimated at about 29,900,000 individuals a year (Hokkaido
90 National Fisheries Research Institute, Fisheries Research Agency, 2009), equivalent to
91 2590 tons of total nitrogen. The size of the Okhotsk coastal region of Hokkaido is about
92 24,000 km², which corresponds to that the mean total nitrogen input from the ocean is
93 about 108 kg km⁻² yr⁻¹ if we assume that all salmon run up rivers and the total nitrogen
94 is distributed into the river basins completely. Shiretoko is located on the northeast
95 coast of Hokkaido, Japan (approximately 43°57' N to 44°21' N and 144°58' E to 145°
96 23'E), and has a width, length and maximum altitude of about 15 km, 50 km and 1660
97 m, respectively (Fig. 1). The Rausu River basin was selected as a study area because its
98 watershed is the largest in the region and it is considered a representative watershed in
99 the Shiretoko Peninsula. The watershed area, river length, and the mean river slope are
100 32.5 km², 7 km, 1/7, respectively (Fig. 2). Because of the steep slope, nutrient flux due
101 to suspended sediments is larger than dissolved nutrient flux in the Rausu River basin
102 (Nakayama et al. 2011). Field experiments were carried out over 5 years from 2008 to
103 2012.

104

105 **3. Methods**

106 **3.1 Nitrogen from a river basin to the ocean**

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

118

119 **3.2 Salmon runs**

120 To evaluate the contribution of salmon to soil organic matter (SOM), salmon runs were
121 investigated in the Rausu River. Salmon were caught at the river mouth for artificial
122 incubation and release, providing an estimate of the number of salmon caught by the
123 apparatus (Hokkaido National Fisheries Research Institute, Fisheries Research Agency,
124 2009). The apparatus for catching salmon consisted of lattice fence, which does not
125 obstruct flood flow or completely block the runs of salmon. Therefore, it was necessary

A-10

126 to quantify the capture rate of the apparatus in order to estimate the actual volume of
127 salmon runs. Field observations were conducted in the Tokorohoronai River, which is
128 located in the same region of Hokkaido and has a custom to remove its apparatus before
129 and after salmon run seasons, allowing us to monitor the salmon escapement from the
130 apparatus and the salmon run under the open condition at the same place. The capture
131 rate of the apparatus was calculated with numbers of salmons passing the observation
132 point which has a channel section of 3 m in width and 0.2 m in depth, instead of the
133 Rausu River because its river width (about 15 m) is too wide to cover the entire width.
134 We used two infrared cameras (SM-AVIR-602S, Hero Corp., Izumo, Japan) placed 2 m
135 above the river surface and recorded videos in all day to monitor the individual salmon
136 passing this 3 m section. Videos were taken from the 25th to 28th of November (before
137 removal of the apparatus) and from the 4th to 7th of December (after removal of the
138 apparatus) in 2013. There was no influence of rainfall during the observation period.

139

140 **3.3 Stable isotope analysis**

141 MDN, such as nitrogen, are generally supplied from the ocean to surface soils in a river
142 basin as SOM, which includes feces of mammals, droppings of birds, and the remains of
143 salmon preyed upon by mammals, birds and insects. To focus on the influence of SOM
144 on particulate nitrogen in the river basin soils, soil particles with diameter of less than

145 500 μm after rinsing in 1N-HCL solution were used in the analysis. In general, some
146 proportion of the nitrogen is reduced into the atmosphere due to denitrification, which
147 indicates the difficulty evaluating total amount of supplied nitrogen. In this study, it
148 cannot be allowed to evaluate how much dissolved nitrogen is decomposed from SOM.
149 However, TPN export from an entire river basin is revealed to be larger than TDN in the
150 Rausu River basin due to the steep slope (Nakayama et al., 2011). Therefore, we made
151 an attempt to estimate the contribution of MDN to SOM as a sequel to an accumulation,
152 which directly corresponds to the suspended sediments transporting particulate nutrient
153 through a river to the ocean, by sampling surface soils across the Rausu River basin (Fig.
154 2). Surface soil samples were taken at 12, 20 and 21 stations in 2008, 2009 and 2012,
155 respectively. In 2008, fewer samples were taken as we did not have permission to
156 sample surface soils in special protection zones. Surface soils were sampled from three
157 different points at each station in a volume of 15 cm \times 15 cm \times 5 cm (height \times width \times
158 depth). Surface soil sampling stations in 2012 are shown in Fig. 2. Since previous
159 studies have revealed that surface soil transport is related to the spatial distribution of
160 surface soil type, land-use type and vegetation (Ishida et al., 2010), the location of each
161 sampling station was selected by dividing the river basin into 21 domains (sub-basin
162 areas) that vary in soil type and vegetation. The spatial distribution of surface soil type
163 is divided into 6 categories. Although the spatial pattern in vegetation is complicated,

A-12

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164 the vegetation can generally be categorized in terms of altitude. Since Shiretoko is
165 protected as natural World Heritage area, all areas studied are classified as forest and
166 have high vegetation cover. Stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$)
167 were measured using a Delta Plus Advantage mass spectrometer (Thermo Electron)
168 coupled with an elemental analyzer (Flash EA 1112, Thermo Electron) at the Port and
169 Airport Research Institute, Japan. Stable isotope ratios are expressed in δ notation as the
170 deviation from standards in parts per thousand (‰) according to the following equation:

171

$$172 \quad \delta^{13}\text{C}, \delta^{15}\text{N} = [R_{\text{sample}} / R_{\text{standard}} - 1] \quad (1)$$

173

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

175

176 PeeDee Belemnite and atmospheric nitrogen were used as the isotope standards of
177 carbon and nitrogen, respectively. The analytical precision in the mass spectrometer
178 system based on the standard deviation of the internal reference (L-histidine) replicates
179 was $<0.15\text{‰}$ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. The contribution of MDN to SOM in surface soils
180 was evaluated by applying a two source mixing model based on stable isotope analysis
181 (SIA) of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) (Kline et al., 1998; Moore and Semmens,
182 2008; Hossler and Bauer, 2012). Three soil samples were collected at each sampling

A-13

183 station in order to account for small scale variability in SOM (Fig. 2). Salmon tissue
 184 isotopes were considered representative of the isotope composition of ocean
 185 productivity. To isotopically characterize terrestrial productivity, we considered one
 186 terrestrial end-members (sources): Soil Samples exhibiting the Lowest values of $\delta^{13}\text{C}$
 187 and $\delta^{15}\text{N}$ (hereafter SSL), and thus assumed to have the highest terrestrial contribution
 188 to SOM. SSL was collected close to the top of the mountain, where MDN is not
 189 expected to influence isotope values. Representative soil samples collected in the same
 190 river basin were chosen because they have isotopically similar characteristics to the
 191 target soil samples in this study.

A-16

192
 193 The contribution of MDN to SOM was evaluated using a two sources mixing model
 194 based on the measured $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. The average contribution in the Rausu River
 195 basin was computed using each sub-basin area obtained from the Thiessen method.

197 $f_{C_MDN} + f_{C_LDN} = 1$ (2)

198 $f_{C_MDN} \delta^{13}\text{C}_{salmon} + f_{C_LDN} \delta^{13}\text{C}_{SSL} = \delta^{13}\text{C}_{soil}$ (3)

199 $f_{N_MDN} + f_{N_LDN} = 1$ (4)

200 $f_{N_MDN} \delta^{15}\text{N}_{salmon} + f_{N_LDN} \delta^{15}\text{N}_{SSL} = \delta^{15}\text{N}_{soil}$ (5)

201

202 where f_{C_MDN} and f_{C_LDN} are the contributions of MDN and land-derived nutrient (LDN)
203 by carbon, $\delta^{13}C_{salmon}$, $\delta^{13}C_{SSL}$ and $\delta^{13}C_{soil}$ are the stable isotope ratios of carbon for
204 salmon, SSL and soil samples, respectively, f_{N_MDN} and f_{N_LDN} are the contributions of
205 MDN and LDN by nitrogen, $\delta^{15}N_{salmon}$, $\delta^{15}N_{SSL}$ and $\delta^{15}N_{soil}$ are the stable isotope ratios
206 of nitrogen for salmon, SSL and soil samples, respectively.

207

208 As bamboo grass (*Sasa senanensis*) is the dominant species in the study area, bamboo
209 grass was collected at 6 soil sampling points. Furthermore, to investigate the
210 contribution of the other typical mammals and birds in Shiretoko to SOM, and to
211 roughly understand these animals diets, droppings of sea eagles (*Haliaeetus* spp.) and
212 feces of brown bear (*Ursus arctos*) were collected (Kuwae et al., 2008; Kuwae et al.,
213 2012). Chum salmon tissues and droppings of sea eagles were collected at the river
214 mouth and feces of brown bear were collected at St.14. Then those samples were used
215 into SIA after rinsing in chloroform-methanol solution (2:1). Dropping and feces
216 samples can reduce the uncertainty in terms of SIA fractionation factors when compared
217 to tissue samples (e.g., muscle, liver, and blood). As fractionation occurs during the
218 making or breaking of bonds in small molecules, we might not expect fractionation
219 during food assimilation, i.e., uptake of large molecules, in the absence of the breaking
220 of nitrogen bonds (Fry, 2006). Thus, while tissue samples have variability and

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221 uncertainty related to fractionation factors (body conditions such as fasting), we
222 consider that feces and dropping samples do not. However, in the case of multiple food
223 sources, feces and dropping are likely to be enriched in relatively indigestible food
224 sources, when compared with stomach contents or assimilated materials (Sponheimer et
225 al., 2003; Kuwae et al., 2008). A further advantage of using droppings, as opposed to
226 tissues, is that no killing and/or damage to wildlife is involved in collecting samples.
227 Multivariate analysis of variance (MANOVA) and post hoc tests by Tukey Kramer
228 were used to investigate differences in surface soil samples.

A-20

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230 4. Results and Discussion

231 4.1 Estimation of nitrogen export to the ocean

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

B-7

A-22

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$$239 \text{ TPN} = 0.0032 \times Q^{1.771} \quad (6)$$

240

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

242 The validity of the storage function method model was confirmed using the observed

243 river discharge from April to September of 2009, which resulted in a Coefficient of

244 Determination (CoD) of 0.61. The reliability of the model has been shown to be high

245 enough for the analysis of river discharge when the CoD is more than 0.6 (Dutta and

246 Nakayama, 2010). Annual mean export of TDN, TPN and TN from 2008 to 2012 were

247 5210 kg yr^{-1} , 14750 kg yr^{-1} and 19960 kg yr^{-1} , respectively. Since the size of the Rausu

248 River basin of Shiretoko is 32.5 km^2 , the annual mean exports of TDN, TPN and TN per

249 unit catchment area were $160 \text{ kg km}^{-2} \text{ yr}^{-1}$, $454 \text{ kg km}^{-2} \text{ yr}^{-1}$ and $614 \text{ kg km}^{-2} \text{ yr}^{-1}$,

250 respectively (Table 1). The average concentrations of TDN and TPN from 2008 to 2012

251 were 0.090 mg L^{-1} and 0.216 mg L^{-1} , which agrees with a previous study at the site

252 (Nakayama et al., 2011).

253

254 **4.2 Contribution of salmon runs to nitrogen input from the ocean**

255 The average number of salmon passing the cameras in the Tokorohoronai River during

256 the 4 days while the apparatus for catching salmon was present was 0.49 hr^{-1} . The

257 average numbers for 4 days after the apparatus was removed from the river was 0.61

A-25

258 hr⁻¹, so the rate of capture of salmon by the apparatus was estimated as 20 %:

259 $(0.61-0.49) / 0.61 = 0.20$.

260

261 In the Rausu River of Shiretoko, the annual average numbers of salmon caught by the

262 apparatus at the river mouth were 3075 and 10580 for chum and pink salmon,

263 respectively, from 2001 to 2009. By assuming that all apparatuses have the same rate of

264 capture, the potential for chum and pink salmon runs can be estimated as 15375 and

265 52900, respectively. The average weight of chum and pink salmon are 3.3 kg and 2.0 kg,

266 respectively (Makiguchi et al., 2007), with a nitrogen content of about 30.4 g kg⁻¹

267 (Larkin and Slaney, 1997). Therefore, annual TN potentially transported by chum and

268 pink salmon is estimated to be 1542 kg yr⁻¹ and 3216 kg yr⁻¹, respectively. Finally, the

269 annual TN transported by chum and pink salmon per unit catchment area can be

270 estimated as 146 kg km⁻² yr⁻¹ (SD 19 kg km⁻² yr⁻¹), which corresponds to the

271 contribution of TN by salmon, 23.8 % (SD 3.1 %), relative to the annual outflow of TN

272 per unit area (considered to be 100 %) (Table 1).

273

274 **4.3 Contribution of MDN to SOM in the Rausu River basin**

275 ~~The 2012 field experiment suggested that stable isotope ratios, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, were~~

276 ~~relatively higher close to the ocean (stations 20 and 21) compared to the top of the~~

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277 mountain (station 19), which has been also demonstrated by Kline et al. (1998) (Fig. 2).
278 Tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were highest in the sea eagles (n=8) and lowest in the bamboo
279 grass (n=3). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of SOM (n=53) lay between sea eagles and bamboo
280 grass (Fig. 4). MANOVA suggested that there was no isotopic ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$)
281 difference between salmon tissue (n=12) and sea eagle droppings. Feces from brown
282 bears (n=7), which were previously thought to be the major terrestrial consumer of
283 spawning salmon, were significantly lower than those of salmon and sea eagles (bear vs.
284 salmon ($\delta^{13}\text{C}$): $P < 0.001$; bear vs. salmon ($\delta^{15}\text{N}$): $P < 0.001$; bear vs. sea eagle ($\delta^{13}\text{C}$):
285 $P < 0.001$; bear vs. sea eagle ($\delta^{15}\text{N}$): $P < 0.001$). The stable isotope ratios in sea eagle
286 droppings were the highest among the animals measured. Also, the contributions of the
287 other predators may impact re-export of nutrient from the ocean across the river basin;
288 their role in marine derived nitrogen input and re-export, such as through release of
289 MDN-rich feces, should be investigated in more detail in future studies.

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291 Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of SOM were lower than those of salmon (Fig. 4). Interestingly,
292 SSL has almost the same value of the mean value of bamboo grass, which may suggest
293 that bamboo grass can be considered to be as LDN. The stable isotope ratios in sea
294 eagle droppings and brown bear feces were higher than LDN. Since brown bears are
295 previously thought to be the major terrestrial consumer of spawning salmon, they may

296 impact re-export of nutrient from the ocean across the river basin, such as through
297 release of MDN-rich urine and feces (Hilderbrand et al. 1999). Although fractionation
298 occurs due to breaking of bonds in small molecules, we might not expect fractionation
299 during food assimilation in the absence of the breaking of nitrogen bonds (Fry, 2006).
300 Therefore, feces and dropping are likely to be enriched in relatively indigestible food
301 sources, when compared with stomach contents or assimilated materials (Sponheimer et
302 al., 2003; Kuwae et al., 2008). However, it is difficult from Fig. 4 to decide that sea
303 eagles eat more oceanic fish than bears because bears release more MDN as urine
304 compared to dropping (Hilderbrand et al. 1999).

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A-3

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306 The isotopic composition of salmon as representative of oceanic $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were
307 10.99 and -20.54, respectively. The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of SSL were -3.19 and -29.48,
308 respectively. Therefore, the three-year average estimate of the contribution of MDN to
309 SOM for $\delta^{15}\text{N}$ depending on the choice of terrestrial isotope values was obtained e.g.
310 22.9 % (SD 3.6%) by using a two sources mixing model (Fig. 5). As the reference, the
311 three-year average estimate of the contribution of MDN to SOM for $\delta^{13}\text{C}$ was 17.7 %
312 (SD 1.1%) (Fig. 5). Since annual export of TPN per unit area from the Rausu River
313 basin to the ocean was $454 \text{ kg km}^{-2} \text{ yr}^{-1}$, annual re-export of TPN originally derived
314 from the ocean is estimated to be $104 \text{ kg km}^{-2} \text{ yr}^{-1}$ ($= 454 \text{ kg km}^{-2} \text{ yr}^{-1} * 22.9\%$) (SD 16

315 $\text{kg km}^{-2} \text{ yr}^{-1} = 454 \text{ kg km}^{-2} \text{ yr}^{-1} * 3.6\%$) based on the contribution of MDN to SOM
316 (Table 1 and Fig. 5). However, it should be noted that this value for MDN re-export is
317 estimated without contribution of marine derived TDN and thus should be considered
318 the minimum annual MDN re-export from the viewpoint of TN.

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320 **5. Conclusions**

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

334

335 Annual TN transport estimated for pink salmon was twice that for chum salmon, which
336 suggests that pink salmon play a greater role in the input of TN across the Rausu River
337 basin. The potential contribution of TN by salmon was 23.8 % (SD 3.1 %), while the
338 contribution of MDN to SOM was 22.9 % (SD 3.6 %). Therefore, the annual potential
339 contribution of salmon to TN may be $146 \text{ kg km}^{-2} \text{ yr}^{-1}$ (= 23.8 %), which provides
340 valuable support for a strong influence of MDN on the ecological systems across this
341 river basin (Table 1 and Fig. 6).

342

343

344 **Author contribution**

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

354

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A-35

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515 Fig. captions:

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517 Fig. 1. Coastline around the Shiretoko Peninsula and the Rausu River basin.

A-36

518 Fig. 2. (a) Elevation of the Rausu River basin. Green circles indicate surface soil

A-37

519 sampling stations in September of 2012. Red circles indicates a field observation station

520 for discharge, TDN (total dissolved nitrogen) and TPN (total particulate nitrogen). (b)

521 $\delta^{15}\text{N}$ and sampling stations in 2012. (c) $\delta^{13}\text{C}$ and sampling stations in 2012.

522 Fig. 3. River discharge, total particulate nitrogen and suspended sediment at the river

523 mouth of Rausu River. (a) River discharge and concentration of total particulate

524 nitrogen. (b) Concentration of suspended sediment and concentration of total particulate

525 nitrogen.

A-39

526 Fig. 4. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of bamboo grass (*Sasa senamensis*), SSL (Soil Samples

A-41

527 exhibiting the Lowest values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), soil samples, bear feces (*Ursus arctos*),

528 salmon (*Oncorhynchus keta*), and sea eagles droppings (*Haliaeetus spp.*). The bars

529 indicate the standard deviation.

B-8

530 Fig. 5. Contribution of MDN (marine derived nitrogen) from the ocean to the Rausu

531 River basin in 2008, 2009 and 2012 using the two sources mixing model. (a) Average

532 contributions of MDN based on SSL (Soil Samples exhibiting the Lowest values of

533 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) for $\delta^{15}\text{N}$ were 22.9 %. (b) Average contributions of MDN based on SSL

534 for $\delta^{13}\text{C}$ were 17.7 %.

535 Fig. 6. Annual input of TN (total nitrogen) per unit area from the Rausu River basin to

536 the ocean, and annual TN transported by salmon per unit area, relative to the annual

537 outflow of TPN per unit area (considered to be 100 %).

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540 Table captions:

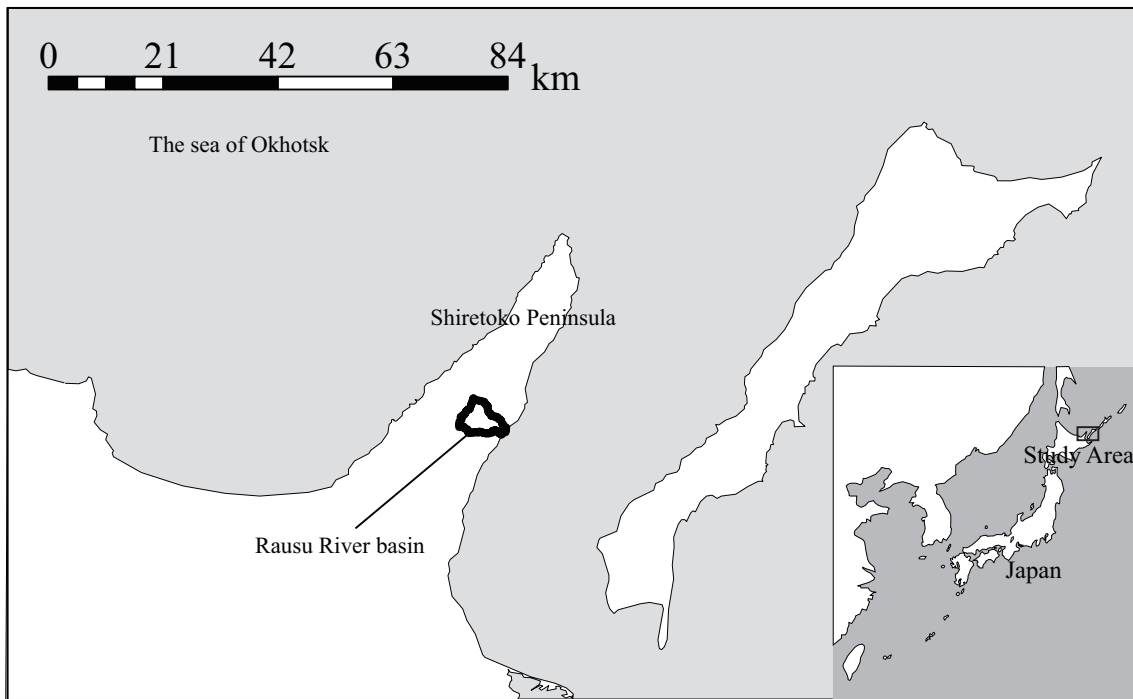
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542 Table 1. Summary of annual export and re-export of nitrogen per unit area.

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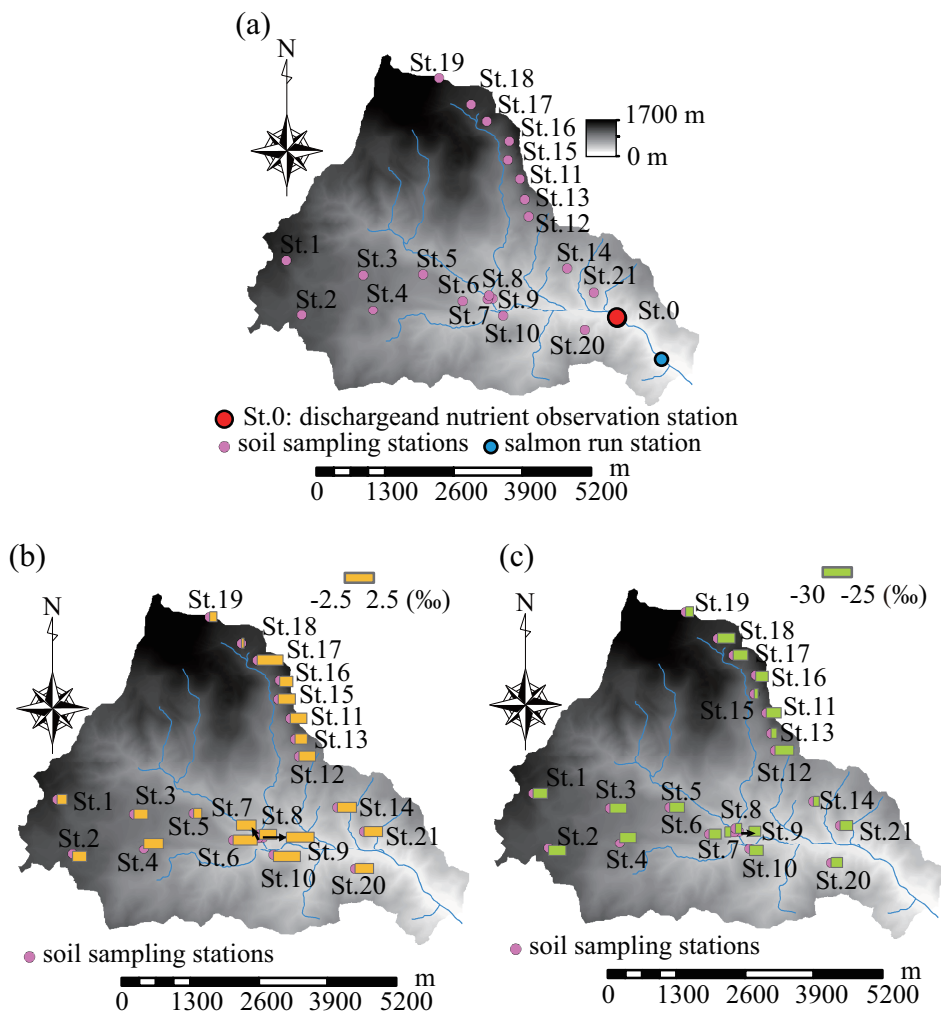
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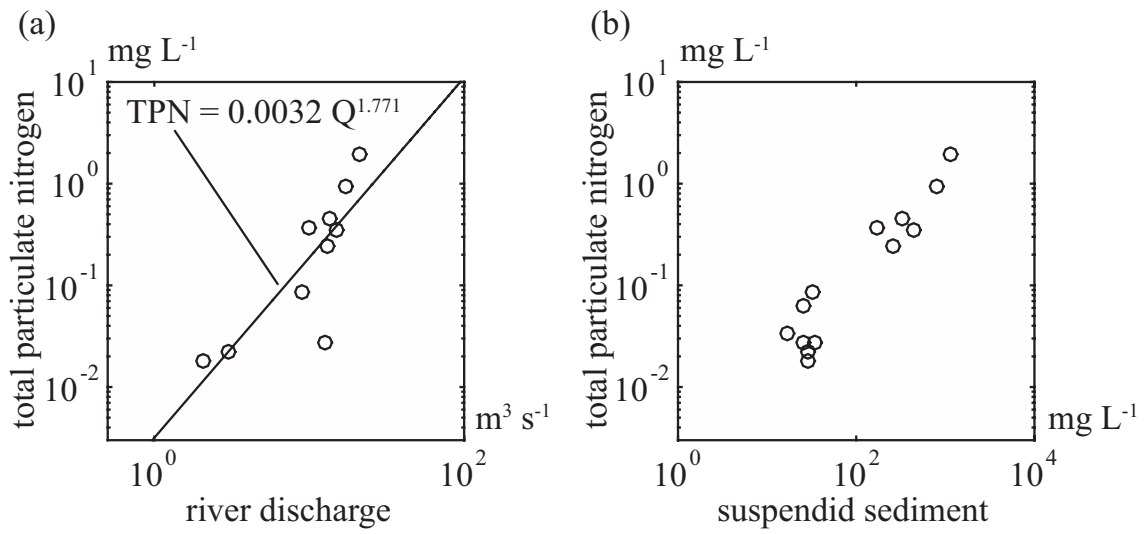
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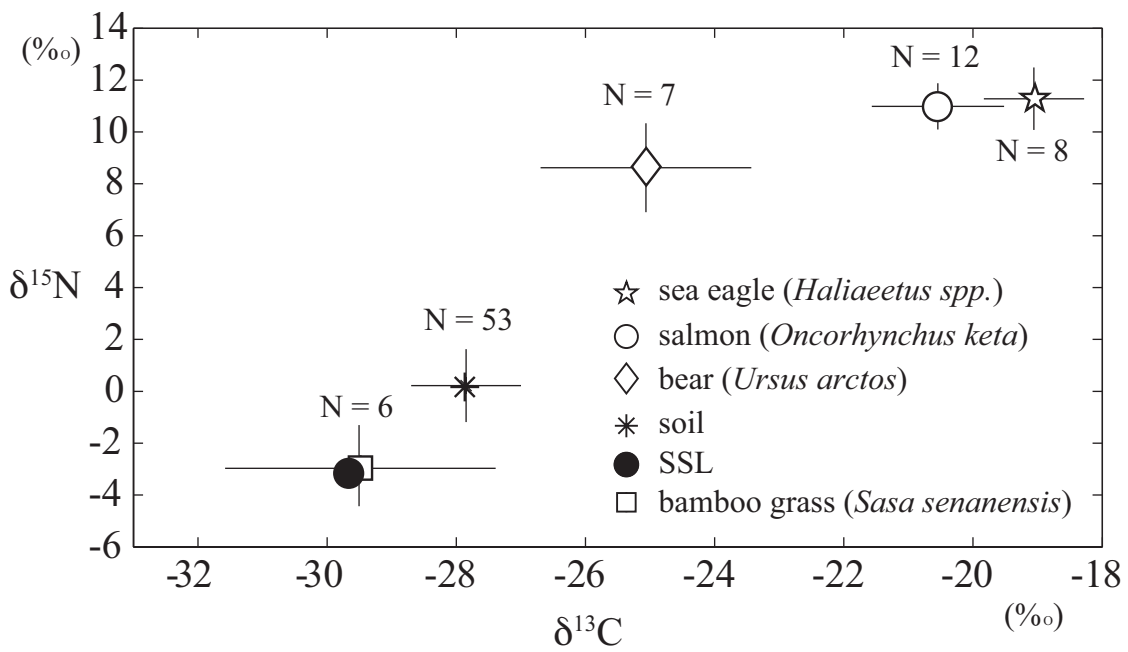
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564 nitrogen.

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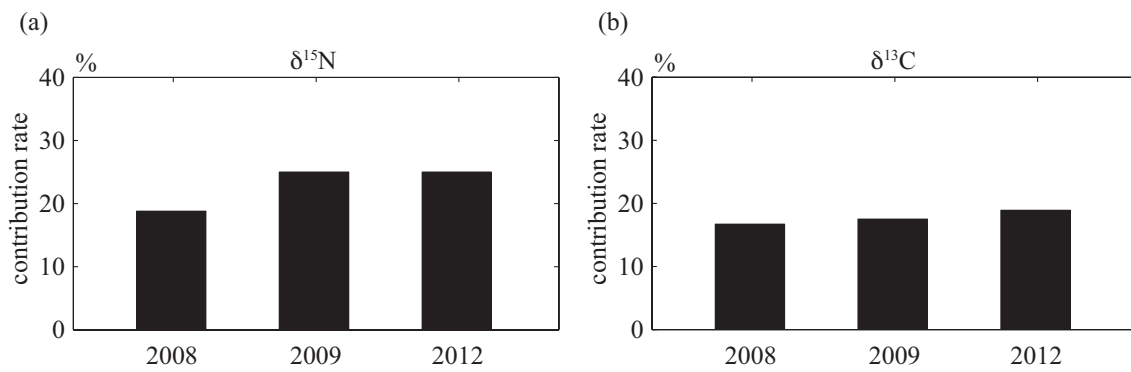
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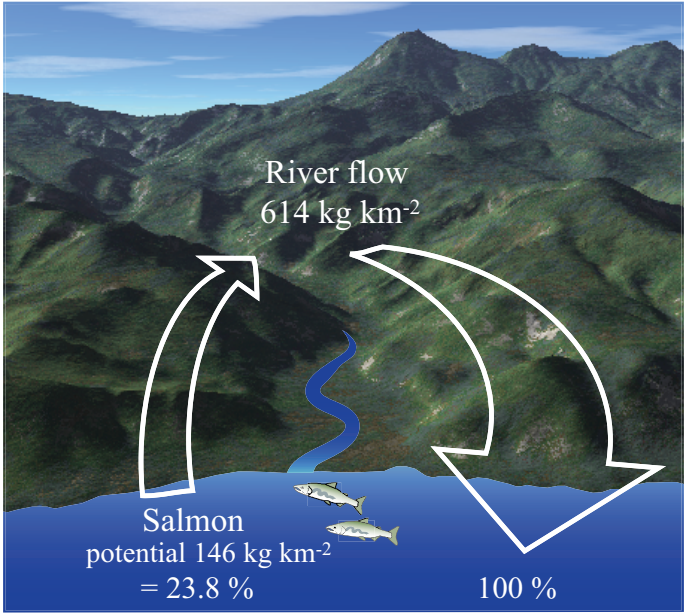
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594 Table 1. Summary of annual export and re-export of nitrogen per unit area.

	N export		N re-export	
	N kg· y ⁻¹	N kg· km ⁻² · y ⁻¹	Salmon run (%*)	MDN input (%)**
			N kg· km ⁻² · y ⁻¹	N kg· km ⁻² · y ⁻¹
TDN	5210	160	-	-
TPN	14750	454	-	104 (22.9)
TN	19960	614	146 (23.8)	-

595 * = (Salmon run)/(N export)

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