**Working title:** Effects of long term enrichment of riparian areas with salmon carcasses on inorganic soil nitrogen pools, nitrogen transformations, and stable isotopic ratios.

Megan Feddern

Gordon Holtgrieve

Julia Hart

Hyejoo Ro

Tom Quinn

**Abstract** *(350 words for ecology)*

**Introduction**

The migration of Pacific salmon (*Oncorhynchus* spp.) from marine environments to freshwater spawning grounds contributes nutrients and organic material to freshwater and terrestrial ecosystems (Gende et al. 2002). Given a majority of salmon growth occurs in the ocean, these annual events provide an influx of marine derived nutrients (MDN). Carcasses and roe are prey for a variety of organisms, and this resource pulse is linked to variable responses in periphyton density, stream biofilms, freshwater nutrient concentration, and insect abundance (Mitchell and Lamberti 2005; Rüegg et al. 2011). A majority of these nutrients are directly deposited to freshwater systems, and aquatic ecosystems demonstrate strong influences on their riparian counterparts. Flooding, erosion, and sediment deposition can alter nutrient dynamics and soil composition in adjacent ecosystems (Naiman 1998; Naiman and Decamps 1997). Transport of salmon carcasses by bears increases soil ammonium (NH4+) concentrations (Holtgrieve et al. 2009), and the spatial distribution of MDN in foliar material is linked to flooding and piscivorous predator activity (Ben-David et al. 1998).

The body of research focused on the contributions of MDN from anadromous, semelparous, fish species to surrounding ecosystems is primarily based on stable isotope analysis of δ15N (15N/14N). Salmon tend to be enriched in 15N relative to 14N when compared to freshwater nitrogen; this elevation of the heavier isotope can be used to trace the fate and distribution of MDN (Schindler et al. 2003). Higher salmon returns have demonstrated elevated δ15N in zooplankton and periphyton (Finney et al. 2000; Holtgrieve et al. 2010). Stable isotope analysis is used to understand utilization of MDN by juvenile and adult salmon, plankton, and piscivorous predators (Hicks et al. 2005; Chaloner et al 2002; Claeson et al. 2006; Rinella et al. 2013)*.* Additionally, salmon has been identified as an important source of terrestrial nitrogen and a contributor to plant growth and distribution (Bilby et al. 2003; Helfield and Naiman 2001, 2006; Ben-David et al. 1998).

Studies using δ15N as an MDN tracer for plants frequently assume nitrogen from salmon is directly assimilated by plants by using salmon δ15N as a mixing model end member, an assumption that is demonstrably false. The nitrogen cycle in soils contains a number of potentially fractionating step (Figure 1). Organic material from decomposing salmon must undergo mineralization, or the conversion of organic compounds to ammonium by nitrogen fixing bacteria. Ammonium then must then be converted to nitrate (NO3-) by nitrifying bacteria before it can be assimilated into plant tissues. Therefore, processes such as mineralization and nitrification control plant available nitrogen pools and ultimately its corresponding δ15N. These conversions also have the potential to enrich nitrogen pools in 15N (Gende et al. 2002; Schindler et al. 2003) which is likely to provide misleading estimates of the contributions of MDN to terrestrial flora and its consumers. Additionally, there is evidence δ15N and nitrogen availability are correlated, with foliar δ15N becoming more enriched when plant available nitrogen is abundant. Therefore, foliar δ15N is a signature of both the source δ15N of inorganic nitrogen in soils and nitrogen availability (Crane et al. 2009). Given plants assimilate nitrogen that has been converted to inorganic forms in soil rather than direct uptake of organic matter from salmon, fractionation in soils and nitrogen availability must be accounted for when calculating the relative contribution of MDN to terrestrial communities via pathways that do not involve direct consumption of salmon.

Experiments examining the contributions of MDN are often limited by short timescales and few experiments investigate plant available nitrogen pools. Collins et al. 2015 demonstrated the need to evaluate ecosystem responses over longer periods of time to understand the magnitude and timescale of the effects of salmon on ecosystems. Studies examining spatial and temporal impacts of salmon on inorganic nitrogen pools have identified responses are highly localized (effects only observed less than 30 cm from carcasses), and NH4+ and NO3- become biologically available on the order of weeks to months respectively (Gende et al. 2007; Drake et al. 2005). Predator exclosure experiments determined impacts to soil N processing only persist for a year (Holtgrieve et al. 2009). While these results provide a framework of the importance of soil processing of MDN on inorganic nitrogen pools, no experiment has examined the impacts of salmon removal on soil nitrogen pools on long time scales

To resolve the extent to which salmon carcasses impact plant available nitrogen pools (NH4+ and NO3-) and the rate of supply of these pools (via nitrification and mineralization), we present the results of a long-term fertilization experiment in southwestern Alaska. We also evaluate the impacts of microbial processing on δ15N by examining the δ15N of NH4+ and NO3- in soils. Finally, we assess the spatial scale of these response variables.

**Methods**

*Sample Collection*

This study was conducted on Hansen Creek, a small stream flowing from a beaver pond to Lake Aleknagik in the Wood River system of Bristol Bay, AK. For the last 20 years adult spawned out sockeye salmon (*Oncorhynchus nerka*) were removed from Hansen Creek and its river right bank (decreasing salmon density) and tossed on the river left bank (increasing salmon density). Unlike some watershed in the region, Hansen Creek has a low density of alder (*Alnus crispa*) which can be a source of nitrogen via symbiotic fixation of atmospheric N2, making it a useful system for studying MDN in plant available nitrogen in soils.

Soil samples were collected from on July 13th 2017 along nine sets of paired transects on the river left and river right banks at 1m, 3m, 6m, 10m, and 20m from the proper bank. Sites were selected to be representative of typical riparian vegetation and high spawning intensity. An approximate 5cm x 5cm x 10cm soil column was taken for each sample and plant matter was removed from the samples. Samples were stored at 4 degrees C in airtight plastic bags for 48 hours prior to processing. To determine soil water content 50 – 100 g of soil (wet weight) were dried at 105°C until dry soil reached a constant mass, and gravimetric water content was calculated.

*Soil nutrient concentrations (NH4+ and NO3-) and N transformations*

Soil nutrient concentration and N transformations were conducted according to Holtgrieve and Schindler 2009. Briefly, to determine the initial NH4+ and NO3- concentrations, subsamples (10-12 g wet weight) of sieved soils were extracted within 48 hours of collection in 100 ml of 2 M potassium chloride (KCl) (Hart et al. 1994). Soil extracts were shaken for 60 s then left to settle for 24-48 h before being filtered through Whatman #1paper filters pre-leached with 100 ml of KCl. Approximately 8 ml of filtered extracts were frozen and later analyzed colorimetrically with an Alpkem Flow Solution IV (OI Analytical, College Station, TX, USA). All leftover filtered extracts were frozen and stored for inorganic nitrogen stable isotope analysis. A second 10-12 g soil subsample was incubated aerobically in the dark for 15 days at 12-16°C prior to extraction with KCl as above, an 8ml were frozen and stored for colormetric analysis. Net mineralization calculated as the change in inorganic N concentration (NH4+ and NO3-) divided by the incubation duration, while net nitrification was calculated as the change in NO3- concentration over the incubation.

*Stable isotope analysis*

Soil subsamples were freeze dried for 48 hours, sieved, and ground into a uniform powder (< 212 μm) using a ball mill grinder for bulk isotope analysis. 2 mg of soil were packed in tin capsules and analyzed for nitrogen stable isotope ratios (15N/14N) using Air-N2 for reference and carbon (13C/12C) stable isotope ratios using VPDB for reference. Samples were analyzed at University of Washington’s IsoLab using a Costech Elemental Analyzer, Conflo III MAT253 for continuous flow-based measurements. Measurements are reported as δ15N or δ13C which represent the per mil deviation in heavy isotope abundance from the isotopic standard (Schoeninger et al. 1983).

For inorganic stable isotope analysis of NH4+ and NO3-, total volume of formerly frozen filtered extracts were measured and placed in Erlenmeyer flasks. To retrieve NH4+,300 mg of MgO and an acid trap (1 cm glass fiber filter treated with KHSO4 and sealed in Teflon tape) were added to each flask, which were immediately stoppered, sealed with parafilm, and placed on a shaker table. Samples shook continuously for six days after which acid traps were removed and stored in desiccator for 3-4 days before filters were removed and packed in tin capsules for analysis. Extracts were returned to the shaker table and left open to the atmosphere to remove remaining NH4+. To retrieve NO3-, 300 mg of MgO were added to each extract and immediately followed with 75 mg of Devarda’s alloy and an acid trap. Extracts were immediately stoppered, sealed with parafilm, and placed on a shaker table for six days before acid traps were removed and filters were packed as described above.

*Statistical analyses*

Our study design resulted in samples being collected at 9 sites (transects) across three reaches of Hansen Creek. Samples were also collected at 5 distances from the stream edge on two different banks for a total of 90 samples. Linear models and mixed effects models were fit to the data for each response variables: initial NH4+ and NO3- concentration, net mineralization and net nitrification, δ15N and δ13C of bulk isotopes, and δ15N of NH4+ and NO3-. For all response variables, the interaction between bank and distance from rivers edge were included. Reach and site were considered as nested random effects and gravimetric water content was considered as a fixed effect. NH4+ concentration was considered as a fixed effect for net nitrification, and organic nitrogen concentration was considered as a fixed effect for net mineralization, given they function as the substrate for the corresponding reactions. The best model was selected from the candidate models set using Akaike’s information criterion (AIC) for each response variable. Under circumstances where multiple models had a difference in AIC value of < 2 relative to the best model, the most parsimonious model was selected. To test the hypothesis MDN provided by salmon carcasses impacts plant available nitrogen, the significance of the bank effect was tested.

**Results**

**Discussion**

Given global declines in Pacific salmon populations caused by anthropogenic impacts (overharvest, habitat degradation, hydropower dams), concern has grown for the preservation of this ecosystem service and has been a focal point for many management and mitigation strategies (Collins et al. 2015; Lichatowich 1999). – put elsewhere?

**References**

**Tables**

Table 1:

|  |  |
| --- | --- |
| **Response Variable** | **Model Selected** |
| Bulk δ15N | δ15N = bank\*log10(distance)+I(log10(distance)2)\*bank |
| Bulk δ13C | δ13C = bank\*log10(distance)+I(log10(distance)2)\*bank |
| NO3- concentration | NO3- concentration = bank\*log10(distance)+I(log10(distance)2)\*bank |
| NH4+ concentration | NH4+ concentration = bank\*log10(distance) |
| Net Mineralization | Net Mineralization = bank\*log10(distance) + organic nitrogen concentration |
| Net Nitrification | Net Nitrification = bank\*log10(distance) + NH4+ concentration + gravimetric water content |
| δ15N of NO3- | δ15N of NO3- = bank\*log10(distance)+I(log10(distance)2) |
| δ15N of NH4+ | δ15N of NH4+ = bank\*log10(distance)+I(log10(distance)2) |

**Figures**

Figure 1: Soil processing pathways where MDN enters terrestrial systems via decay of salmon organic tissues or excretion from direct consumers such as bears. Arrows represent conversion pathways with the potential to impart a fractionation on plant available nitrogen (NH4+ or NO3-).

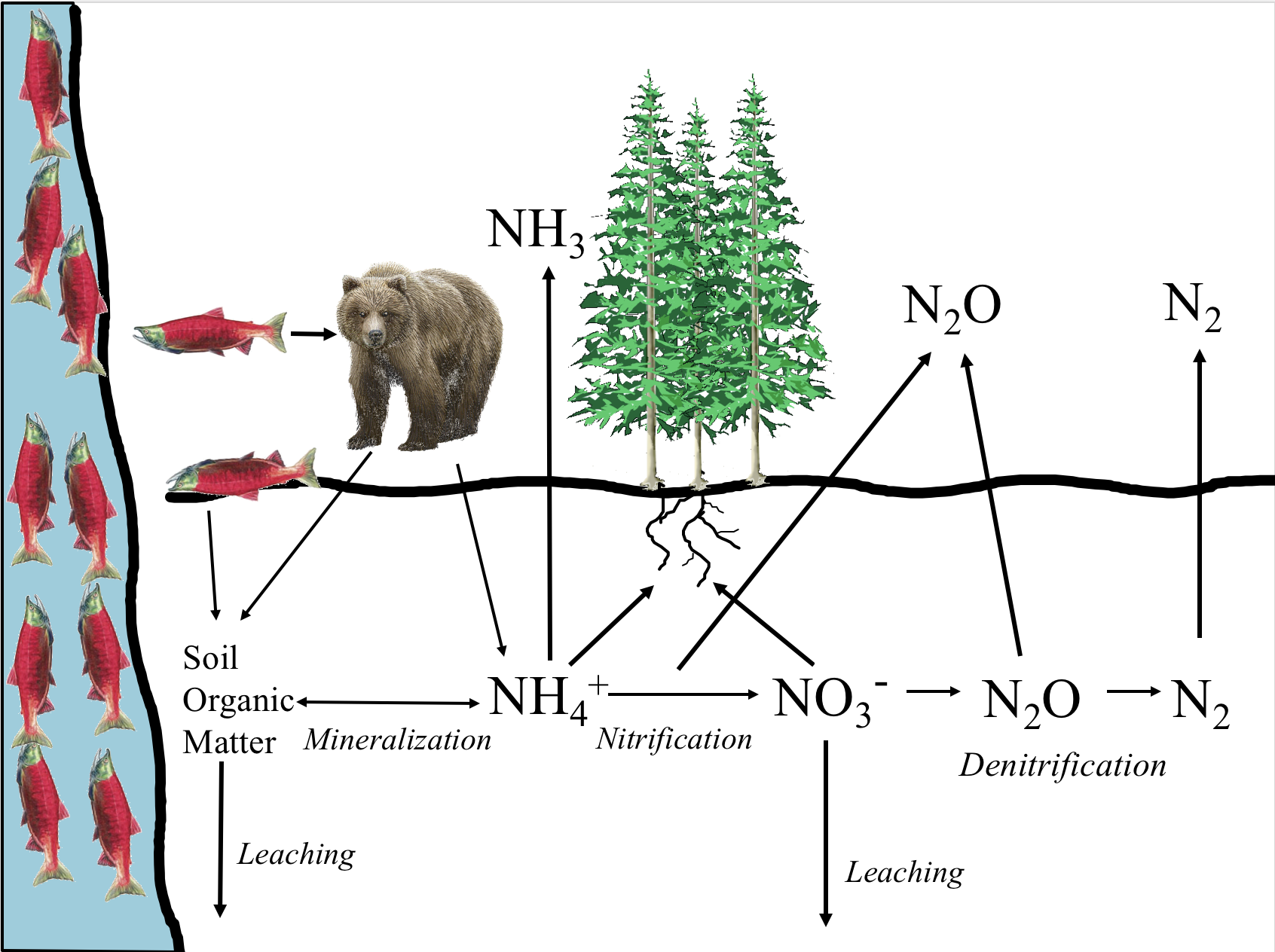
****

Figure 2:

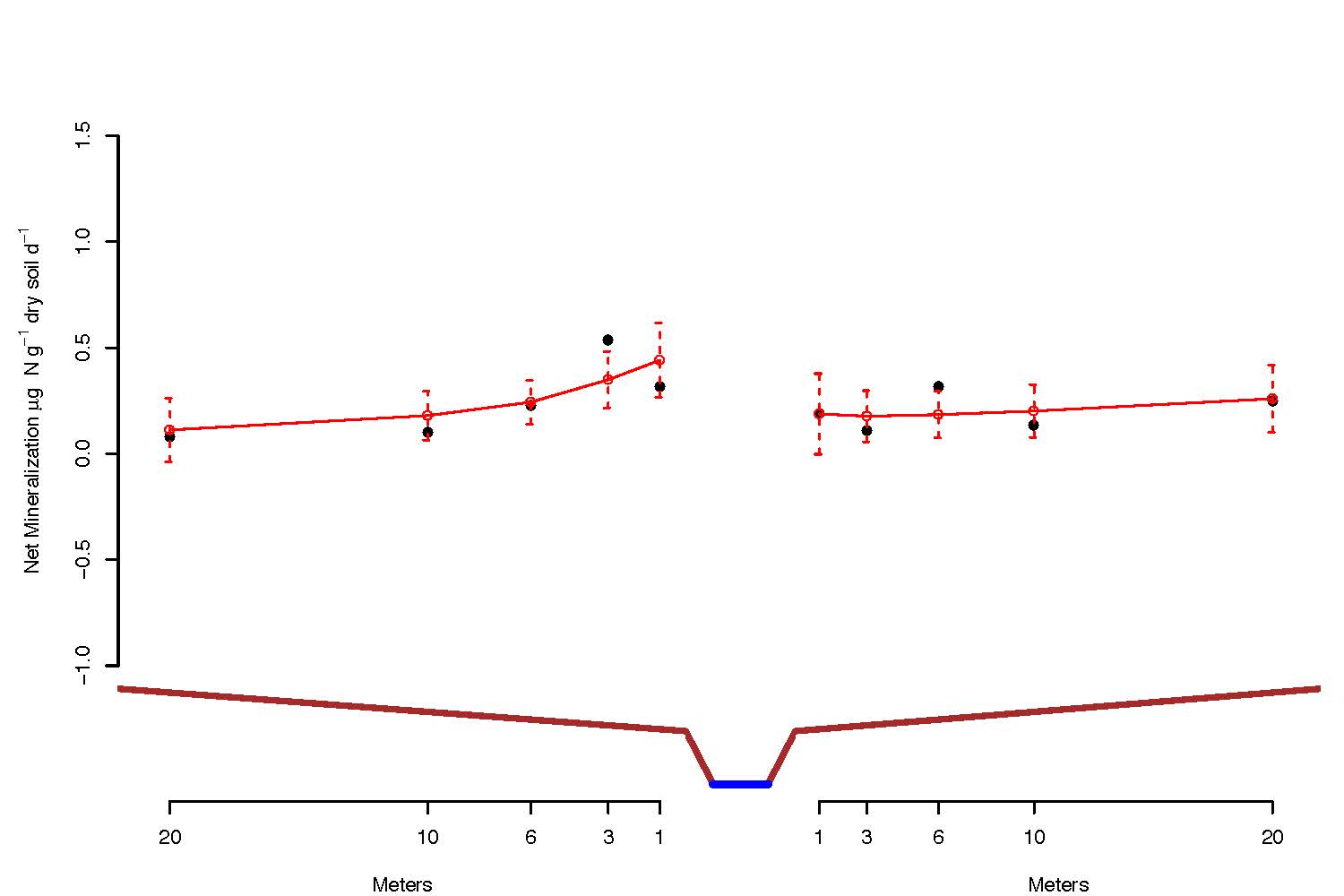
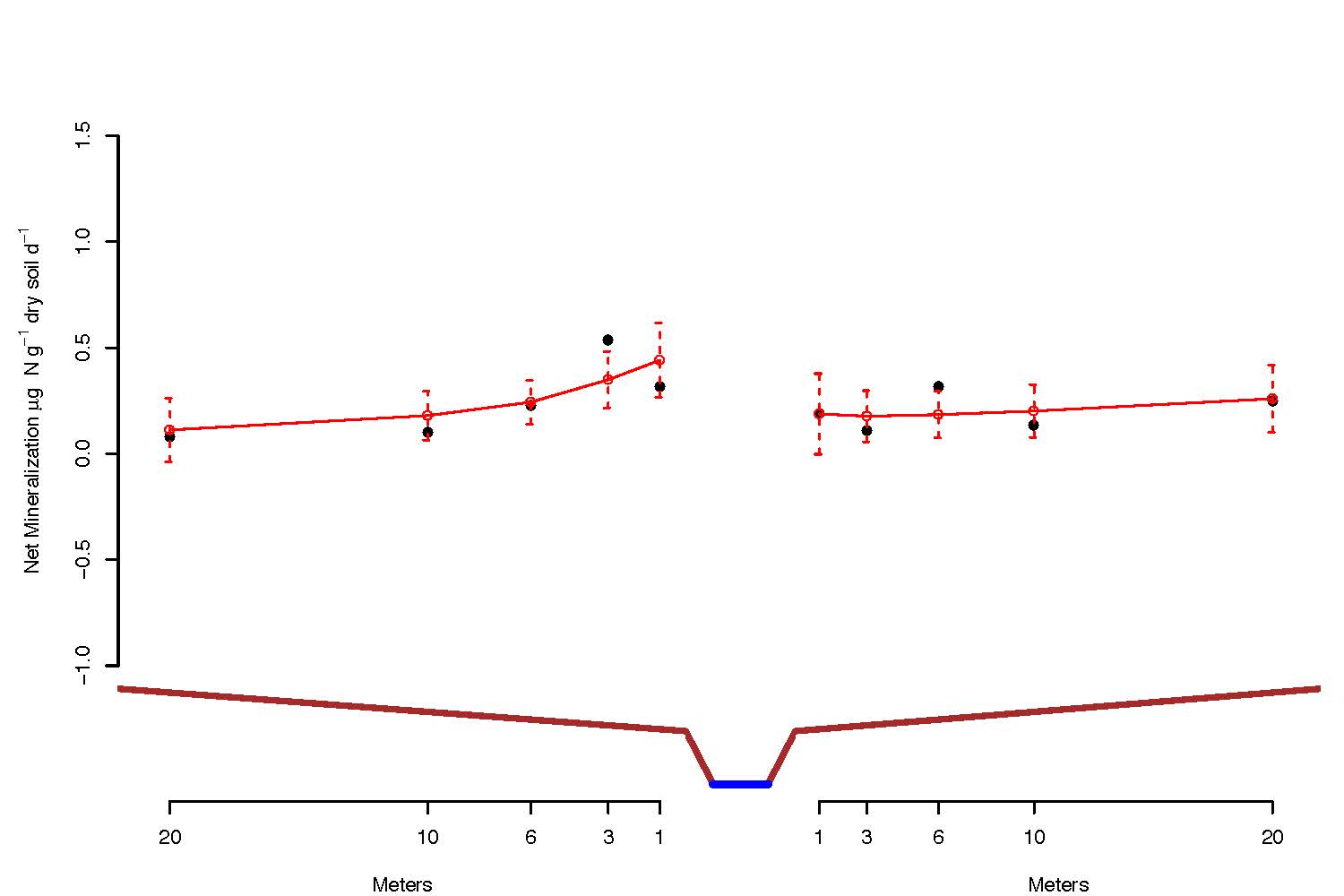
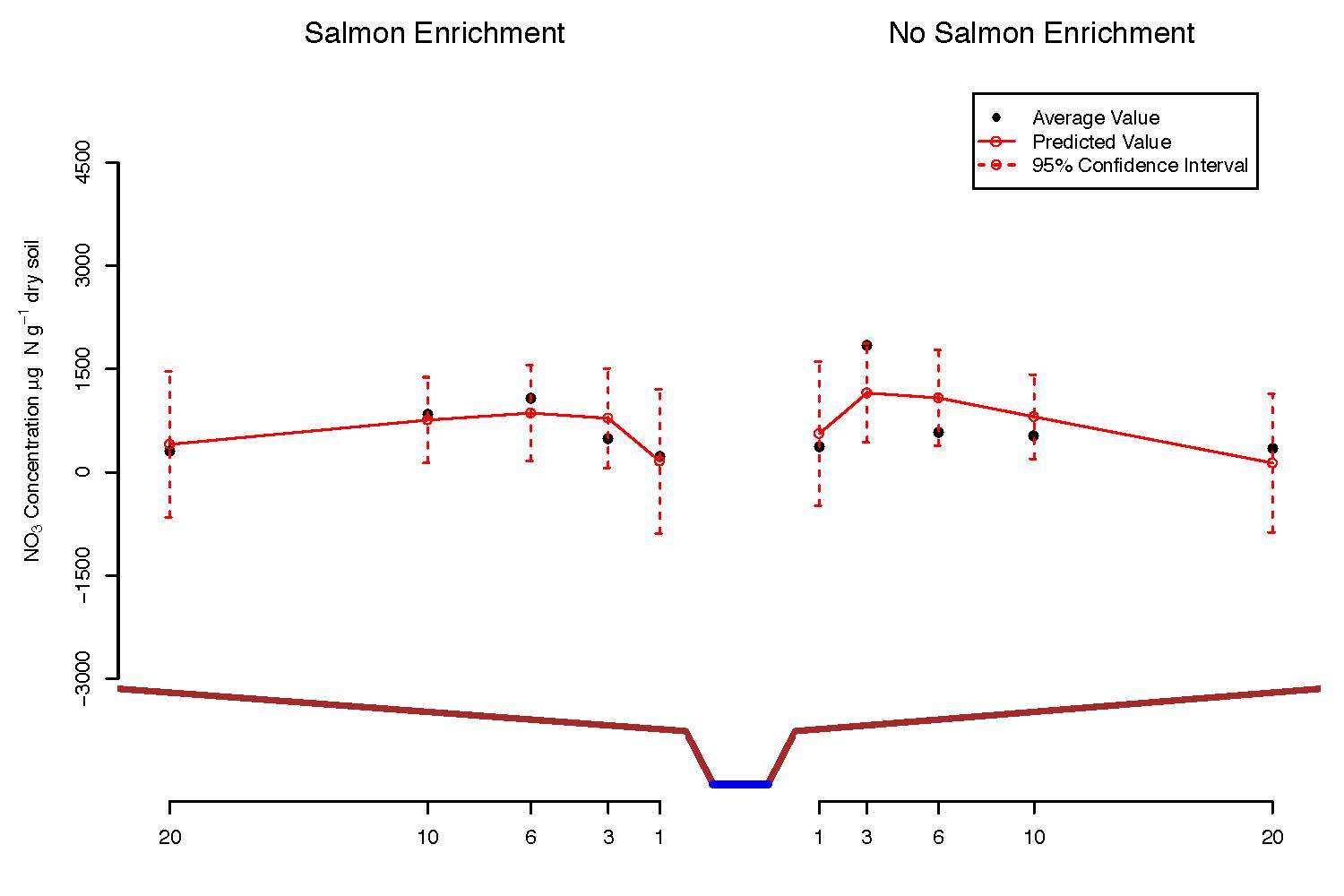
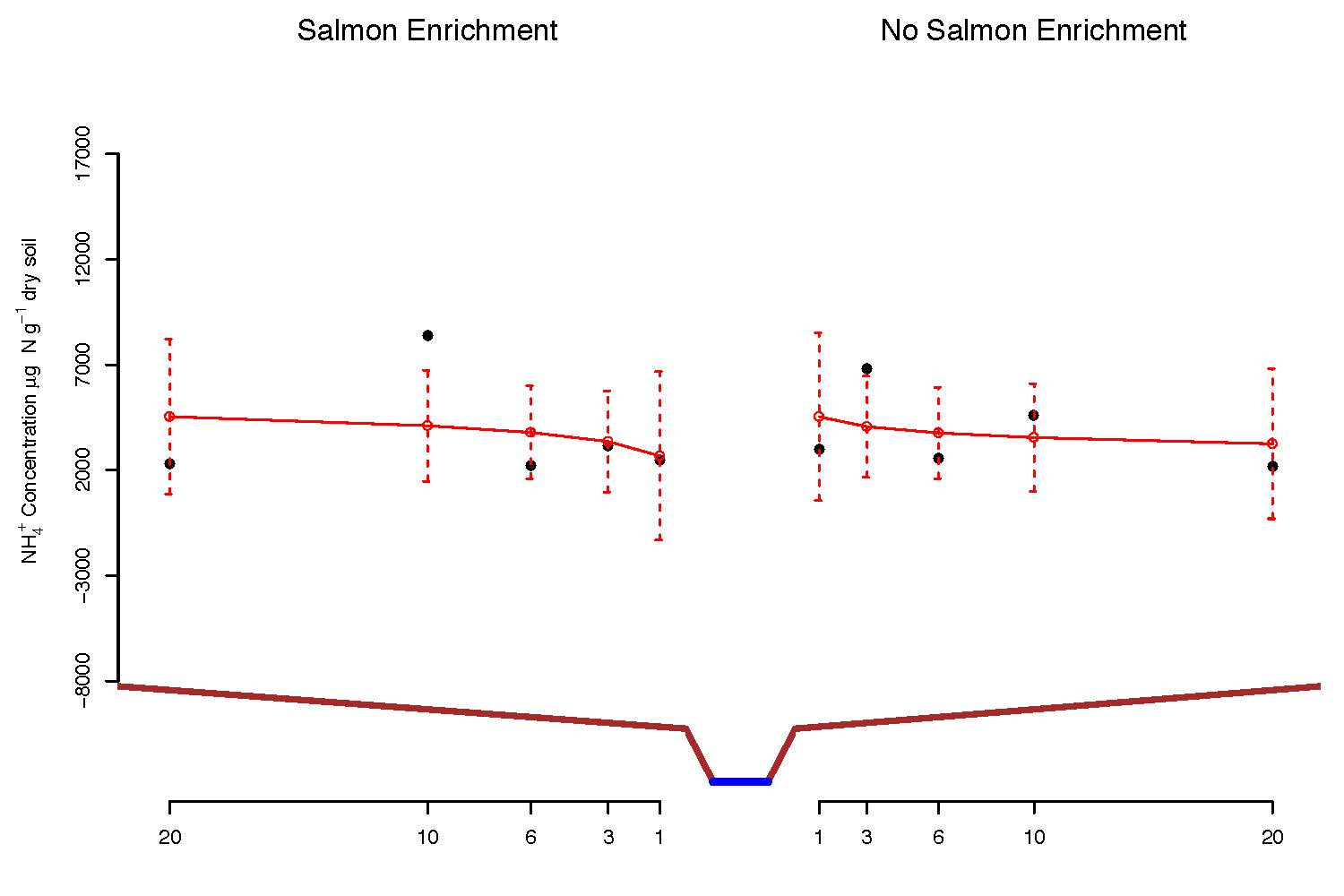


Figure 3:

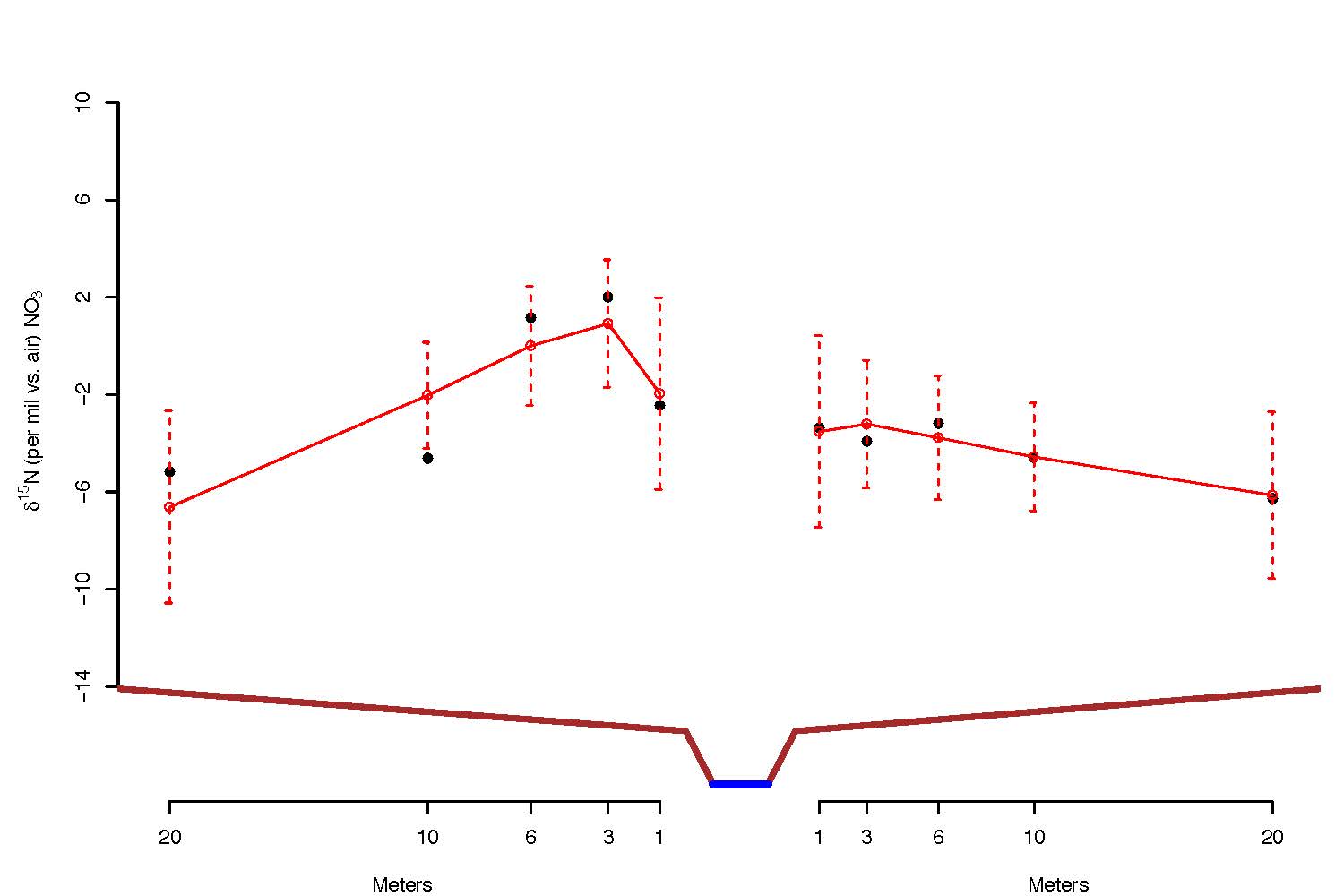
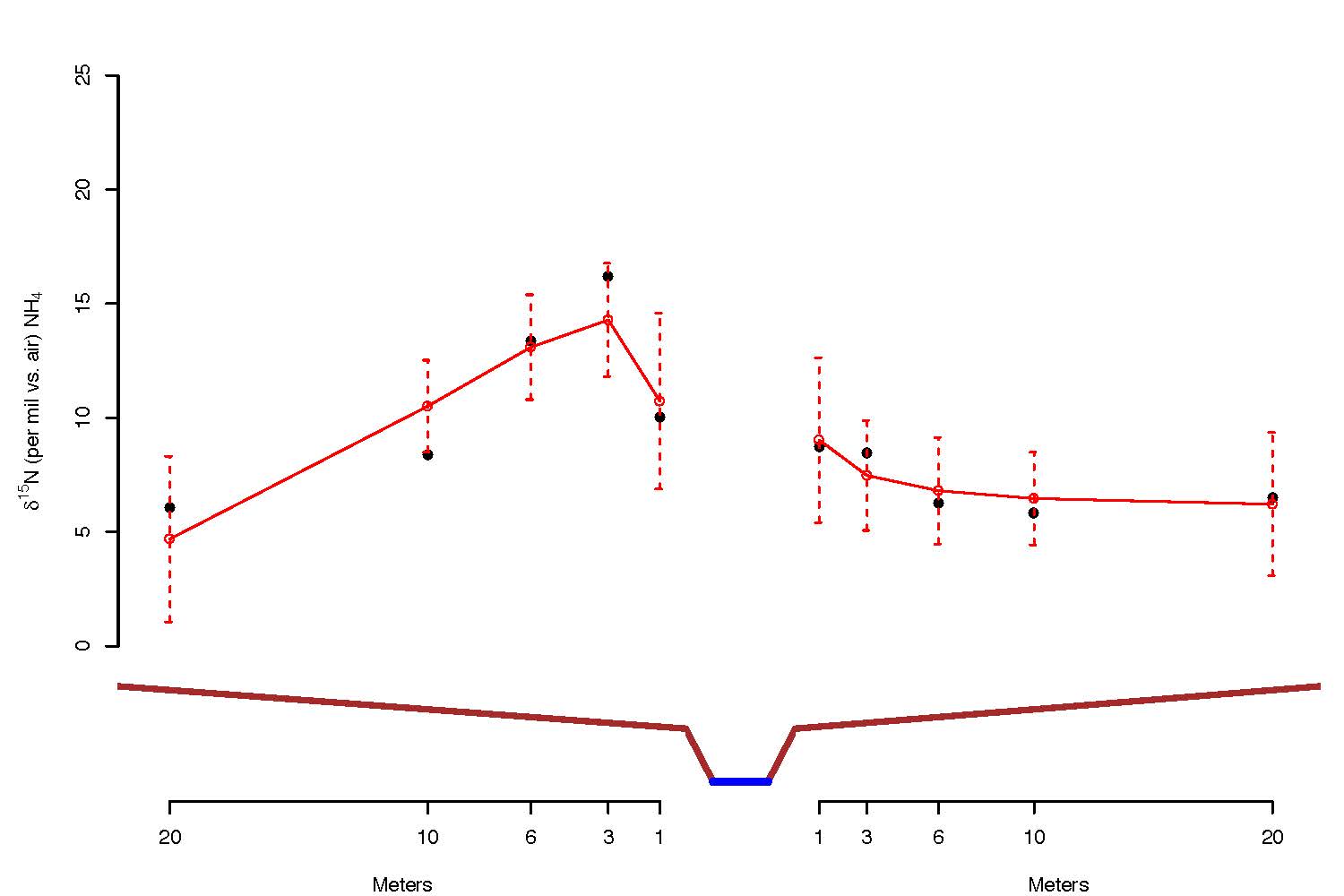
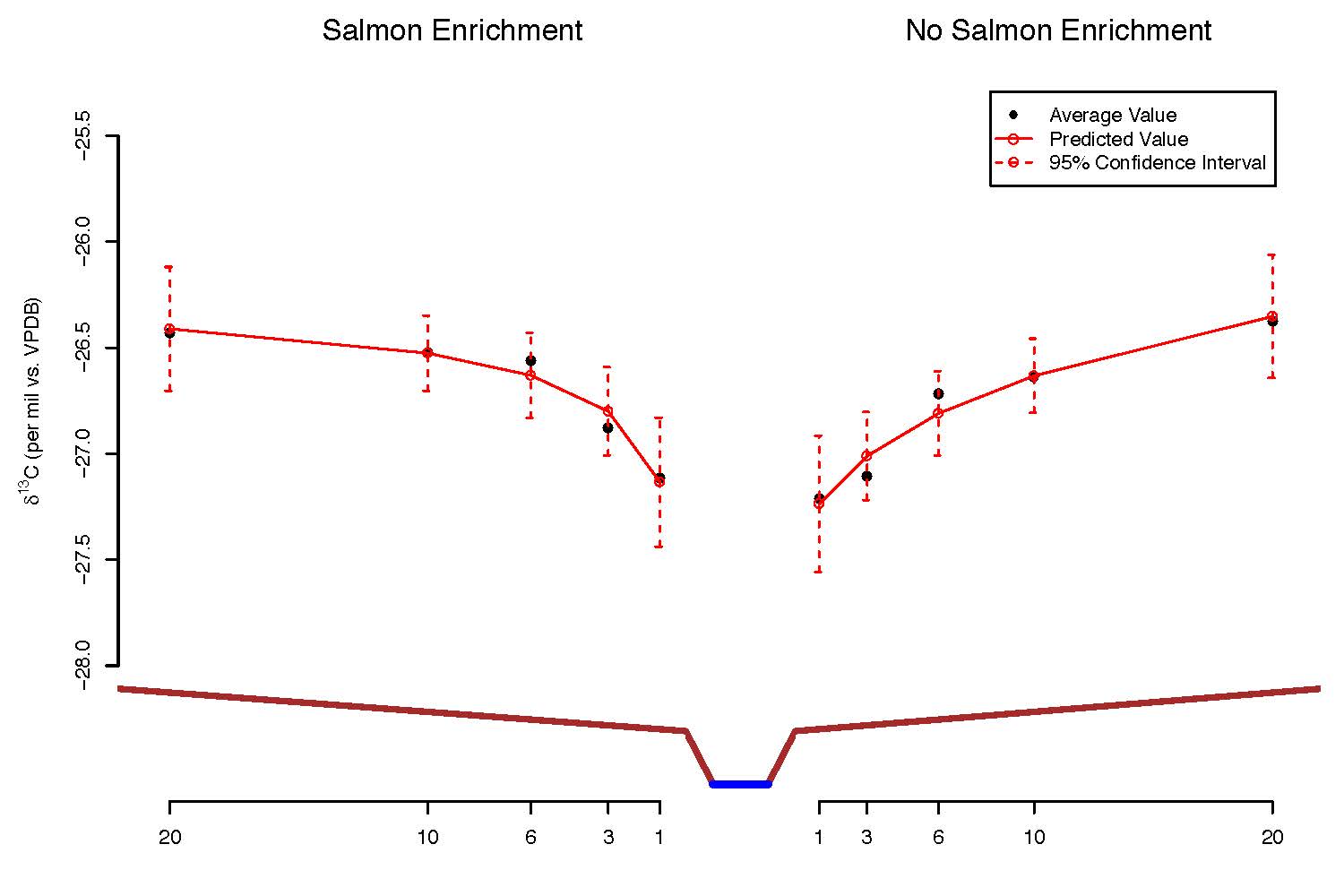
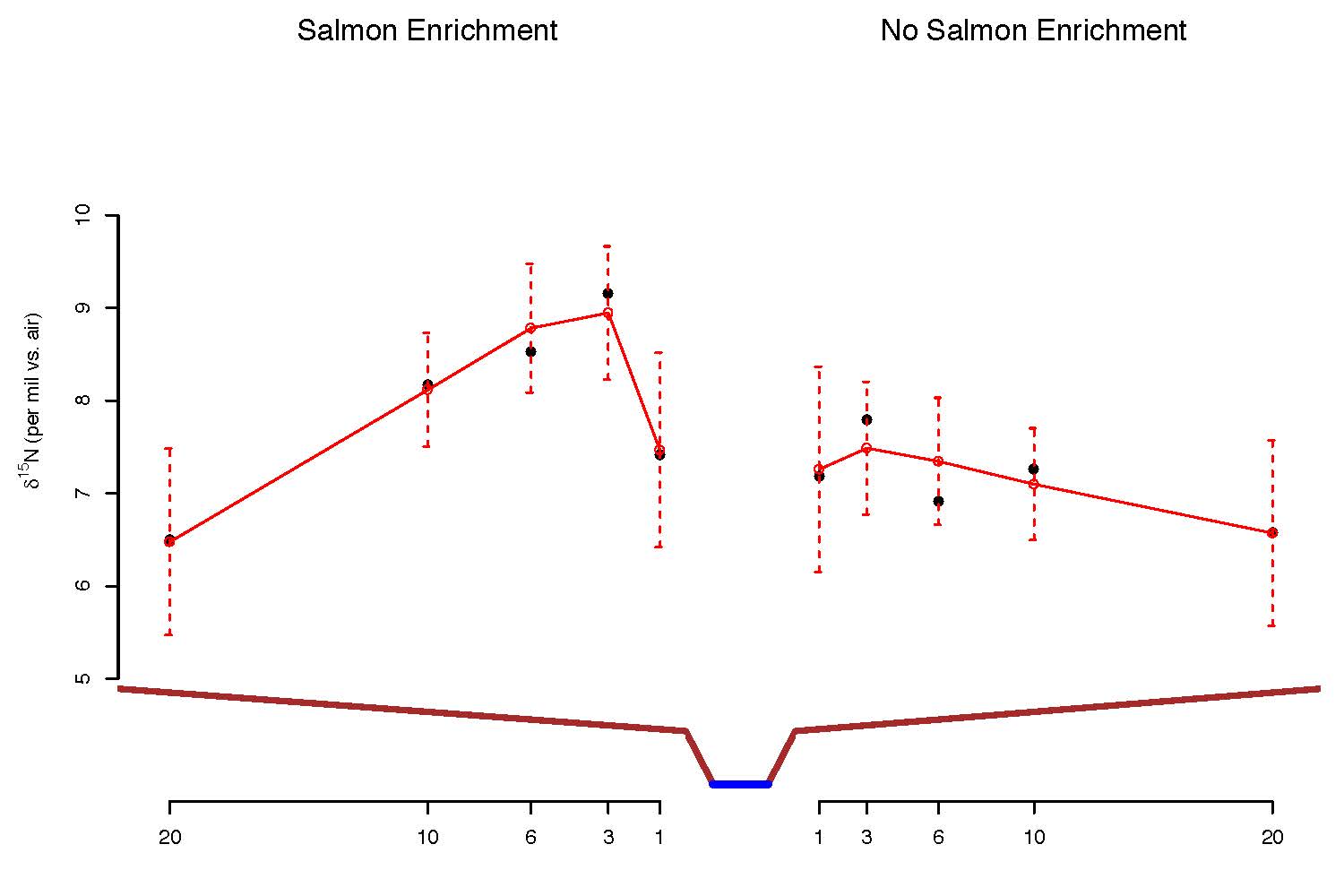
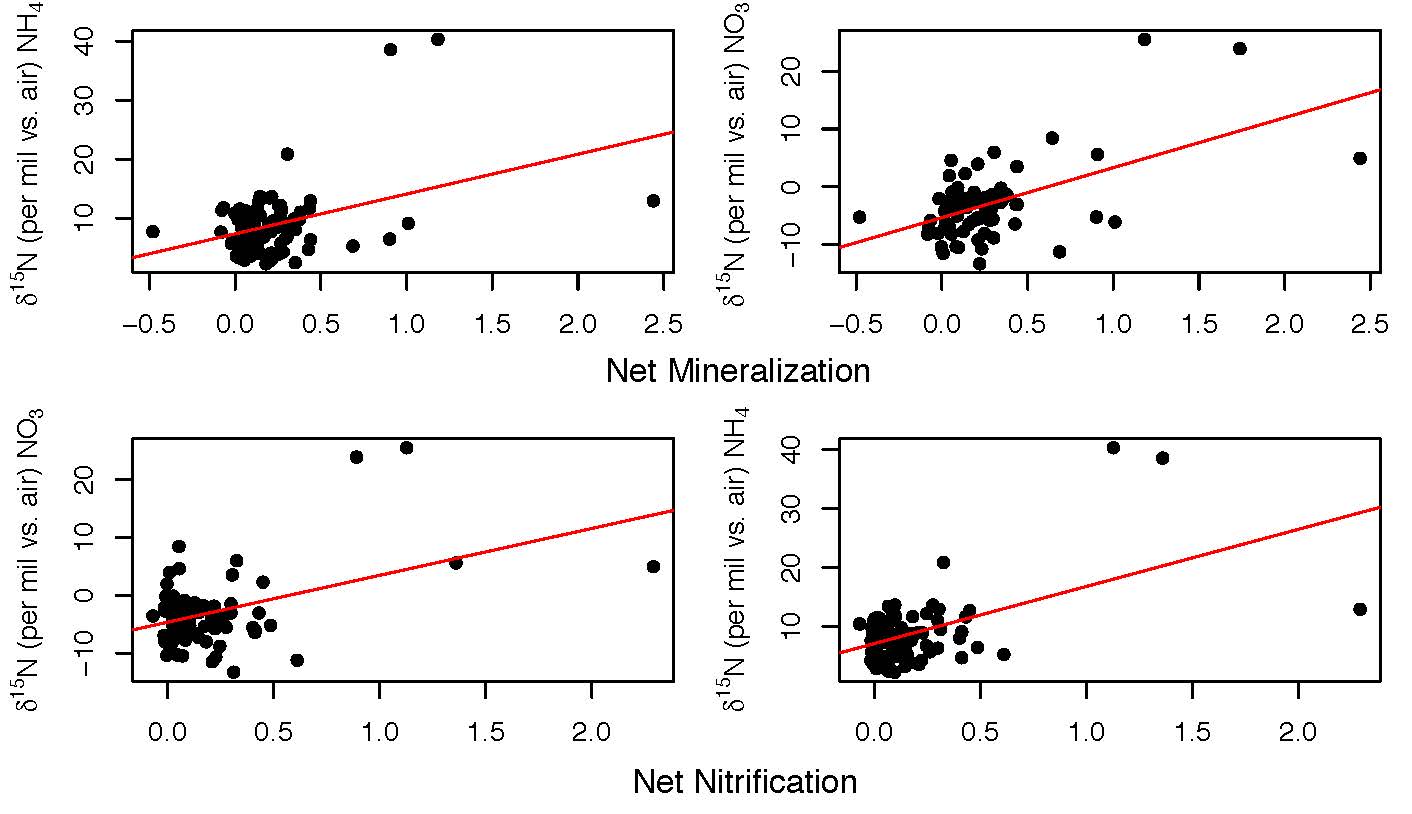


Figure 4:



**Acknowledgements**

FRI, Jackie Carter, Katie McElroy, Max ???(field sampling), Eric Ward (advice with models)