Riparian soil nitrogen cycling and isotopic enrichment in response to a long-term salmon

carcass manipulation experiment

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1   **Abstract**

2   Pacific salmon acquire most of their biomass in the ocean before returning to spawn

3   and die in coastal streams and lakes, thus providing subsidies of marine-derived nitrogen

4   (MDN) to freshwater and terrestrial ecosystems. Recent declines in salmon abundance have

5   raised questions of whether managers should mitigate for losses of salmon MDN subsidies.

6   To test the long-term importance of salmon subsidies to riparian ecosystems we measured

7   soil nitrogen cycling in response to a 20-year manipulation where salmon carcasses were

8   systematically removed from one bank and deposited on the opposite bank along a 2 km

9   stream in southwestern Alaska. Soil samples were taken at different distances from the stream

10 bank along nine paired transects and measured for organic and inorganic nitrogen

11 concentrations, and nitrogen transformation rates. MDN was measured using $^{15}N/^{14}N$ for bulk

12 soils, and NH_4^+ and NO_3^- soil pools. Stable isotope analyses confirmed $^{15}N/^{14}N$ was elevated

13 on the salmon enhanced bank compared to the salmon depleted bank. However, $^{15}N/^{14}N$

14 values of plant-available inorganic nitrogen exceeded the $^{15}N/^{14}N$ of salmon inputs,

15 highlighting nitrogen isotope fractionation in soils that raises significant methodological

16 issues with standard MDN assessments in riparian systems. Surprisingly, despite 20 years of

17 salmon supplementation, the presence of MDN did not cause a long-term increase in soil

18 nitrogen availability. This finding indicates the importance of MDN to ecosystem nitrogen

19 biogeochemistry and riparian vegetation may be overestimated for some systems. Given that

20 essential nutrients can also be pollutants, we urge more critical analyses of the role of MDN

21 to inform compensatory mitigation programs targeting salmon nutrient enhancement.

22

23 Keywords: salmon, stable isotopes, marine-derived nitrogen, riparian, soil biogeochemistry,

24 boreal forest, Alaska

Introduction

25

26   Pacific salmon (*Oncorhynchus* spp.) migration from marine environments to

27   freshwater spawning grounds is a textbook case of cross-ecosystem nutrient subsidies.

28   Dozens of studies have identified the presence of marine-derived nitrogen (MDN) from

29   salmon cross ecosystem boundaries from oceans to freshwaters and into the terrestrial

30   environment (sensu, Polis et al. 2004, Gende et al. 2002, Schindler et al. 2003). Declines in

31   Pacific salmon populations in many areas, caused by human activities (overharvest, habitat

32   degradation, dams) (Gustafson et al. 2007), and the concern over loss of MDN to coastal

33   watersheds has made restoration of salmon nutrients a focal point for many management and

34   mitigation strategies. For example, in the Columbia River Basin where Pacific salmon

35   populations have declined, legislation requiring compensatory mitigation has led to nutrient

36   enhancement programs, on the foundation that habitats have lost critical nutrients from

37   salmon and therefore augmentation is necessary to maintain ecosystem function (Collins et

38   al. 2015).

39   Salmon bring nutrients, including phosphorus (P) and other compounds in addition to

40   nitrogen (N), into freshwater and terrestrial food webs through two pathways: 1) direct

41   consumption of tissues by predators and scavengers, and 2) autotrophic or heterotrophic

42   assimilation of nutrients released as salmon spawn, die, and eventually decay (Gende et al.

43   2002). Salmon are enriched in the heavy isotope of nitrogen (^{15}N) relative to the light isotope

44   (^{14}N) when compared to terrestrial and watershed-derived N. This isotopic enrichment has

45   been used to quantitatively trace the presence of salmon derived nutrients into watersheds

46   (Schindler et al. 2003). For example, the proportion of N derived from salmon ranges from

47   approximately 30% – 75% in fish and aquatic invertebrates (Naiman et al. 2002), 10 – 90% in

48   piscivorous mammals such as bears, and 20 – 40% in piscivorous fishes near salmon

49 spawning grounds (Bilby et al. 1996, Hilderbrand et al. 1999, Chaloner et al. 2002, Claeson

50 et al. 2006).

51   The annual return of this predictable and abundant, yet temporally limited, high

52 quality resource drives the foraging ecology of both terrestrial and aquatic consumers (Quinn

53 2018, Schindler et al. 2013). Carcasses and roe are documented food sources for over 22

54 species of mammals, birds (Cenderholm et al. 1989), fishes (Scheuerell et al. 2007), and

55 invertebrates (Minakawa et al. 2002, Meehan et al. 2005; Winder et al. 2005). Bear

56 population density, body size, and reproductive output has been correlated with meat

57 (primarily salmon) consumption, with piscivorous populations having 55 times higher density

58 than their meat-limited counterparts (Hilderbrand et al. 1999). In aquatic ecosystems, salmon

59 carcass abundance has been correlated with elevated growth rates of invertebrates, and with

60 size, density, and condition factor of juvenile salmonids (Bilby et al. 1998, Minakawa et al.

61 2002, Wipfli et al. 2003).

62   The presence of MDN has been documented in aquatic primary producers, though its

63 overall ecological importance remains ambiguous. Via this bottom-up pathway, salmon

64 supply critical limiting nutrients that can increase primary and/or bacterial productivity,

65 which are subsequently transferred to consumers and up through the food web (Wipfli et al.

66 1998, Chaloner et al. 2002, Holtgrieve and Schindler 2011). Higher salmon returns are

67 correlated with MDN signatures in lower trophic levels including zooplankton and

68 periphyton (Kline et al. 1993, Finney et al. 2000, Holtgrieve et al. 2010). Both direct

69 ecological and paleolimnological evidence suggest MDN and P positively influence primary

70 production in lakes (Moore et al. 2007). For example, commercial fisheries remove upwards

71 of two-thirds of MDN which would otherwise enter some freshwater lakes in Alaska,

72 resulting in a 3-fold decline in algal production (Schindler et al. 2005). In stream ecosystems,

73 the decomposition of salmon increases dissolved organic and inorganic nutrients, including

74   highly available forms such as orthophosphate (PO_4^{3-}) and ammonia/ammonium (NH_3/NH_4^+).

75   These nutrients can stimulate epilithon growth (bacteria and algae), though the magnitude of

76   this response is highly variable, and dependent on other growth limiting factors such as

77   sunlight and disturbance (Johnston et al. 2004, Mitchell and Lamberti 2005, Janetski et al.

78   2009).

79   In the terrestrial realm, bottom-up effects of MDN from salmon are also thought to be

80   ecologically important, though this has been difficult to demonstrate rigorously. Studies

81   across the range of salmon in North America have inferred that up to 26% of foliar N in

82   riparian plants is marine derived, with foliar N levels often correlating with salmon

83   abundance and distance from the salmon spawning location (e.g., Hocking and Reynolds

84   2012, Reimchen and Fox 2013). While MDN is clearly present in terrestrial producers, direct

85   evidence of the importance of MDN for ecosystem function and productivity is much less

86   evident. Helfield and Naiman (2001) measured tree growth increments in areas with and

87   without salmon and found higher growth in one species (Sitka spruce) in areas where salmon

88   nutrients were present, although these findings were later contested on statistical grounds

89   (Kirchoff 2003). Hocking and Reynolds (2012) observed decreased understory plant diversity

90   with increasing salmon abundance, though this pattern was largely attributed to increased

91   dominance of a single N tolerant species (salmonberry). Reimchen and Fox (2013) suggested

92   that salmon abundance increased tree growth, but tree ring $^{15}N/^{14}N$ values were not related to

93   salmon abundance; other growth limiting factors such as temperature and location were

94   important covariates. Most recently, Quinn et al. (2018) examined tree growth increments in

95   the riparian zone of a small Alaskan stream before and after a 20-year, > 200,000 kg, salmon

96   carcass manipulation. In the two decades prior to manipulation, white spruce (*Picea glauca*)

97   on average grew faster on one bank compared to the other. The subsequent decades of carcass

98   manipulation enriched the naturally slower growing side, and were associated with increased

99   growth. However, the growth effect of the carcasses was smaller than the natural side-to-side

100   variation, and other important site and landscape factors such as forest demography, climate,

101   aspect, and water availability were not fully considered, a common trend in MDN studies of

102   riparian vegetation.

103   Interpreting the contributions of MDN to terrestrial producers using stable isotopes is

104   often highly simplified, and does not consider how variability of N sources and overall N

105   availability may confound results. MDN analyses apply simple two-source mixing models to

106   infer the proportion of total N derived from salmon. When applied to terrestrial vegetation,

107   the terrestrial end-member for the mixing models is typically determined by sampling the

108   $^{15}N/^{14}N$ of the same species of plant either laterally away from the stream (where MDN

109   contribution is expected to be small), upstream of barriers to salmon migration, or in

110   watersheds without salmon. For the salmon end-member, a single value equal to the average

111   $^{15}N/^{14}N$ of salmon (12.62 ± 0.31 per mil for sockeye salmon) is typically used (Appendix S1:

112   Equation S1). Inherent assumptions with these models therefore include: 1) reference sites

113   are biogeochemically similar to salmon sites and 2) the isotopic signature of salmon is

114   unchanged in the soils prior to plant uptake. N cycling in soils is strongly controlled by

115   position in the landscape and contains a number of chemical reactions which fractionate N

116   isotopically (Högberg 1997, Wheeler et al. 2014, Figure 1), therefore these assumptions may

117   not be valid.

118   Experiments examining the contributions of MDN are often limited by short

119   timescales, and relatively few experiments investigate changes in plant-available soil N pools

120   important to plant nutrient uptake and growth (Collins et al. 2015). Studies examining spatial

121   and temporal impacts of salmon on soil inorganic N have identified highly localized

122   responses (effects only observed < 30 cm from carcasses) where soil ammonium (NH_4^+) and

123   nitrate (NO_3^-) increase for weeks to months (Drake et al. 2005, Gende et al. 2007, Holtgrieve

124   et al. 2009) and rarely consider long-term N retention in the system. Experiments typically

125   examine the contributions of MDN by nutrient addition not nutrient removal; however,

126   nutrient removal is important for understanding the effects of lower numbers of salmon

127   returning to coastal watersheds due to fishing, habitat reduction, and climate change. In

128   addition, previous research observed a strong effect of watershed slope on $^{15}N/^{14}N$ in riparian

129   plants and attributed this to topography concentrating carcasses near streams (Hockings and

130   Reynolds, 2012). However, watershed topography also influences soil water content and N

131   cycling, which affect N isotopes (Högberg 1997) and therefore complicates MDN

132   assessments.

133   To resolve the extent to which salmon carcasses contributed MDN to plant-available

134   N pools and the long-term ecological response to this subsidy, we present a second study of

135   the 20-year carcass manipulation experiment described in Quinn et al. (2018). While Quinn et

136   al. focused on tree growth before and after the manipulation, the objective of this work was to

137   determine whether prolonged enhancement and reduction of salmon subsidies altered long-

138   term soil N cycling, similar to that documented in forests receiving N fertilizer additions

139   (Prescott et al. 1992, Prescott et al. 1995, Lu et al, 2010). If long-term changes in N

140   availability due to salmon enhancement or reduction were observed, compensatory nutrient

141   subsidies may be valuable for maintaining critical ecosystem functions in riparian areas with

142   reduced salmon returns. If not, then the addition of nutrients as a management response to

143   low salmon returns may have unintended negative consequences (sensu Compton et al.

144   2006). Specifically, the importance of MDN to riparian ecosystems was assessed by 1)

145   evaluating the presence of MDN in soils enhanced and depleted in salmon carcasses through

146   bulk stable isotope analysis of N, 2) quantifying the response of plant-available N pools

147   ($[NH_4^+]$ and $[NO_3^-]$) and their rate of supply via mineralization and nitrification, 3)

148   considering how fractionation in soils may impact mixing model results by measuring

149     $^{15}N/^{14}N$ of NH_4^+ and 4) comparing these results to the vegetation responses measured by

150   Quinn et al. 2018 at the same site. This research fills key knowledge gaps by examining the

151   long-term legacy of inorganic N pools, both salmon addition and removal, and considering

152   site variability that may impact the assumption of biogeochemical similarity between test and

153   control sites, following a 20-year manipulation.

154   **Methods**

155   *Site Description and Sample Collection*

156   This study was conducted on Hansen Creek, a ~2 km long, 2nd order tributary to Lake

157   Aleknagik in the Wood River system of Bristol Bay, AK and uses the same carcass

158   manipulation described in Quinn et al. (2018). Briefly, from 1997-2016 an average of 10,853

159   sockeye salmon returned to the stream annually. Overstory vegetation is dominated by white

160   spruce and paper birch (*Betula papyrifera*), and unlike many other watersheds in the region, it

161   has a low density of symbiotic N_2-fixing alder (*Alnus spp.* Helfield and Naiman 2002). From

162   1997-2016 the stream was surveyed daily during the annual sockeye salmon (*Oncorhynchus*

163   *nerka*) run and all dead salmon were removed from the creek and the river right bank to a

164   distance of about 5 m and tossed onto the river left bank. To avoid double counting carcasses

165   on the river left bank, carcasses naturally occurring on the river left bank were also relocated

166   to a distance of about 5 m, thus all carcasses (with the exception of those moved by wildlife,

167   see Quinn et al. 2018) were located between 3 – 6 m on the river left bank. Therefore, the

168   right side of the stream experienced a reduction in carcass density (depletion) while the left

169   bank received an increase in carcasses (enhancement). Quinn et al. (2018) calculated that

170   prior to manipulation the both banks averaged 4545.6 kg of salmon annually and after

171   manipulation the river left bank averaged 13,381 kg of salmon and the river right bank

172   averaged 2,260 kg of salmon annually, a 9.6-fold difference. Approximately 108,530

173   individual fish (in many cases partially consumed by bears) were translocated over the 20-

174   year period representing a total of 267,620 kg of salmon, 8,028 kg of N and 1,356 kg of

175   phosphorus (P) (Quinn et al. 2018). To estimate the mass of nitrogen added per m^2 we

176   assumed all salmon were tossed within 6 m of the creek's edge along the entire 2 km creek,

177   thus within a 12,000 m^2 area.

178   Soil samples were collected from the riparian zone on 13 July, 2017 (prior to arrival

179   of salmon and any carcass manipulation that season) along nine sets of paired transect sites.

180   Paired transects were used to control for naturally occurring salmon density. Transects

181   covered the full 2 km length of the stream and were selected to represent typical riparian

182   vegetation and high annual carcass abundance. Each transect included sampling sites at 1, 3,

183   6, 10, and 20 m from the bank-full point. Sampling occurred during peak growing season

184   (i.e., during the typical time of maximum normalized difference vegetation index (NDVI);

185   Kasischke and French 1995), approximately one week prior to the arrival of the first salmon

186   in the creek. Thus, our sampling was intended to capture the long-term legacy of MDN

187   manipulations and to avoid short-term pulses following salmon return that may not represent

188   a system-level change in N availability, retention, and recycling in soils, which has already

189   been documented in multiple short-term studies. A 5 cm x 5 cm x 10 cm soil column was

190   taken for each sample site and the litter layer was removed before storing at 4 °C in airtight

191   plastic bags for 48 hours prior to processing. Nitrogen cycling decreases dramatically with

192   depth, sampling at this depth includes the O and A horizons where a majority of nitrogen

193   cycling occurs (Sparks et al. 1996).

194   *Soil nitrogen concentrations and transformations*

195   Soil [NH_4^+], [NO_3^-], and N transformations were measured according to Holtgrieve

196   and Schindler (2009). Briefly, we extracted 10 to 12 g of field-moist sieved (< 2 mm) soil

197   with 100 mL of 2 M potassium chloride (KCl) by shaking for 60 s, followed by settling for

198   24 hours prior to filtration through pre-leached Whatman #1 filter papers. Approximately 8

199   mL of filtered extracts were frozen and later analyzed colorimetrically for $[NH_4^+]$ and $[NO_3^-]$

200   with an Auto-Analyzer 500 Model (Perstorp Analytical Co, Analytical Service Station,

201   Seattle, WA, USA). The remaining extract was frozen prior to stable isotope analyses (see

202   *Stable isotope analysis*). To estimate inorganic N transformation rates, a second 10 to 12 g

203   soil subsample was incubated aerobically in the dark for 15 d at 20°C prior to extraction,

204   filtration, and analysis as above. Net mineralization was calculated as the sum of the change

205   in $[NH_4^+]$ and $[NO_3^-]$ divided by the incubation duration, and net nitrification was calculated

206   as the change in $[NO_3^-]$ over the incubation duration and represents the conversion of NH_4^+ to

207   NO_3^- (Hart 1994). $[N_{org}]$ was calculated by taking total soil N concentration, $[N_{tot}]$ determined

208   by elemental analysis (see *Stable isotope analysis*) and subtracting $[NH_4^+]$ and $[NO_3^-]$. All

209   soil N values were corrected for gravimetric soil water content (g H_2O/g dry soil) determined

210   by drying 50 to 100 g of field-moist soil at 105°C for 48 h (Gardner, 1986).

211   *Stable isotope analysis*

212   Fresh soil was freeze dried for 48 h and ground into a uniform powder (< 212 μm)

213   using a ball mill prior to analysis for nitrogen ($^{15}N/^{14}N$) and carbon ($^{13}C/^{12}C$) stable isotope

214   ratios at the University of Washington's IsoLab using a Costech Elemental Analyzer, Conflo

215   III MAT253 for continuous flow-based measurements. This procedure also provided total

216   carbon and nitrogen concentrations, $[C_{tot}]$ and $[N_{tot}]$, and percent C and N, of the soil samples.

217   Data are reported using standard delta notation, which describes the per mil deviation in the

218   ratio of heavy to light isotope relative to accepted international standards, in this case air and

219   Vienna Pee Dee Belemite (VPDB) for N and C respectively (Schoeninger et al. 1983).

220   For $^{15}N/^{14}N$ stable isotope analysis of NH_4^+ and NO_3^-, KCl extracts were placed in

221   Erlenmeyer flasks for diffusion using modified methods from Sigman et al. (1997) and

222   Holmes et al. (1998). To retrieve NH_4^+ as gaseous NH_3, 300 mg of MgO and an acid trap (1

223   cm glass fiber filter treated with $KHSO_4$ and sealed in Teflon) were added to each flask,

224   immediately stoppered, sealed with parafilm, and shaken for six days prior to removal of acid

225   traps to a desiccator for 3 to 4 days. The same extracts were then shaken uncovered for one

226   day to remove any remaining NH_4^+. To retrieve NO_3^- as NH_3, another 300 mg of MgO were

227   added to each extract and immediately followed with 75 mg of Devarda's alloy and an acid

228   trap, then processed as above. Samples were run in four separate batches, for each batch three

229   blanks, KCl with no soil extract, and three reference standards, NH_4Cl and KNO_3 with known

230    $^{15}N/^{14}N$, were also run. Batch blanks showed quantifiable N from the KCl; therefore, a two-

231   source mixing model correction was applied to both samples and reference standards to

232   calculate the blank corrected value, $\delta^{15}N_{bl,x}$, using equation 1:

233   (1) $\delta^{15}N_{bl,x} = \dfrac{\delta^{15}N_x(N_{bl,b} + N_x) - \left(\delta^{15}N_{bl,b} * N_{bl,b}\right)}{N_x}$

234   Where b represents an individual batch and x represents an individual sample, $N_{bl,b}$ is the

235   average measured mass (µg) of nitrogen in a blank for a given batch, and $\delta^{15}N_{bl,b}$ is the

236   average measured $\delta^{15}N$ of blanks for a given batch. $\delta^{15}N_x$ is the $\delta^{15}N$ value for a given

237   sample, and N_x is the mass of nitrogen (µg) measured in the sample. A standard correction

238   was then applied to the blank corrected measurements using equation 2:

239   (2) $\delta^{15}N_c = \delta^{15}N_{bl,x} - (standard_{m,x} - standard_t)$

240   Where $standard_{m,x}$ is the average measured value of the standard for a given batch and

241    $standard_t$ is the true value of the standard. All reported $\delta^{15}N$-NH_4^+ and NO_3^- values are

242   expressed as the $\delta^{15}N_c$, where a blank and a standard correction have been applied. The

243   internal standard of the $\delta^{15}N$ of NO_3^- had a -23.6 to 9.6 ‰ deviation from its true value,

244   indicating a significant methodological issue. Given there was not enough sample to refine

245   these methods and the potential for standard corrections of this magnitude to be misleading,

246    $\delta^{15}N$ of NO_3^- data are not reported here.

247   C:N ratio, percent nitrification, and percent carbon were also calculated to evaluate N

248   availability and retention across the sites. C:N ratios were calculated on a mass basis Percent

249   nitrification was calculated as:

250   (3) Percent Nitrification = $100 * \dfrac{\text{Net Nitrification}}{\text{Net Mineralization}}$

251   *Statistical analyses*

252   We used multi-model selection procedures via Akaike's information criterion (AIC)

253   to identify how salmon carcass treatment governed a suite of response variables using the

254   stats v3 and lme4 packages in R. These response variables were: $\delta^{15}N$ and $\delta^{13}C$ of bulk soil,

255      $\delta^{15}N$ of NH_4^+, $[NH_4^+]$ and $[NO_3^-]$, net mineralization and net nitrification, $[N_{org}]$, gravimetric

256   water content (GW), and C:N. For all response variables, candidate models (Appendix S1:

257   Table S1) included bank (left vs. right) and distance from river's edge. A linear and quadratic

258   interaction structure for bank and distance were fit for each response variable and these

259   interaction terms allowed the effect of distance to vary by bank and the effect of bank to vary

260   by distance. A $\log_e$ transformation was used for the distance. GW was considered as a

261   covariate for all response variables, soil $[NH_4^+]$ was considered as a covariate for net

262   nitrification, and soil $[N_{org}]$ was considered as a covariate for net mineralization, given $[N_{org}]$

263   and $[NH_4^+]$ function as the substrate for mineralization and nitrification respectively. $[N_{tot}]$

264   was considered as a covariate for $\delta^{15}N$ and $\delta^{13}C$ of bulk soil, and for $\delta^{15}N$ of NH_4^+. The best

265   model was selected from the candidate model set using AIC for each response variable.

266   Two model parameters – bank (left vs. right) and distance from the stream – were

267   used to test salmon carcass and site variability impacts to soil N cycling. Changing the

268   number of salmon carcasses on each bank was the primary goal of the manipulation;

269   however, the two banks potentially differ in aspect, soil type, and drainage, which can affect

270   nutrient cycling and generate a bank effect unrelated to salmon manipulation (Chapin et al.

271   2011). Notably, the salmon enhanced bank has a northwest facing slope approximately 20 m

1

272   of the creek edge. Distance from the stream reflects the magnitude of salmon manipulation

273   because carcasses were placed primarily 3 – 6 m from the stream's edge. Other factors such

274   as vegetation, soil type, and water availability can also change with distance laterally from

275   the stream edge, though such changes are expected to be more continuous, rather than

276   focused on the same 3 – 6 m band where salmon were placed. These differences in expected

277   lateral patterns in soil properties due to salmon (focused at 3 – 6 m) verse other factors (more

278   continuous) provide a means to test whether salmon significantly altered soil patterns in our

279   experiment.

280   We inferred that salmon significantly influenced a soil property when that soil

281   property met the following conditions: (a) the property differed between the study banks, (b)

282   varied with distance from the stream edge, and (c) displayed a peak response at 3 - 6 m on the

283   salmon addition bank. All conditions (a, b, c) are required to infer that salmon significantly

284   altered the soils on the treatment bank. In contrast, we inferred that support for only one of

285   these parameters demonstrates underlying site variability in the system. Effects of natural site

286   variability on soil properties is also an important component to test. Control sites are typically

287   assumed to be biogeochemically similar to carcass sites without validating this assumption,

288   despite control sites often being located at different stream reaches or on different streams

289   altogether. For each of the nine response variables, three competing hypotheses were

290   compared, that the differences in response variables were due to H1) a bank and/or distance

291   effect that does not demonstrate a peak response between 3 – 6 m indicating site variability

292   not caused by salmon manipulation, H2) a bank and distance effect as a quadratic interaction

293   with a peak between 3 – 6 m indicting a response to salmon manipulation or H3) no

294   difference caused by distance and bank indicating support for the other covariates tested.

295   These hypotheses were tested by categorizing each candidate model into one of the three

296   hypotheses (Table 1; Appendix S1: Table S1) and considering the hypothesis categorization

297   for the model with the most support, and any additional competing models with relative

298   support (ΔAIC value of < 2) (Burnham and Anderson 2002) for each response variable (e.g.,

299     $[NH_4^+]$, $[NO_3^-]$, $\delta^{15}N$, etc.). If models showed support for H2, the effect of salmon was

300   confirmed by examining whether the response variable peaked at the salmon enhanced bank

301   between 3 – 6 m. If this did not occur, the response is due to site variability and not salmon.

302   **Results**

303   Bulk soil stable isotope analysis indicated that salmon carcasses enriched the N

304   isotope pools (Table 1). $\delta^{15}N$ values peaked between 3 and 6 m from the stream edge, which

305   was the distance salmon were typically relocated to during the experiment and declined at

306   distances greater than 6 m. Maximum $\delta^{15}N$ of bulk soils was 11.8‰ for the salmon enhanced

307   bank and 11.6‰ for the salmon depleted bank and no observations exceeded the sockeye

308   salmon end-member value of 12.6‰ (Figure 2a). $\delta^{13}C$ was more enriched at greater distances

309   from the bank and on average was highest at 20 m (Figure 2b). $\delta^{13}C$ was primarily governed

310   by distance, with some evidence $[N_{tot}]$ and bank also had an effect (Table 1).

311   Salmon carcass manipulation also enriched $\delta^{15}N$ of soil NH_4^+. Stable isotope values

312   were enriched at 3 m from the stream edge on the salmon enhanced bank, and declined at

313   distances > 3 m. On the salmon depleted bank, $\delta^{15}N$ of soil NH_4^+ was most enriched at 1 m

314   and declined with distance (Figure 2c). The only model with support contained a quadratic

315   interaction of distance and bank, which provides strong evidence that $\delta^{15}N$ of NH_4^+ was

316   affected by salmon (Table 1). In contrast to bulk soil N, $\delta^{15}N$ values of NH_4^+ exceeded the

317   salmon endmember of 12.6‰ for 23% of all observations (n=21).

318   Inorganic nitrogen concentrations were primarily governed by bank and GW (Table

319   1). The salmon enhanced bank had a higher mean $[NH_4^+]$ and $[NO_3^-]$ compared to the salmon

320   depleted bank (Figure 3e, f). The most supported models for both $[NH_4^+]$ and $[NO_3^-]$ showed

321   evidence for H1, that observed differences were not caused by salmon. For $[NH_4^+]$ there was

322   substantial model uncertainty, with six competing models receiving relative support (ΔAIC <

323   2) (Table 1) but none of the competing models supported a salmon effect. Two competing

324   models for [NO_3^-] supported a site variability effect and one competing model supported a

325   salmon effect (Table 1) and all three contained gravimetric water content as a covariate. This

326   indicates [NH_4^+] was driven by site factors unrelated to salmon while [NO_3^-] was driven by

327   gravimetric water content with some support for salmon enhancement.

328   Nitrogen transformation rates were unaffected by salmon carcass manipulation. Both

329   net nitrification and net mineralization models with relative support contained N substrate

330   ([NH_4^+] and [N_{org}] respectively), and the models with the most support did not include

331   distance or bank. Net mineralization had some model uncertainty, with four models receiving

332   relative support; however, all of the competing models supported either H1 or H3 with no

333   support for a salmon effect. [N_{org}] was the only covariate included in all of the competing

334   models, indicating [N_{org}] was the most important covariate tested for determining net

335   mineralization. Net nitrification had greater model certainty and both models that received

336   relative support contained [NH_4^+] and gravimetric water content. Similar to net

337   mineralization, these models supported H1 and H3 with no support for H2, the salmon effect,

338   though net nitrification was slightly higher on average between $3 - 6$ m on the salmon

339   enhanced bank (Table 1; Appendix S1: Table S2). Overall, these results demonstrated the

340   manipulation of salmon carcasses did not have clearly detectable effects on N transformation

341   rates.

342   Both [N_{org}] and GW indicated there are site differences caused by distance and bank

343   unrelated to salmon carcass manipulation. On average [N_{org}] was higher on the salmon

344   depleted bank than the salmon enhanced bank. There was model support of H1 for both GW

345   and [N_{org}], indicating these variables decrease with distance (Table 1, Figure 3i, j). While

346   there was some evidence that there was both a distance and bank effect on GW, it was not

347   caused by salmon as the salmon enhanced bank does not show a peak GW at 3 - 6 m from the

348   stream, which was where there was the highest observed isotopic enrichment and expected

349   MDN. However, one competing model for [N_{org}] did support H2, indicating site factors and

350   salmon may both affect [N_{org}]. However, the mean [N_{org}] for the salmon enhanced bank was

351   18.42 mg/g and 18.97 mg/g for the salmon depleted bank indicating salmon decrease [N_{org}],

352   if they affect it at all.

353   C:N, percent nitrification, and percent carbon indicate relatively high nitrogen

354   availability across sampling sites in the Hansen Creek system. Mean percent carbon was 24.2

355   and 24.9 on the enhanced and depleted banks respectively (Appendix S1: Table S2). Soil C:N

356   of bulk isotopes was less than 20 for all sites, with a mean of 15.8 (enhanced) and 14.2

357   (depleted). These values are well below the critical microbial C:N threshold of 29,

358   demonstrating N is more available to meet microbial metabolic demands relative to C (Figure

359   2d). In contrast, percent nitrification was relatively high with a mean of 64% and 62% on the

360   enhanced and depleted banks (Appendix S1: Table S2).

361   **Discussion**

362   This study confirmed that MDN was both present in soils and increased on the bank

363   enhanced with salmon carcasses for 20 years. However, plant-available inorganic N pools

364   and N transformation rates measured in soil during the peak growing season immediately

365   prior to the annual return of salmon were largely unaffected by salmon enhancement. Even

366   though the salmon enhanced bank had increased net nitrification compared to the salmon

367   depleted bank, our analysis found no pattern with distance from the stream, suggesting that

368   elevated nitrification was caused by bank characteristics unrelated to salmon carcass density.

369   Given numerous conventional long-term fertilization experiments worldwide have shown a

370   consistent pattern of elevated soil inorganic N pools and N transformations, (Högberg 2006,

371   Lu et al. 2010), it was surprising that 20 years of MDN inputs did not clearly accelerate soil

372   N cycling in our study. Soils are the dominant (> 70%) sink for added N in forests worldwide

373   (Templer et al. 2012) and tree growth in high latitude conifer forests is often strongly N-

374   limited (Nordin et al. 2001), both of which should have fostered retention of salmon N inputs

375   to our site. Indeed, the 20 years of cumulative salmon N additions in the zone near the stream

376   in our study (~ 6,690 kg N/ha) greatly exceeded typical riparian surface soil N pools (500 to

377   2500 kg N/ha, Walker 1989, Morris and Stanford 2011, Perry et al. 2017), suggesting that

378   even partial retention of salmon N inputs in soils should have increased soil $[N_{org}]$. The lack

379   of increase in soil $[N_{org}]$ due to salmon that we observed is consistent with the lack of

380   increase in N availability, because soil $[N_{org}]$ fuels long-term changes in N availability and

381   recycling via plant uptake, litterfall, and decomposition (Chappell et al. 1999, Perakis and

382   Sinkhorn 2011, Perakis et al. 2012). Combined with observations of low C:N and high

383   percent nitrification, this indicates N from salmon subsidies is not being retained in this

384   system. Overall, the lack of increase in soil organic and inorganic N concentrations and N

385   transformations that we observed following 20-year salmon manipulation raises questions of

386   whether plant growth responses should be expected at our site.

387   Prior work at Hansen Creek inferred that MDN stimulated white spruce growth based

388   on tree ring analyses (Quinn et al. 2018). However, substantial salmon enhancement

389   corresponding to approximately 669 g/m^2 (6,690 kg/ ha) of N and 113 g/m^2 (1,130 kg/ha) of

390   P over the past 20 years was unable to overcome pre-treatment differences in forest growth

391   between banks. For reference, it is estimated white spruce in floodplain stands require

392   approximately 1.35 $g/m^2/y$ of N (Chapin et al. 2006), which was far exceeded by the mean

393   change of 33.45 $g/m^2/y$ of N added from this manipulation. Additionally, fertilization

394   experiments apply N on the order of 100 - 1,000 kg/ha with clear results (Chappell et al.

395   1999), a much lower application rate than in this study. Factors such as climate, stand

396   demography, and site variability also must affect tree growth in this system. Indeed, white

397   spruce growth response to recent warming across southwest Alaska depends strongly on tree

398   density (Wright et al. 2018). Basal area density is highly variable across our site, differing on

399   average 40% between salmon-enhanced and salmon-depleted banks, although the difference

400   was not statistically significant (Quinn et al. 2018). Ultimately, the hierarchy of drivers of

401   tree growth in this ecosystem appears to be landscape position (and associated forest

402   demography) followed by climate and thirdly, nutrients. All told, a lack of long-term changes

403   in soil nutrient dynamics and only marginal response in tree growth (Quinn et al. 2018)

404   indicates that salmon nutrients are not a strong bottom-up force in northern riparian forest

405   dynamics.

406   Our $^{15}N/^{14}N$ stable isotope data raise further questions of assessing MDN subsidies to

407   tree growth. Vegetation typically takes up only 17% of added N to forests, with soils instead

408   being the dominant N sink (Templer et al. 2012). Thus, elevated bulk soil $^{15}N/^{14}N$ in our

409   study suggests a potentially significant MDN sink in soil. On the other hand, elevated bulk

410   soil $^{15}N/^{14}N$ may also reflect increases in soil N fractionation during N cycling and loss under

411   salmon. Highly localized N pulses (as occur with MDN and other N subsidies) temporarily

412   exceed plant and soil N sinks, leading to accelerated N loss via ammonia volatilization,

413   nitrification and nitrate leaching, and/or denitrification (Perakis 2002). All of these N loss

414   pathways favor ^{14}N and discriminate against ^{15}N (in some cases with a fractionation up to

415   30‰), and effects are strongest at high N availability, leading to high values of residual soil

416   ^{15}N (Högberg et al. 1997). Prior work has shown that MDN inputs accelerate N losses from

417   soil, particularly gaseous N losses (Holtgrieve et al. 2009) that are associated with large

418   isotope fractionation (Högberg et al. 1997). Our finding that $\delta^{15}N$ of soil NH_4^+ was greater

419   than bulk soil $\delta^{15}N$ for 95% of observations on the salmon enhanced bank and 84% of

420   observations on the salmon depleted bank, further confirms that isotopic fractionation is

421   important at Hansen Creek and likely elsewhere.

422   There is a global trend for higher foliar $\delta^{15}N$ with increased soil N supply (Craine et

423 al. 2009) indicating accelerated soil N cycling and $\delta^{15}N$ fractionation due to exogenous N

424 (from salmon or elsewhere) will alter plant foliar $\delta^{15}N$. This has important implications for

425 using two-source mixing models to assess salmon N subsidies to riparian forests. Typical

426 MDN mixing models assume 1) the isotopic signature of salmon is unchanged in the soils

427 prior to plant uptake, and 2) reference sites are biogeochemically similar to salmon sites.

428 However, our data suggest that both of these assumptions are violated at Hansen Creek, and

429 are likely violated at all salmon-influenced riparian ecosystems. First, we observed that $\delta^{15}N$

430 of NH_4^+, the dominant form of inorganic N in our soils, exceeded the 12.6‰ salmon end-

431 member for 26% of our observations from the salmon-enhanced bank and 9% of observations

432 from the salmon depleted bank, thus violating assumption (1) above. Our soil N data indicate

433 Hansen Creek is a site of intermediate fertility relative to other boreal forests, so that soil

434 NH_4^+ (rather than organic N or NO_3^-) is most likely the dominant N source taken up by plants

435 (Chapin et al. 2011). Second, $[N_{Org}]$, C:N, $\delta^{13}C$, and GW varied with distance from the stream

436 independent of salmon enhancement indicating site variability is a dominant driver of N

437 cycling in this system. This presents a challenge for selecting control sites to calculate

438 terrestrial end members, as key N cycling factors vary longitudinally away from streams and

439 simply selecting reference sites that are beyond the reach of salmon would likely violate the

440 mixing model assumption of biogeochemical similarity. Additionally, observations of $\delta^{13}C$

441 increasing and GW decreasing from the creek edge are consistent with higher water use

442 efficiency and less ^{13}C discrimination by vegetation, resulting in higher $\delta^{13}C$ in soil due to

443 litterfall (Gabriel and Phillip 2016). These data identify systematic differences between

444 salmon-enhanced vs. salmon-depleted banks that cannot be attributed to salmon, and which

445 likely reflect landscape or soil differences. Previous studies examining contributions of MDN

446   to riparian vegetation have not tested biogeochemical similarity across sites, an assumption

447   that is violated beyond Hansen Creek specifically.

448   Violation of mixing model assumptions can lead to significant bias in calculations of

449   MDN sources. To illustrate this point, we applied a typical mixing model framework to our

450   maximum observed $\delta^{15}N$ of NH_4^+ values to calculate the percent MDN contribution of

451   salmon to NH_4^+ for the most extreme observation, representing the greatest possible bias in

452   calculations. Assuming soil processes have no effect on the isotopic signature yielded

453   impossible result of 298% MDN contribution. To account for isotopic fractionation in soils,

454   we applied our mean observed $\delta^{15}N$ of soil NH_4^+ at the 3 m distance (19.25‰) as the marine

455   endmember to mean foliar $\delta^{15}N$ data at the same site from Quinn et al. (2018) and estimate

456   59.24% MDN on the salmon bank, which is 27.6% lower than the original estimate of 86.8%

457   using salmon $\delta^{15}N$ as the marine endmember. Repeating this with our maximum observed

458   value for $\delta^{15}N$ of NH_4 (41.2‰), we estimate only 28.9% of foliar N on salmon enhanced

459   bank was MDN (a 57.9% reduction from Quinn et al. 2018 estimates). Thus, failure to

460   account for isotopic enrichment associated with soil N transformations can lead to

461   overestimates of MDN contributions to plants, and observed variability in $\delta^{15}N$ of NH_4^+ can

462   produce a wide range of MDN estimates not previously considered. Given that our elevated

463    $\delta^{15}N$ of NH_4^+ values are consistent with expected changes during soil N transformation

464   (Högberg 1997), there is a distinct possibility that previous MDN studies have overestimated

465   the amount of MDN by not considering the effects of $^{15}N/^{14}N$ fractionation in mixing model

466   calculations. Wheeler and Kavanagh (2017) found similar results in a semi-arid ecosystem of

467   central Idaho, where accounting for fractionation from decomposition resulted in a 16%

468   reduction in estimated N deposition rates from salmon carcasses. The effects of fractionation

469   on soil N pools is occurring in both of these systems, and likely elsewhere, and needs to be

470   considered when applying mixing models to MDN data to avoid overestimations of salmon N

471   contributions to riparian systems.

472   Our study is comprehensive in terms of the number of ecosystems factors considered

473   but limited in that it includes only one seasonal timeframe. As much as 40% of the annual

474   inorganic N flux is released during the eight-month dormant season (September-May) and it

475   has been posited spring and fall may be important for many biogeochemical processes in

476   boreal forests (Hobbie and Chapin 1996, Chapin et al. 2006, Drake et al. 2006). While MDN

477   inputs do not affect the N pools and transformation rates during the summer growth period

478   based on our results, N concentrations and transformations may be elevated in this system on

479   shorter timescales (weeks to months after salmon return). The objective of this study was to

480   identify the long-term legacy of salmon subsidies; short-term effects were both beyond the

481   scope of this study and have been previously investigated in this system (Holtgrieve et al.

482   2009). Considering long-term effect of N subsidies as opposed to short-term provides new

483   information on sustained N use and retention in the ecosystem and whether these salmon

484   nutrients have lasting impacts on ecosystem function meaningful in a restoration context.

485   While this study is limited to one system, the results that N transformations cause a

486   fractionation that can bias MDN mixing model estimates and that landscape factors are the

487   primary driver of long-term N retention and use, are relevant to other systems where

488   anadromous, semelparous salmon are abundant (Pacific, Atlantic, Great Lakes) (Quinn 2018).

489   This result also agrees with related research examining fractionation of mineralization and

490   nitrification (Högberg 1997), and fertilization studies (Lu et al. 2010). Additionally, it

491   demonstrates salmon N subsidies may have a short-term and likely small spatial scale (Drake

492   et al. 2005) legacy in soils. While the importance of site variability relative to salmon

493   subsidies may vary by system, this work demonstrates the importance of considering site

494   variability and demonstrating biogeochemical similarity when selecting control sites for

495   riparian MDN studies.

496   Salmon provide critical food resources to many of terrestrial and aquatic consumers

497   (Cederholm et al. 1999, Gende et al. 2002, Schindler et al. 2003), but the evidence that MDN

498   stimulate terrestrial primary production is less certain. The salmon carcass manipulation

499   experiment described here and in Quinn et al. (2018) represents an extreme case of carcass

500   addition and depletion to riparian areas, as measured by bulk $\delta^{15}N$ and estimated percent

501   contribution was approximately twice previous studies for both trees and soils (Helfield and

502   Naiman 2002, Bartz and Naiman 2005). Generally, results of this manipulation were

503   equivocal for soils and a statistically significant but ecologically small effect on trees (Quinn

504   et al. 2018). Simultaneously, other recent changes to boreal forest systems, such as moisture

505   and temperature, appear to have a greater potential than MDN to alter biogeochemical

506   pathways and primary production in these systems (Chapin et al. 2006, Yarie 2008, Lloyd et

507   al. 2010, Wright et al. 2018). This study also demonstrates the importance of testing

508   biogeochemical and site similarity between experimental and control sites in nutrient subsidy

509   studies, as even banks on the same creek can have landscape and soil variability that alter N

510   concentrations, transformations, and thus vegetative growth. Altogether, while salmon have

511   clear benefits for consumers, management of salmon populations or application of

512   compensatory restoration strategies based on terrestrial productivity response to salmon

513   inputs may be unfounded for some systems, and at least, hard to predict.

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Table 1: Competing models with relative support (ΔAIC < 2) using AIC analysis for each response variable, where the models with the most support are shown in bold. Reported are ΔAIC and the hypothesis supported by each model: 1) a bank and/or distance effect caused by site variability and not salmon, 2) a bank and distance effect as a quadratic interaction indicting a response to salmon manipulation, and 3) no difference caused by distance and bank indicating support for the other covariates tested.

Response Variable	Model Hypothesis	ΔAIC	Covariates Included in Models with Relative Support
Bulk $\delta^{15}N$	**2**	**0.00**	**Bank, ln(Distance), Bank:ln(Distance), Bank:ln(Distance)^2**
	2	0.41	Bank, ln(Distance), Bank:ln(Distance), Bank:ln(Distance)^2, [N_{tot}]
Bulk $\delta^{13}C$	**1**	**0.00**	**ln(Distance)**
	1	0.22	Bank, ln(Distance)
	1	0.62	ln(Distance), [N_{tot}]
	1	1.23	Bank, ln(Distance), [N_{tot}]
$\delta^{15}N$ of NH_4^+	**2**	**0.00**	**Bank, ln(Distance), Bank:ln(Distance), Bank:ln(Distance)^2**
[NH_4^+]	**1**	**0.00**	**Bank, ln(Distance)**
	1	0.69	Bank, ln(Distance), Bank:ln(Distance)
	1	0.69	Bank
	1	0.95	Bank, GW
	1	1.10	Bank, ln(Distance), GW
	1	1.87	Bank, ln(Distance), Bank:ln(Distance), GW
[NO_3^-]	**1**	**0.00**	**Bank, GW**
	1	1.72	Bank, ln(Distance), GW
	2	1.87	Bank, ln(Distance), Bank:ln(Distance), Bank:ln(Distance)^2, GW
Net Mineralization	**3**	**0.00**	**[N_{Org}]**
	3	0.61	GW, [N_{Org}]
	1	0.74	Bank, [N_{Org}]
	1	1.61	Bank, GW, [N_{Org}]
Net Nitrification	**3**	**0.00**	**[NH_4^+], GW**
	1	1.02	Bank, [NH_4^+], GW
[N_{Org}]	**1**	**0.00**	**ln(Distance), GW**
	1	0.22	Bank, ln(Distance), Bank:ln(Distance), GW
	2	0.33	Bank, ln(Distance), Bank:ln(Distance), Bank:ln(Distance)^2, GW
	1	1.94	Bank, ln(Distance), GW
Gravimetric Water Content (GW)	**1**	**0.00**	**ln(Distance), Bank**
	1	1.00	ln(Distance)
	1	1.80	Bank, ln(Distance), Bank:ln(Distance)

Figures

Figure 1: Nitrogen pathways in soil where MDN enters terrestrial systems via decay of salmon organic tissues or excretion from direct salmon consumers such as bears. Arrows represent conversion pathways with the potential to impart isotopic fractionations on plant available nitrogen (NH_4^+ or NO_3^-).

Figure 2: Data (closed circles) and predicted values (open circles) for the model with the most support (bolded, Table 1) for soil organic $\delta^{15}N$ and $\delta^{13}C$, $\delta^{15}N$ of NH_4^+, and C:N for both the salmon enhanced and the salmon depleted banks of Hansen creek at 1 m, 3 m, 6 m, 10 m, and 20 m from the edge of the creek bed with 95% confidence intervals (dashed line) for predicted values. Blue (a and c) denotes measures of marine derived nitrogen and green (d) denotes site variable factors.

Figure 3: Data (closed circles) and predicted values (open circles) for the model with the most support (bolded, Table 1) for $[NH_4^+]$ and $[NO_3^-]$, net mineralization and nitrification, $[N_{Org}]$, and gravimetric water content for both the salmon enhanced and the salmon depleted banks of Hansen creek at 1 m, 3 m, 6 m, 10 m, and 20 m from the edge of the creek bed with 95% confidence intervals (dashed line) for predicted values. Red (e, f, g, h) denotes measures of soil productivity and green (i and j) denotes site variable factors.

Ecosphere
Riparian soil nitrogen cycling and isotopic enrichment in response to a long-term salmon
carcass manipulation experiment
Megan L. Feddern, Gordon W. Holtgrieve, Steven S. Perakis, Julia Hart, Hyejoo Ro, Thomas
P. Quinn

Appendix S1

Equation S1: Mixing model calculation applied to Quinn et al. (2018) data where %MDN is
the percentage of MDN in a given sample, TEM is the terrestrial end member (δ^{15}N value
representing 0% MDN), MEM is the marine end member (δ^{15}N value representing 100%
MDN) which is typically 12.65‰ for sockeye salmon. SAM values were the mean enhanced
(10.7‰) and mean depleted (7.65‰) values; TEM was the mean control value from white
spruce >50m from Hansen Creek edge (-1.74‰) from Quinn et al. (2018). MEM were the
maximum and average δ^{15}N of NH_4^+ observed in this study.

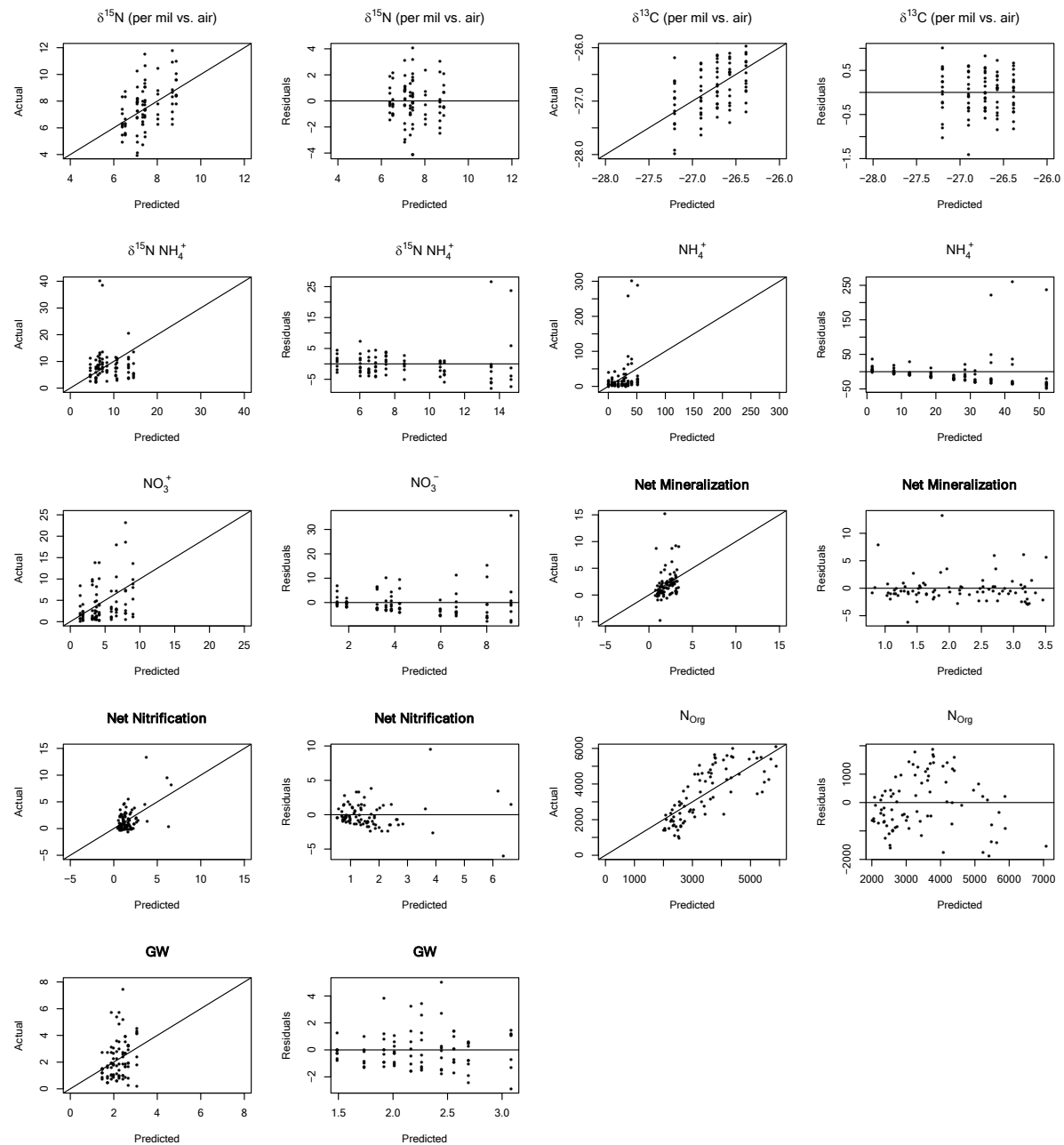$$\%MDN = \frac{SAM - TEM}{MEM - TEM} * 100$$

Table S1: The candidate model set tested for each response variable using AIC analysis. * denotes models used for all response variables, additional models were used for net mineralization and net nitrification where substrate represents organic nitrogen concentration and NH_4^+ concentration, respectively. For $\delta^{15}N$ data, GW was not tested as a covariate and total [N] was tested instead. The three tested hypotheses are: 1) a bank and/or distance effect caused by site variability and not salmon; 2) a bank and distance effect as a quadratic interaction indicting a response to salmon manipulation, and 3) no difference caused by distance and bank indicating support for the other covariates tested. Response variables include: $\delta^{15}N$ and $\delta^{13}C$ of bulk soil, $\delta^{15}N$ of NH_4^+, [NH_4^+] and [NO_3^-], net mineralization and net nitrification, [N_{org}], gravimetric water content (GW), and C:N.

Candidate Model Set	Hypothesis
*Response Variable = bank + ε	1
*Response Variable = bank + GW + ε	1
*Response Variable = ln(distance) + GW + ε	1
*Response Variable = ln(distance) + ε	1
*Response Variable = bank + ln(distance) + bank:ln(distance) + ln(distance)^2:bank + GW + ε	2
*Response Variable = bank + ln(distance) + bank:ln(distance) + ε	1
*Response Variable = bank + ln(distance) + bank:ln(distance) + GW + ε	1
*Response Variable = bank + ln(distance) + bank:ln(distance) + ln(distance)^2:bank + ε	2
*Response Variable = bank + ln(distance) + bank+ ε	1
*Response Variable = bank + ln(distance) + bank + GW+ ε	1
*Response Variable = GW + ε	3
Response Variable = bank + substrate + ε	1
Response Variable = ln(distance) + substrate + ε	1
Response Variable = bank + GW + substrate + ε	1
Response Variable = bank + ln(distance) + bank:ln(distance) + GW + substrate + ε	2
Response Variable = bank + ln(distacne) + bank:ln(distance) + substrate + ε	1
Response Variable = bank + ln(distance) + bank:ln(distance) + GW + substrate + ε	2
Response Variable = bank + ln(distance) + bank:ln(distance) + ln(distance)^2:bank + GW+ substrate + ε	2
Response Variable = bank + ln(distance) + bank:ln(distance) + ln(distance)^2:bank + substrate + ε	2
Response Variable = bank + ln(distance) + GW+ substrate + ε	1
Response Variable = bank + ln(distance) + substrate + ε	1
Response Variable = substrate + ε	3
Response Variable = GW + substrate + ε	3

Table S2: Summary statistics mean (standard deviation) of each response variable

Distance	1 m		3 m		6 m		10 m		20 m	
Bank	Enhanced	Depleted	Enhanced	Depleted	Enhanced	Depleted	Enhanced	Depleted	Enhanced	Depleted
Bulk $\delta^{15}N$ (‰)	7.4(2.3)	7.2(1.9)	9.2(1.0)	7.8(2.2)	8.5(1.9)	6.9(1.2)	8.2(1.5)	7.3(1.6)	6.5(1.0)	6.6(1.2)
Bulk $\delta^{13}C$ (‰)	-27.1(0.6)	-27.2(0.4)	-26.9(0.5)	-27.1(0.6)	-26.6(0.5)	-26.7(0.3)	-26.5(0.5)	-26.6(0.3)	-26.4(0.5)	-26.4(0.4)
$\delta^{15}N$ of NH_4^+ (‰)	10.1(1.8)	8.7(2.8)	16.2(10.7)	8.5(2.5)	13.3(10.5)	6.3(2.8)	8.4(2.5)	5.8(2.9)	6.1(2.3)	6.5(3.3)
$[NH_4^+]$ (μg N g^{-1})	47.5 (91.6)	22.3(16.4)	62.9(101.5)	10.6(9.4)	52.5(82.8)	11.0(12.7)	12.3(13.1)	11.5(8.2)	8.6(4.4)	13.2(11.6)
$[NO_3^-]$ (μg N g^{-1})	6.0(5.4)	3.4(4.4)	10.8(13.5)	4.3(4.7)	7.6(8.0)	3.3(2.8)	2.4(2.3)	4.0(4.2)	2.8(2.8)	1.7(1.2)
Net Mineralization (μg N g^{-1} d^{-1})	2.8(2.0)	1.8(1.2)	4.4(5.2)	1.1(1.0)	2.1(3.6)	3.0(3.6)	1.2(1.1)	1.4(1.0)	1.1(1.5)	2.3(1.9)
Net Nitrification (μg N g^{-1} d^{-1})	1.7(1.6)	1.2(1.4)	3.4(4.5)	0.8(1.2)	2.8(2.9)	1.7(1.9)	1.0(0.9)	1.4(0.8)	0.6(0.7)	1.6(1.9)
$[N_{Org}]$ (mg N g^{-1})	22.0(4.7)	19.11(5.8)	18.0(8.2)	19.7(7.6)	17.7(6.6)	19.5(8.5)	13.0(6.3)	18.4(8.9)	9.5(3.3)	13.9(5.5)
GW	2.6(1.1)	3.2(1.6)	2.4(1.5)	2.2(1.1)	2.2(1.5)	2.8(2.2)	1.5(0.9)	2.6(1.8)	1.4(0.6)	1.9(0.8)
C:N	11.9(1.4)	11.2(1.1)	11.7(1.6)	10.9(1.5)	12.8(2.2)	12.1(2.7)	14.2(1.7)	12.1(1.9)	17.0(2.0)	14.1(3.0)
% Nitrification	54.8(44.7)	67.9(43.8)	75.4(35.5)	49.3(39.7)	75.7(36.2)	53.1(39.0)	65.9(36.4)	87.9(15.8)	50.6(33.5)	56.2(39.2)
% C	30.0(5.5)	25.5(8.8)	26.4(10.1)	24.7(9.7)	25.7(8.2)	27.5(13.3)	21.3(8.8)	25.2(11.7)	19.0(6.7)	21.2(6.7)

Figure S1: Predicted verse observed values and predicted verse residuals for the model with the most support (Table 1, Figure 2) for each the response variables

"Simplified" Soil N Cycle Biosphere

NH_3

N_2O

N_2

Soil Organic Matter $\rightarrow$ Amino Acids $\rightarrow$ NH_4^+ $\rightarrow$ NO_3^- $\rightarrow$ N_2O $\rightarrow$ N_2

Depolymerization   Mineralization   Nitrification   Denitrification

Leaching

Leaching

a. Salmon Enhanced — Salmon Depleted: $\delta^{15}N$ per mil vs. air

b. Salmon Enhanced — Salmon Depleted: $\delta^{13}C$ per mil vs. VPDB

c. $\delta^{15}N$ NH_4^+ per mil vs. air

d. C:N

Data
Predicted Value

Meters

20 10 6 3 1   1 3 6 10 20