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Abstract

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“Here is my abstract”

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ACKNOWLEDGMENTS

“My acknowledgments”

DEDICATION

“My dedication”

INTRODUCTION

Welcome to the *R Markdown* thesis template. This template is based on (and in many places copied directly from) the UW LaTeX template, but hopefully it will provide a nicer interface for those that have never used TeX or LaTeX before. Using *R Markdown* will also allow you to easily keep track of your analyses in **R** chunks of code, with the resulting plots and output included as well. The hope is this *R Markdown* template gets you in the habit of doing reproducible research, which benefits you long-term as a researcher, but also will greatly help anyone that is trying to reproduce or build onto your results down the road.

Hopefully, you won't have much of a learning period to go through and you will reap the benefits of a nicely formatted thesis. The use of LaTeX in combination with *Markdown* is more consistent than the output of a word processor, much less prone to corruption or crashing, and the resulting file is smaller than a Word file. While you may have never had problems using Word in the past, your thesis is likely going to be at least twice as large and complex as anything you've written before, taxing Word's capabilities. After working with *Markdown* and **R** together for a few weeks, we are confident this will be your reporting style of choice going forward.

Why use it?

R Markdown creates a simple and straightforward way to interface with the beauty of LaTeX. Packages have been written in **R** to work directly with LaTeX to produce nicely formatting tables and paragraphs. In addition to creating a user friendly interface to LaTeX, *R Markdown* also allows you to read in your data, to analyze it and to visualize it using **R** functions, and also to provide the documentation and commentary on the results of your project. Further, it allows for **R** results to be passed inline to the commentary of your results. You'll see

more on this later.

Who should use it?

Anyone who needs to use data analysis, math, tables, a lot of figures, complex cross-references, or who just cares about the final appearance of their document should use *R Markdown*. Of particular use should be anyone in the sciences, but the user-friendly nature of *Markdown* and its ability to keep track of and easily include figures, automatically generate a table of contents, index, references, table of figures, etc. should make it of great benefit to nearly anyone writing a thesis project.

Chapter 1

RIPARIAN SOIL NITROGEN CYCLING AND ISOTOPIC ENRICHMENT IN RESPONSE TO A LONG-TERM SALMON CARCASS MANIPULATION EXPERIMENT

1.1 *Abstract*

Pacific salmon acquire most of their biomass in the ocean before returning to spawn and die in coastal streams and lakes, thus providing subsidies of marine-derived nitrogen (MDN) to freshwater and terrestrial ecosystems. Recent declines in salmon abundance have raised questions of whether managers should mitigate for losses of salmon MDN subsidies. To test the long-term importance of salmon subsidies to riparian ecosystems we measured soil N cycling in response to a 20-year manipulation where salmon carcasses were systematically removed from one bank and deposited on the opposite bank along a 2 km stream in southwestern Alaska. Soil samples were taken at different distances from the stream bank along nine paired transects and measured for organic and inorganic nitrogen concentrations, and nitrogen transformation rates. MDN was measured using $^{15}\text{N}/^{14}\text{N}$ for bulk soils, and NH_4^+ and NO_3^- soil pools. Stable isotope analyses confirmed $^{15}\text{N}/^{14}\text{N}$ was elevated on the salmon enhanced bank compared to the salmon depleted bank. However, $^{15}\text{N}/^{14}\text{N}$ values of plant-available inorganic nitrogen exceeded the $^{15}\text{N}/^{14}\text{N}$ of salmon inputs, highlighting N isotope fractionation in soils that raises significant methodological issues with standard MDN assessments in riparian systems. Surprisingly, despite 20 years of salmon supplementation, the presence of MDN did not cause a long-term increase in soil N availability. This finding indicates the importance of MDN to ecosystem N biogeochemistry and riparian vegetation may be overestimated for some systems. Given that essential nutrients can also be pollutants, we

urge more critical analyses of the role of MDN to inform compensatory mitigation programs targeting salmon nutrient enhancement.

1.2 Introduction

Pacific salmon (*Oncorhynchus spp.*) migration from marine environments to freshwater spawning grounds is a textbook case of cross-ecosystem nutrient subsidies, and dozens of studies have identified the presence of marine-derived nitrogen (MDN) from salmon as crossing ecosystem boundaries from oceans to freshwaters and into the terrestrial environment (sensu, (Polis, Power, & Huxel, 2004; Schindler et al., 2003; Scott M, Richard T, Mary F, & Mark S, 2002). Declines in Pacific salmon populations in many areas, caused by human activities (overharvest, habitat degradation, dams) (Richard et al., 2007), and the concern over loss of MDN to coastal watersheds has made restoration of salmon nutrients a focal point for many management and mitigation strategies. For example, in the Columbia River Basin where Pacific salmon populations have declined, legislation requiring compensatory mitigation has led to nutrient enhancement programs, on the foundation that habitats have lost critical nutrients from salmon and therefore augmentation is necessary to maintain ecosystem function (Collins, Marcarelli, Baxter, & Wipfli, 2015).

Salmon bring nutrients, including phosphorus (P) and other compounds in addition to nitrogen (N), into freshwater and terrestrial food webs through two pathways: 1) direct consumption of tissues by predators and scavengers, and 2) autotrophic or heterotrophic assimilation of nutrients released as salmon spawn, die, and eventually decay (Scott M et al., 2002). Salmon are enriched in the heavy isotope of nitrogen (^{15}N) relative to the light isotope (^{14}N) when compared to terrestrial and watershed-derived N. This isotopic enrichment has been used to quantitatively trace the presence of salmon derived nutrients into watersheds (Schindler et al., 2003). For example, the proportion of N derived from salmon ranges from approximately 30% – 75% in fish and aquatic invertebrates (Naiman, Bilby, Schindler, & Helfield, 2002), 10 – 90% in piscivorous mammals such as bears, and 20 – 40% in piscivorous

fishes near salmon spawning grounds (Bilby, Fransen, & Bisson, 1996; Chaloner, Martin, Wipfli, Ostrom, & Lamberti, 2002; Claeson, Li, Compton, & Bisson, 2006; Hilderbrand et al., 1999).

The annual return of this predictable and abundant, yet temporally limited, high quality resource drives the foraging ecology of both terrestrial and aquatic consumers (Quinn, Helfield, Austin, Hovel, & Bunn, 2018; Schindler et al., 2013). Carcasses and roe are documented food sources for over 22 species of mammals, birds (Cederholm, Houston, Cole, & Scarlett, 1989), fishes (Scheuerell, Moore, Schindler, & Harvey, 2007), and invertebrates (Meehan, Seminet-Reneau, & Quinn, 2005; Winder, Schindler, Moore, Johnson, & Palen, 2005). Bear population density, body size, and reproductive output has been correlated with meat (primarily salmon) consumption, with piscivorous populations having 55 times higher density than their meat-limited counterparts (Hilderbrand et al., 1999). In aquatic ecosystems, salmon carcass abundance has been correlated with elevated growth rates of invertebrates, and with size, density, and condition factor of juvenile salmonids (R. E. Bilby, Fransen, Bisson, & Walter, 1998; Wipfli, Hudson, Caouette, & Chaloner, 2003).

The presence of MDN has been documented in aquatic primary producers, though its overall ecological importance remains ambiguous. Via this bottom-up pathway, salmon supply critical limiting nutrients that can increase primary and/or bacterial productivity, which are subsequently transferred to consumers and up through the food web (Chaloner et al., 2002; Holtgrieve & Schindler, 2011; M. S. Wipfli, Hudson, & Caouette, 1998). Higher salmon returns are correlated with MDN signatures in lower trophic levels including zooplankton and periphyton (Finney, 2000; Holtgrieve, Schindler, Gowell, Ruff, & Lisi, 2010; Kline Jr et al., 1993). Both direct ecological and paleolimnological evidence suggest MDN and P positively influence primary production in lakes (Moore et al., 2007). For example, commercial fisheries remove upwards of two-thirds of MDN which would otherwise enter some freshwater lakes in Alaska, resulting in a 3-fold decline in algal production (Schindler, Leavitt, Brock, Johnson, & Quay, 2005). In stream ecosystems, the decomposition of salmon

increases dissolved organic and inorganic nutrients, including highly available forms such as orthophosphate (PO_4^{3-}) and ammonia/ammonium ($\text{NH}_3/\text{NH}_4^+$). These nutrients can stimulate epilithon growth (bacteria and algae), though the magnitude of this response is highly variable, and dependent on other growth limiting factors such as sunlight and disturbance (Janetski, Chaloner, Tiegs, & Lamberti, 2009; Johnston, MacIsaac, Tschaplinski, & Hall, 2004; Mitchell & Lamberti, 2005).

In the terrestrial realm, bottom-up effects of MDN from salmon are also thought to be ecologically important, though this has been difficult to demonstrate rigorously. Studies across the range of salmon in North America have inferred that up to 26% of foliar N in riparian plants is marine derived, with foliar N levels often correlating with salmon abundance and distance from the salmon spawning location (e.g., Hocking & Reynolds, 2012; Reimchen & Fox, 2013). While MDN is clearly present in terrestrial producers, direct evidence of the importance of MDN for ecosystem function and productivity is much less evident. Helfield & Naiman (2001) measured tree growth increments in areas with and without salmon and found higher growth in one species (Sitka spruce) in areas where salmon nutrients were present, although these findings were later contested on statistical grounds (Kirchhoff, 2003). Hocking & Reynolds (2012) observed decreased understory plant diversity with increasing salmon abundance, though this pattern was largely attributed to increased dominance of a single N tolerant species (salmonberry). Reimchen & Fox (2013) suggested that salmon abundance increased tree growth, but tree ring $^{15}\text{N}/^{14}\text{N}$ values were not related to salmon abundance; other growth limiting factors such as temperature and location were important covariates. Most recently, Quinn et al. (2018) examined tree growth increments in the riparian zone of a small Alaskan stream before and after a 20-year, > 200,000 kg, salmon carcass manipulation. In the two decades prior to manipulation, white spruce (*Picea glauca*) on average grew faster on one bank compared to the other. The subsequent decades of carcass manipulation enriched the naturally slower growing side, and were associated with increased growth. However, the growth effect of the carcasses was smaller than the natural side-to-side

variation, and other important site and landscape factors such as forest demography, climate, aspect, and water availability were not fully considered, a common trend in MDN studies of riparian vegetation.

Interpreting the contributions of MDN to terrestrial producers using stable isotopes is often highly simplified, and does not consider how variability of N sources and overall N availability may confound results. MDN analyses apply simple two-source mixing models to infer the proportion of total N derived from salmon using equation (1.1):

$$MDN = \frac{SAM - TEM}{MEM - TEM} * 100 \quad (1.1)$$

MDN is the percentage of marine derived nitrogen in a given sample, *TEM* is the terrestrial end member ($\delta^{15}N$ value representing 0% MDN), *MEM* is the marine end member ($\delta^{15}N$ value representing 100% MDN) which is typically 12.65‰ for sockeye salmon. *SAM* values are the values in a salmon area and *TEM* is derived from a non-salmon control. When applied to terrestrial vegetation, the terrestrial end-member for the mixing models is typically determined by sampling the $^{15}N/^{14}N$ of the same species of plant either laterally away from the stream (where MDN contribution is expected to be small), upstream of barriers to salmon migration, or in watersheds without salmon. For the salmon end-member, a single value equal to the average $^{15}N/^{14}N$ of salmon (12.62 ± 0.31 per mille for sockeye salmon) is typically used.

Inherent assumptions with these models therefore include: 1) reference sites are biogeochemically similar to salmon sites and 2) the isotopic signature of salmon is unchanged in the soils prior to plant uptake. N cycling in soils is strongly controlled by position in the landscape and contains a number of chemical reactions which fractionate N isotopically (Högberg, 1998; Wheeler, Kavanagh, & Daanen, 2014) (Figure 1.1), therefore these assumptions may not be valid.

Figure 1.1: 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^-).

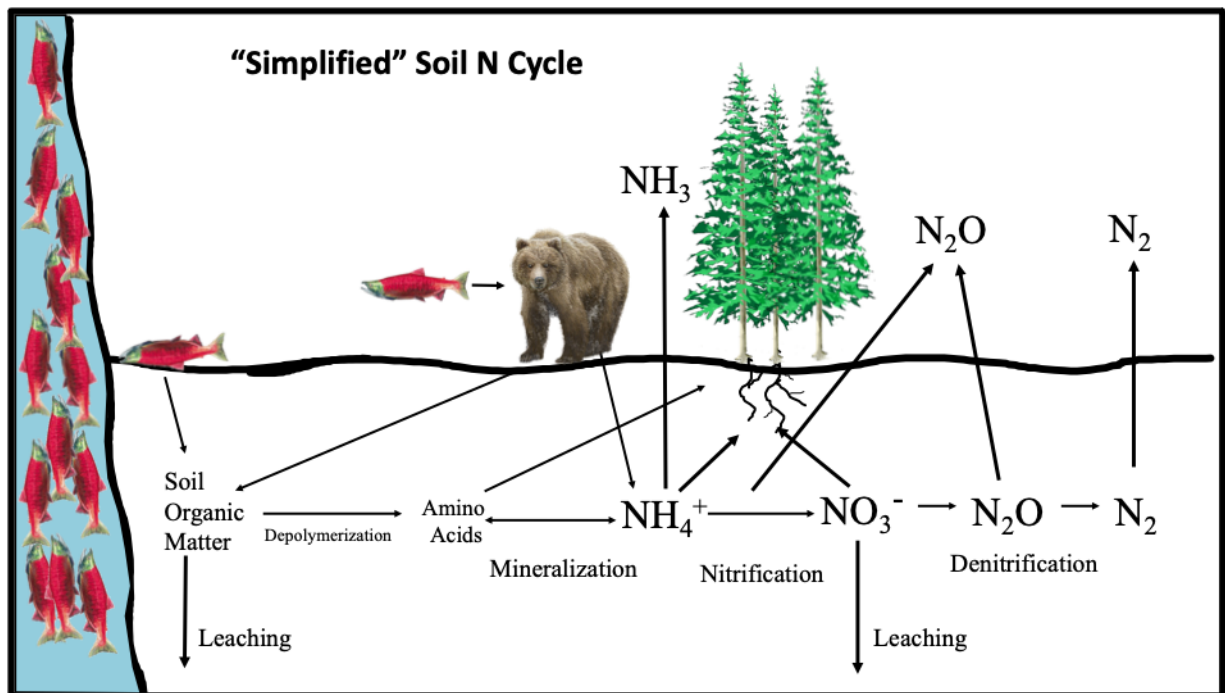


Figure 1.1: Nitrogen pathways in soil

Experiments examining the contributions of MDN are often limited by short timescales, and relatively few experiments investigate changes in plant-available soil N pools important to plant nutrient uptake and growth (Collins et al., 2015). Studies examining spatial and temporal impacts of salmon on soil inorganic N have identified highly localized responses (effects only observed < 30 cm from carcasses) where soil ammonium (NH_4^+) and nitrate (NO_3^-) increase for weeks to months (Drake, Naiman, & Bechtold, 2006; Gende, Miller, & Hood, 2007; Holtgrieve, Schindler, & Jewett, 2009) and rarely consider long-term N retention in the system. Experiments typically examine the contributions of MDN by nutrient addition not nutrient removal; however, nutrient removal is important for understanding the effects of

lower numbers of salmon returning to coastal watersheds due to fishing, habitat reduction, and climate change. In addition, previous research observed a strong effect of watershed slope on $^{15}\text{N}/^{14}\text{N}$ in riparian plants and attributed this to topography concentrating carcasses near streams (Hocking & Reynolds, 2012). However, watershed topography also influences soil water content and N cycling, which affect N isotopes (Högberg, 1998) and therefore complicates MDN assessments.

To resolve the extent to which salmon carcasses contributed MDN to plant-available N pools and the long-term ecological response to this subsidy, we present a second study of the 20-year carcass manipulation experiment described in Quinn et al. (2018). While Quinn et al. (2018) focused on tree growth before and after the manipulation, the objective of this work was to determine whether prolonged enhancement and reduction of salmon subsidies altered long-term soil N cycling, similar to that documented in forests receiving N fertilizer additions (Lu et al., 2011; Prescott, Corbin, & Parkinson, 1992; Prescott, Kishchuk, & Weetman, 1995). If long-term changes in N availability due to salmon enhancement or reduction were observed, compensatory nutrient subsidies may be valuable for maintaining critical ecosystem functions in riparian areas with reduced salmon returns. If not, then the addition of nutrients as a management response to low salmon returns may have unintended negative consequences (*sensu* Compton et al., 2006). Specifically, the importance of MDN to riparian ecosystems was assessed by 1) evaluating the presence of MDN in soils enhanced and depleted in salmon carcasses through bulk stable isotope analysis of N, 2) quantifying the response of plant-available N pools ($[\text{NH}_4^+]$ and $[\text{NO}_3^-]$) and their rate of supply via mineralization and nitrification, 3) considering how fractionation in soils may impact mixing model results by measuring $^{15}\text{N}/^{14}\text{N}$ of NH_4^+ and 4) comparing these results to the vegetation responses measured by Quinn et al. (2018) at the same site. This research fills key knowledge gaps by examining the long-term legacy of inorganic N pools, both salmon addition and removal, and considering site variability that may impact the assumption of biogeochemical similarity between test and control sites, following a 20-year manipulation.

1.3 Methods

1.3.1 Site Description and Sample Collection

This study was conducted on Hansen Creek, a ~2 km long, 2nd order tributary to Lake Aleknagik in the Wood River system of Bristol Bay, AK and uses the same carcass manipulation described in Quinn et al. (2018). Briefly, from 1997-2016 an average of 10,853 sockeye salmon returned to the stream annually. Overstory vegetation is dominated by white spruce and paper birch (*Betula papyrifera*), and unlike many other watersheds in the region, it has a low density of symbiotic N₂-fixing alder (*Alnus spp.*) (Helfield & Naiman, 2002). From 1997-2016 the stream was surveyed daily during the annual sockeye salmon (*Oncorhynchus nerka*) run and all dead salmon were removed from the creek and the river right bank to a distance of about 5 m and tossed onto the river left bank. To avoid double counting carcasses on the river left bank, carcasses naturally occurring on the river left bank were also relocated to a distance of about 5 m, thus all carcasses (with the exception of those moved by wildlife, see Quinn et al. (2018)) were located between 3 – 6 m on the river left bank. Therefore, the right side of the stream experienced a reduction in carcass density (depletion) while the left bank received an increase in carcasses (enhancement). Quinn et al. (2018) calculated that prior to manipulation the both banks averaged 4545.6 kg of salmon annually and after manipulation the river left bank averaged 13,381 kg of salmon and the river right bank averaged 2,260 kg of salmon annually, a 9.6-fold difference. Approximately 108,530 individual fish (in many cases partially consumed by bears) were translocated over the 20-year period representing a total of 267,620 kg of salmon, 8,028 kg of N and 1,356 kg of phosphorus (P) (Quinn et al., 2018). To estimate the mass of nitrogen added per m² we assumed all salmon were tossed within 6 m of the creek's edge along the entire 2 km creek, thus within a 12,000 m² area.

Soil samples were collected from the riparian zone on 13 July, 2017 (prior to arrival of salmon and any carcass manipulation that season) along nine sets of paired transect sites. Paired

transects were used to control for naturally occurring salmon density. Transects covered the full 2 km length of the stream and were selected to represent typical riparian vegetation and high annual carcass abundance. Each transect included sampling sites at 1, 3, 6, 10, and 20 m from the bank-full point. Sampling occurred during peak growing season approximately one week prior to the arrival of the first salmon in the creek. Thus, our sampling was intended to capture the long-term legacy of MDN manipulations and specifically avoid short-term pulses following salmon return that may not represent a system-level change in N availability, retention, and recycling in soils, and has already been documented in multiple short-term studies. A 5 cm x 5 cm x 10 cm soil column was taken for each sample site and the litter layer was removed before storing at 4°C in airtight plastic bags for 48 hours prior to processing. Nitrogen cycling decreases dramatically with depth, sampling at this depth includes the O and A horizons where a majority of nitrogen cycling occurs (Sparks, Soil Science Society of, & American Society of, [1996](#)).

1.3.2 Soil nitrogen concentrations and transformations

Soil $[\text{NH}_4^+]$, $[\text{NO}_3^-]$, and N transformations were measured according to Holtgrieve et al. ([2009](#)). Briefly, we extracted 10 to 12 g of field-moist sieved (< 2 mm) soil with 100 mL of 2 M potassium chloride (KCl) by shaking for 60 s, followed by settling for 24 hours prior to filtration through pre-leached Whatman #1 filter papers. Approximately 8 mL of filtered extracts were frozen and later analyzed colorimetrically for $[\text{NH}_4^+]$ and $[\text{NO}_3^-]$ with an Auto-Analyzer 500 Model (Perstorp Analytical Co, Analytical Service Station, Seattle, WA, USA). The remaining extract was frozen prior to stable isotope analyses (see below). To estimate inorganic N transformation rates, a second 10 to 12 g soil subsample was incubated aerobically in the dark for 15 d at 20°C prior to extraction, filtration, and analysis as above. Net mineralization was calculated as the sum of the change in $[\text{NH}_4^+]$ and $[\text{NO}_3^-]$ divided by the incubation duration, and net nitrification was calculated as the change in $[\text{NO}_3^-]$ over the incubation duration and represents the conversion of NH_4^+ to NO_3^- (???). $[\text{N}_{\text{org}}]$ was

calculated by taking total soil N concentration, $[N_{\text{tot}}]$ determined by elemental analysis (see below) and subtracting $[NH_4^+]$ and $[NO_3^-]$. All soil N values were corrected for gravimetric soil water content (g H₂O/g dry soil) determined by drying 50 to 100 g of field-moist soil at 105°C for 48 h (???)

1.3.3 Stable isotope analysis

Fresh soil was freeze dried for 48 h and ground into a uniform powder ($< 212 \mu\text{m}$) using a ball mill prior to analysis for nitrogen ($^{15}\text{N}/^{14}\text{N}$) and carbon ($^{13}\text{C}/^{12}\text{C}$) stable isotope ratios at the University of Washington's IsoLab using a Costech Elemental Analyzer, Conflo III MAT253 for continuous flow-based measurements. This procedure also provided total carbon and nitrogen concentrations, $[C_{\text{tot}}]$ and $[N_{\text{tot}}]$, and percent C and N, of the soil samples. Data are reported using standard delta notation, which describes the per mil deviation in the ratio of heavy to light isotope relative to accepted international standards, in this case air and Vienna Pee Dee Belemite (VPDB) for N and C respectively (???)

For $^{15}\text{N}/^{14}\text{N}$ stable isotope analysis of NH_4^+ and NO_3^- , KCl extracts were placed in Erlenmeyer flasks for diffusion using modified methods from Sigman et al. (1997) and Holmes, McClelland, Sigman, Fry, & Peterson (1998). To retrieve NH_4^+ as gaseous NH_3 , 300 mg of MgO and an acid trap (1 cm glass fiber filter treated with $KHSO_4$ and sealed in Teflon) were added to each flask, immediately stoppered, sealed with parafilm, and shaken for six days prior to removal of acid traps to a desiccator for 3 to 4 days. The same extracts were then shaken uncovered for one day to remove any remaining NH_4^+ . To retrieve NO_3^- as NH_3 , another 300 mg of MgO were added to each extract and immediately followed with 75 mg of Devarda's alloy and an acid trap, then processed as above. Samples were run in four separate batches, for each batch three blanks (KCl with no soil extract) and three reference standards, NH_4Cl and KNO_3 with known $^{15}\text{N}/^{14}\text{N}$, were also run. Batch blanks showed quantifiable N from the KCl; therefore, a two-source mixing model correction was applied to

both samples and reference standards using (1.2):

$$\delta^{15}N_{blankcorrected} = \frac{\delta^{15}N_{measured} * (N_{blank,x} + N_{extracted}) - (\delta^{15}N_{blank,x} * N_{blank,x})}{N_{extracted}} \quad (1.2)$$

Where x represents an individual batch, $N_{blank,x}$ is the average measured mass (μg) of nitrogen in a blank for a given batch, and $\delta^{15}N_{blank,x}$ is the average measured $\delta^{15}N$ of blanks for a given batch. $\delta^{15}N_{measured}$ is the $\delta^{15}N$ value for a given sample, and $N_{extracted}$ is the mass of nitrogen (μg) measured in the sample. A standard correction was then applied to the blank corrected measurements with (1.3):

$$\delta^{15}N_{corrected} = \delta^{15}N_{blank,corrected} - (Standard_{measured,x} - Standard_{true}) \quad (1.3)$$

Where $Standard_{measured,x}$ is the average measured value of the standard for a given batch. All reported $\delta^{15}N - NH_4^+$ and NO_3^- values are expressed as the $\delta^{15}N_{corrected}$, where a blank and standard correction has been applied. The internal standard of the $\delta^{15}N$ of NO_3^- had a -23.6 to 9.6‰ deviation from its true value, indicating a significant methodological issue. Given there was not enough sample to refine these methods and the potential for standard corrections of this magnitude to be misleading, $\delta^{15}N$ of NO_3^- data are not reported here.

C:N ratio, percent nitrification, and ‰C were also calculated to evaluate N availability and retention across the sites. C:N ratios were calculated on a mass basis Percent nitrification was calculated as (1.4):

$$PercentNitrification = 100 * NetNitrification / NetMineralization \quad (1.4)$$

1.3.4 Statistical analyses

We used multi-model selection procedures via Akaike's information criterion (AIC) to identify how salmon carcass treatment governed a suite of response variables using the stats v3 and lme4 packages in R. These response variables were: $\delta^{15}N$ 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. For all response variables, candidate models Table 1.1 included bank

(left vs. right) and distance from river's edge. A linear and quadratic interaction structure for bank and distance were fit for each response variable and these interaction terms allowed the effect of distance to vary by bank and the effect of bank to vary by distance. A \log_e transformation was used for the distance. GW was considered as a covariate for all response variables, soil $[\text{NH}_4^+]$ was considered as a covariate for net nitrification, and soil $[\text{N}_{\text{org}}]$ was considered as a covariate for net mineralization, given $[\text{N}_{\text{org}}]$ and $[\text{NH}_4^+]$ function as the substrate for mineralization and nitrification respectively. $[\text{N}_{\text{tot}}]$ was considered as a covariate for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of bulk soil, and for $\delta^{15}\text{N}$ of NH_4^+ . The best model was selected from the candidate model set using AIC for each response variable.

Table 1.1: The candidate model set tested for each response variable using AIC analysis where $\{*\}$ 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 $\delta^{15}\text{N}$ data, GW was not tested as a covariate and total mass of N was tested instead. The four tested hypotheses are 1) bank effect, 2) distance effect, 3) bank and distance effect (salmon effect), and 4) no effect of bank and distance. Response variables include: $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of bulk soil, $\delta^{15}\text{N}$ of NH_4^+ , $[\text{NH}_4^+]$ and $[\text{NO}_3^-]$, net mineralization and net nitrification, $[\text{N}_{\text{org}}]$, gravimetric water content (GW), and C:N.

Table 1.1: The candidate model set tested for each response variable using AIC analysis

Candidate Model Set	Hypothesis
*Response Variable = bank +	1
*Response Variable = bank + GW +	1
*Response Variable = $\ln(\text{distance})$ + GW +	1
*Response Variable = $\ln(\text{distance})$ +	1
*Response Variable = bank + $\ln(\text{distance})$ + bank: $\ln(\text{distance})$ + $\ln(\text{distance})^2$:bank + GW +	2
*Response Variable = bank + $\ln(\text{distance})$ + bank: $\ln(\text{distance})$ +	1
*Response Variable = bank + $\ln(\text{distance})$ + bank: $\ln(\text{distance})$ + GW +	1
*Response Variable = bank + $\ln(\text{distance})$ + bank: $\ln(\text{distance})$ + $\ln(\text{distance})^2$:bank +	2
*Response Variable = bank + $\ln(\text{distance})$ + bank+	1
*Response Variable = bank + $\ln(\text{distance})$ + bank + GW+	1
*Response Variable = GW +	3
Response Variable = bank + substrate +	1
Response Variable = $\ln(\text{distance})$ + substrate +	1
Response Variable = bank + GW + substrate +	1

Response Variable = bank + ln(distance) + bank:ln(distance) + GW + substrate +	2
Response Variable = bank + ln(distance) + bank:ln(distance) + substrate +	1
Response Variable = bank + ln(distance) + bank:ln(distance) + GW + substrate +	2
Response Variable = bank + ln(distance) + bank:ln(distance) + ln(distance) ² :bank + GW + substrate +	2
Response Variable = bank + ln(distance) + bank:ln(distance) + ln(distance) ² :bank + substrate +	2
Response Variable = bank + ln(distance) + GW + substrate +	1
Response Variable = bank + ln(distance) + substrate +	1
Response Variable = substrate +	3
Response Variable = GW + substrate +	3

Two model parameters – bank (left vs. right) and distance from the stream – were used to test salmon carcass and site variability impacts to soil N cycling. Changing the number of salmon carcasses on each bank was the primary goal of the manipulation; however, the two banks potentially differ in aspect, soil type, and drainage, which can affect nutrient cycling and generate a bank effect unrelated to salmon manipulation (I. Chapin F. Stuart, Matson, Vitousek, & Chapin, 2011). Notably, the salmon enhanced bank has a northwest facing slope within 20 m of the creek edge. Distance from the stream reflects the magnitude of salmon manipulation because carcasses were placed primarily 3 – 6 m from the stream’s edge. Other factors such as vegetation, soil type, and water availability can also change with distance laterally from the stream edge, though such changes are expected to be more continuous, rather than focused on the same 3 – 6 m band where salmon were placed. These differences in expected lateral patterns in soil properties due to salmon (focused at 3 – 6 m) verse other factors (more continuous) provide a means to test whether salmon significantly altered soil patterns in our experiment.

We inferred that salmon significantly influenced a soil property when that soil property met the following conditions: (a) the property differed between the study banks, (b) varied with distance from the stream edge, and (c) displayed a peak response at 3 - 6 m on the salmon addition bank. All conditions (a, b, c) are required to infer that salmon significantly altered the soils on the treatment bank. In contrast, we inferred that support for only one of these parameters demonstrates underlying site variability in the system. Effects of natural site variability on soil properties is also an important component to test. Control

sites are typically assumed to be biogeochemically similar to carcass sites without validating this assumption, despite control sites often being located at different stream reaches or on different streams altogether. For each of the nine response variables, three competing hypotheses were compared, that the differences in response variables were due to H1) a bank and/or distance effect that does not demonstrate a peak response between 3 – 6 m indicating site variability not caused by salmon manipulation, H2) a bank and distance effect as a quadratic interaction with a peak between 3 – 6 m indicting a response to salmon manipulation or H3) no difference caused by distance and bank indicating support for the other covariates tested. These hypotheses were tested by categorizing each candidate model into one of the three hypotheses (Table 1.1) and considering the hypothesis categorization for the model with the most support, and any additional competing models with relative support (ΔAIC value of < 2) [(???) for each response variable under consideration (e.g., $[\text{NH}_4^+]$, $[\text{NO}_3^-]$, $\delta^{15}\text{N}$, etc.). If models showed support for H2, the effect of salmon was confirmed by examining whether the response variable peaked at the salmon enhanced bank between 3 – 6 m. If this did not occur, the response is due to site variability and not salmon.

1.4 Results

Bulk soil stable isotope analysis indicated that salmon carcasses enriched the N isotope pools (Table 1). $\delta^{15}\text{N}$ values peaked between 3 and 6 m from the stream edge, which was the distance salmon were typically relocated to during the experiment and declined at distances greater than 6 m. Maximum $\delta^{15}\text{N}$ of bulk soils was 11.8‰ for the salmon enhanced bank and 11.6‰ for the salmon depleted bank and no observations exceeded the sockeye salmon end-member value of 12.6‰ (Figure 2a). $\delta^{13}\text{C}$ was more enriched at greater distances from the bank and on average was highest at 20 m (Figure 2b). $\delta^{13}\text{C}$ was primarily governed by distance, with some evidence $[\text{N}_{\text{tot}}]$ and bank also had an effect (1.2).

Table 1.2: Competing models with relative support ($\Delta\text{AIC} < 2$) using AIC analysis for each response variable, where the most parsimonious models with the most support are

shown in bold. Reported are ΔAIC and the hypothesis supported by each model: H1 is a bank effect not caused by salmon manipulation, H2 is a distance effect not caused by salmon manipulation, H3 is both a bank and distance effect indicating a response to salmon manipulation, and H4 indicates support for the other covariates tested.

Table 1.2: Competing models with relative support ($\Delta AIC < 2$) using AIC analysis for each response variable

Response Variable	Model Hypothesis	ΔAIC	Covariates Included in Models with Relative Support
Bulk 15N	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 , [Ntot]
Bulk 13C	1	0.00	ln(Distance)
	1	0.22	Bank, ln(Distance)
	1	0.62	ln(Distance), [Ntot]
	1	1.23	Bank, ln(Distance), [Ntot]
15N of NH4+ [NH4+]	2	0.00	Bank, ln(Distance), Bank:ln(Distance), Bank:ln(Distance) ²
	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
	NA	NA	
[NO3-]	1	1.87	Bank, ln(Distance), Bank:ln(Distance), GW
	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	[NOrg]
	3	0.61	GW, [NOrg]
	1	0.74	Bank, [NOrg]
	1	1.61	Bank, GW, [NOrg]
Net Nitrification	3	0.00	[NH4+], GW
	NA	NA	
	1	1.02	Bank, [NH4+], GW
[NOrg]	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)

Salmon carcass manipulation also enriched $\delta^{15}\text{N}$ of soil NH_4^+ . Stable isotope values were enriched at 3 m from the stream edge on the salmon enhanced bank, and declined at distances > 3 m. On the salmon depleted bank, $\delta^{15}\text{N}$ of soil NH_4^+ was most enriched at 1 m and declined with distance (Figure @ref(fig:modsupp1.2)C). The only model with support contained a quadratic interaction of distance and bank, which provides strong evidence that $\delta^{15}\text{N}$ of NH_4^+ was affected by salmon (Table 1.2). In contrast to bulk soil N, $\delta^{15}\text{N}$ values of NH_4^+ exceeded the salmon endmember of 12.6‰ for 23% of all observations (n=21).

Inorganic nitrogen concentrations were primarily governed by bank and GW (Table 1.2). The salmon enhanced bank had a higher mean $[\text{NH}_4^+]$ and $[\text{NO}_3^-]$ compared to the salmon depleted bank (Figure 2d, e). The most supported models for both $[\text{NH}_4^+]$ and $[\text{NO}_3^-]$ showed evidence for H1, that observed differences were not caused by salmon. For $[\text{NH}_4^+]$ there was substantial model uncertainty, with six competing models receiving relative support ($\Delta\text{AIC} < 2$) (Table 1.2) but none of the competing models supported a salmon effect. Two competing models for $[\text{NO}_3^-]$ supported a site variability effect and one competing model supported a salmon effect (Table 1.2) and all three contained gravimetric water content as a covariate. This indicates $[\text{NH}_4^+]$ was driven by site factors unrelated to salmon while $[\text{NO}_3^-]$ was driven by gravimetric water content and with some support for salmon enhancement.

Figure 1.2: Data (closed circles) and predicted values (open circles) for the model with the most support (Table 1.2) for soil organic $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ of NH_4^+ , and C:N for both the salmon-enhanced and the salmondepleted banks of Hansen Creek at 1, 3, 6, 10, 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 (b and d) denotes site variable factors.

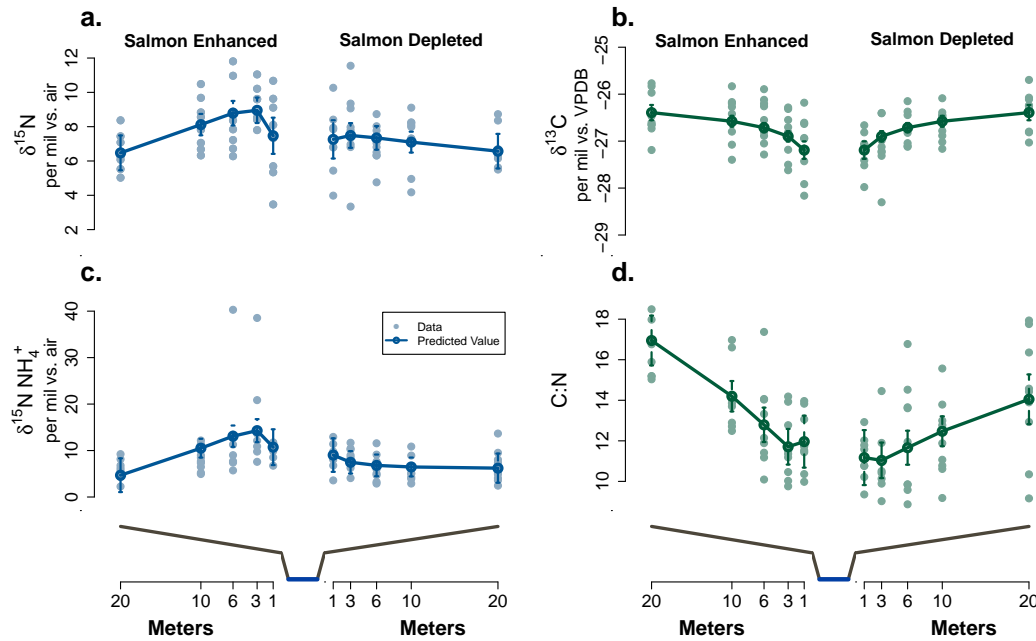


Figure 1.2: Data and predicted values for the model with the most support: Stable Isotopes

Nitrogen transformation rates were unaffected by salmon carcass manipulation. Both net nitrification and net mineralization models with relative support contained N substrate ($[\text{NH}_4^+]$ and $[\text{Norg}]$ respectively), and the models with the most support did not include distance or bank. Net mineralization had some model uncertainty, with four models receiving relative support; however, all of the competing models supported either H1 or H3 with no support for a salmon effect. $[\text{Norg}]$ was the only covariate included in all of the competing models, indicating $[\text{Norg}]$ was the most important covariate tested for determining net mineralization. Net nitrification had greater model certainty and both models that received relative support contained $[\text{NH}_4^+]$ and gravimetric water content. Similar to net mineralization, these models supported H1 and H3 with no support for H2, the salmon effect, though net nitrification was slightly higher on average between 3 – 6 m on the salmon enhanced bank (Table 1). Overall, these results demonstrated the manipulation of salmon carcasses

did not have clearly detectable effects on N transformation rates.

Figure 1.3: Data (closed circles) and predicted values (open circles) for the model with the most support (Table 1.2) for NH_4^+ and NO_3^- , net mineralization and nitrification, $[\text{N}_{\text{org}}]$, and gravimetric water content for both the salmon-enhanced and the salmon-depleted banks of Hansen Creek at 1, 3, 6, 10, and 20 m from the edge of the creek bed with 95% confidence intervals (dashed line) for predicted values. Red (a, b, c, d) denotes measures of soil productivity, and green (e and f) denotes site variable factors.

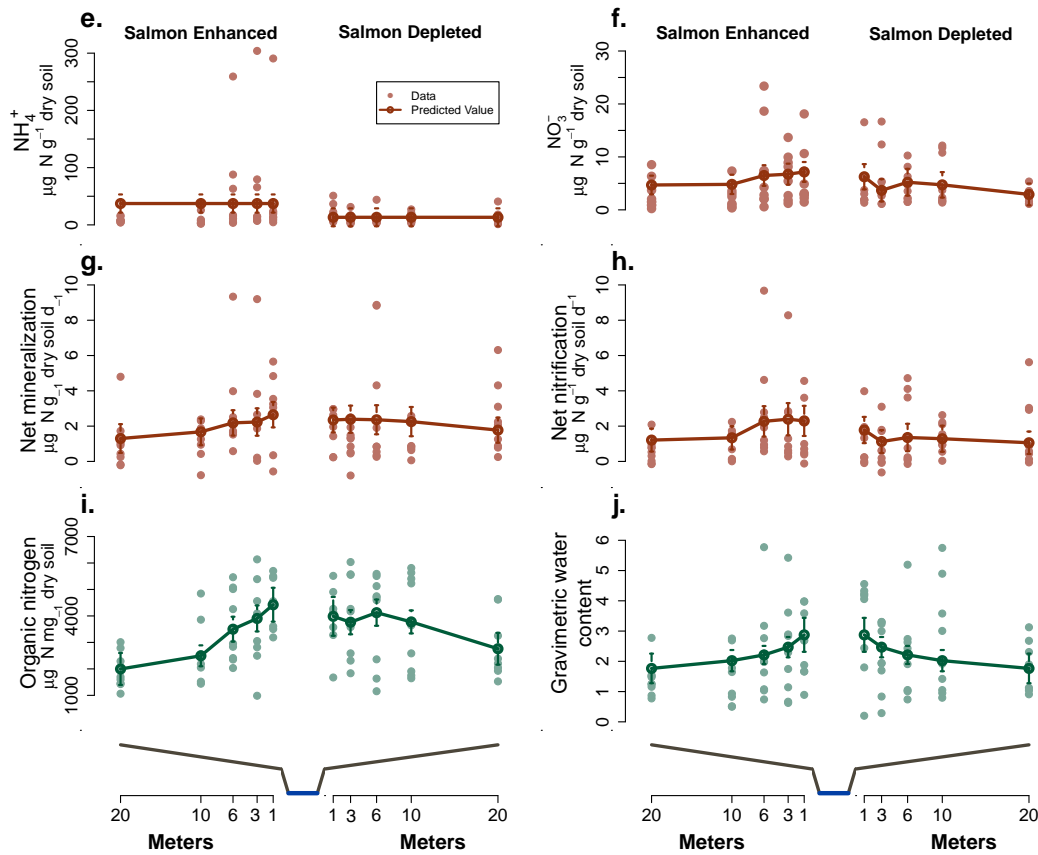


Figure 1.3: Data and predicted values for the model with the most support: Concentrations and Transformations

Both [Norg] and GW indicated there are site differences caused by distance and bank unrelated to salmon carcass manipulation. On average [Norg] was higher on the salmon depleted bank than the salmon enhanced bank. There was model support of H1 for both GW and [Norg], indicating these variables decrease with distance (Table 1, Figure 2 h, i). While there was some evidence that there was both a distance and bank effect on GW, it was not caused by salmon as the salmon enhanced bank does not show a peak GW at 3 - 6 m from the stream, which was where there was the highest observed isotopic enrichment and expected MDN. However, one competing model for [Norg] did support H2, indicating site factors and salmon may both affect [Norg]. However, the mean [Norg] for the salmon enhanced bank was 18.42 mg/g and 18.97 mg/g for the salmon depleted bank indicating salmon decrease [Norg], if they affect it at all. C:N, percent nitrification, and percent carbon indicate relatively high nitrogen availability across sampling sites in the Hansen Creek system. Mean percent carbon was 24.2 and 24.9 on the enhanced and depleted banks respectively (S3). Soil C:N of bulk isotopes was less than 20 for all sites, with a mean of 15.8 (enhanced) and 14.2 (depleted). These values are well below the critical microbial C:N threshold of 29, demonstrating N is more available to meet microbial metabolic demands relative to C (Figure 2j). In contrast, percent nitrification was relatively high with a mean of 64% and 62% on the enhanced and depleted banks (S3).

Chapter 2

MATHEMATICS AND SCIENCE

2.1 *Math*

T_EX is the best way to typeset mathematics. Donald Knuth designed T_EX when he got frustrated at how long it was taking the typesetters to finish his book, which contained a lot of mathematics. One nice feature of *R Markdown* is its ability to read LaTeX code directly.

If you are doing a thesis that will involve lots of math, you will want to read the following section which has been commented out. If you're not going to use math, skip over or delete this next commented section.

2.2 *Chemistry 101: Symbols*

Chemical formulas will look best if they are not italicized. Get around math mode's automatic italicizing in LaTeX by using the argument `$\mathrm{formula here}$` , with your formula inside the curly brackets. (Notice the use of the backticks here which enclose text that acts as code.)

So, Fe₂²⁺Cr₂O₄ is written `$\mathrm{Fe_2^{2+}Cr_2O_4}$` .

Exponent or Superscript: O⁻

Subscript: CH₄

To stack numbers or letters as in Fe₂²⁺, the subscript is defined first, and then the superscript is defined.

Bullet: CuCl • 7H₂O

Delta: Δ

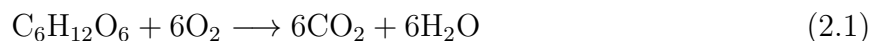
Reaction Arrows: \longrightarrow or $\xrightarrow{\text{solution}}$

Resonance Arrows: \leftrightarrow

Reversible Reaction Arrows: \rightleftharpoons

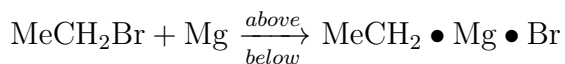
2.2.1 *Typesetting reactions*

You may wish to put your reaction in an equation environment, which means that LaTeX will place the reaction where it fits and will number the equations for you.



We can reference this combustion of glucose reaction via Equation (2.1).

2.2.2 *Other examples of reactions*



2.3 *Physics*

Many of the symbols you will need can be found on the math page <http://web.reed.edu/cis/help/latex/math.html> and the Comprehensive LaTeX Symbol Guide (<http://mirror.utexas.edu/ctan/info/symbols/comprehensive/symbols-letter.pdf>).

2.4 *Biology*

You will probably find the resources at <http://www.lecb.ncifcrf.gov/~toms/latex.html> helpful, particularly the links to bst's for various journals. You may also be interested in

TeXShade for nucleotide typesetting (<http://homepages.uni-tuebingen.de/beitz/txe.html>). Be sure to read the proceeding chapter on graphics and tables.

Chapter 3

TABLES, GRAPHICS, REFERENCES, AND LABELS

3.1 Tables

By far the easiest way to present tables in your thesis is to store the contents of the table in a CSV or Excel file, then read that file in to your R Markdown document as a data frame. Then you can style the table with the `kable` function, or functions in the `kableExtra` package.

In addition to the tables that can be automatically generated from a data frame in **R** that you saw in [R Markdown Basics] using the `kable` function, you can also create tables using *pandoc*. (More information is available at <http://pandoc.org/README.html#tables>.) This might be useful if you don't have values specifically stored in **R**, but you'd like to display them in table form. Below is an example. Pay careful attention to the alignment in the table and hyphens to create the rows and columns. Generally I don't recommend this approach of typing the table directly into your R Markdown document.

Table 3.1: Correlation of Inheritance Factors for Parents
and Child

Factors	Correlation between Parents & Child	Inherited
Education	-0.49	Yes
Socio-Economic Status	0.28	Slight
Income	0.08	No
Family Size	0.18	Slight

Factors	Correlation between Parents & Child	Inherited
Occupational Prestige	0.21	Slight

We can also create a link to the table by doing the following: Table [3.1](#). If you go back to [Loading and exploring data] and look at the `kable` table, we can create a reference to this max delays table too: Table `??`. The addition of the `(\#tab:inher)` option to the end of the table caption allows us to then make a reference to Table `\@ref(tab:label)`. Note that this reference could appear anywhere throughout the document after the table has appeared.

3.2 Figures

If your thesis has a lot of figures, *R Markdown* might behave better for you than that other word processor. One perk is that it will automatically number the figures accordingly in each chapter. You'll also be able to create a label for each figure, add a caption, and then reference the figure in a way similar to what we saw with tables earlier. If you label your figures, you can move the figures around and *R Markdown* will automatically adjust the numbering for you. No need for you to remember! So that you don't have to get too far into LaTeX to do this, a couple **R** functions have been created for you to assist. You'll see their use below.

In the **R** chunk below, we will load in a picture stored as `uw.png` in our main directory. We then give it the caption of "UW logo", the label of "uwlogo", and specify that this is a figure. Make note of the different **R** chunk options that are given in the R Markdown file (not shown in the knitted document).

```
knitr::include_graphics(path = "figure/uw.png")
```

Here is a reference to the UW logo: Figure 3.1. Note the use of the `fig:` code here. By naming the **R** chunk that contains the figure, we can then reference that figure later as done in the first sentence here. We can also specify the caption for the figure via the R chunk option `fig.cap`.



Figure 3.1: UW logo

Below we will investigate how to save the output of an **R** plot and label it in a way similar to that done above. Recall the `flights` dataset from Chapter ?? (Note that we've shown a different way to reference a section or chapter here.) We will next explore a bar graph with the mean flight departure delays by airline from Portland for 2014. Note also the use of the `scale` parameter which is discussed on the next page.

```
flights %>% group_by(carrier) %>%  
  summarize(mean_dep_delay = mean(dep_delay)) %>%  
  ggplot(aes(x = carrier, y = mean_dep_delay)) +  
  geom_bar(position = "identity", stat = "identity", fill = "red")
```

``summarise()`` ungrouping output (override with ``.groups`` argument)

Here is a reference to this image: Figure 3.2.

A table linking these carrier codes to airline names is available at <https://github.com/ismayc/pnwflights14/blob/master/data/airlines.csv>.

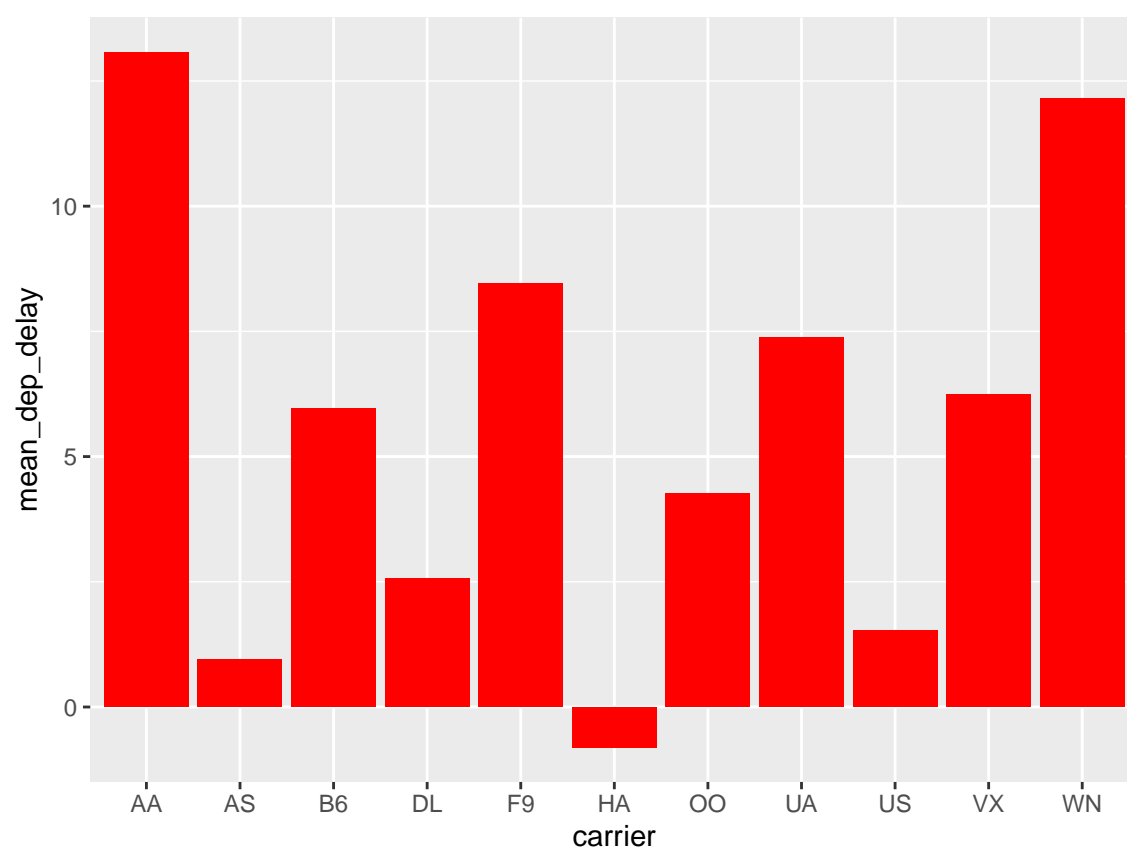


Figure 3.2: Mean Delays by Airline

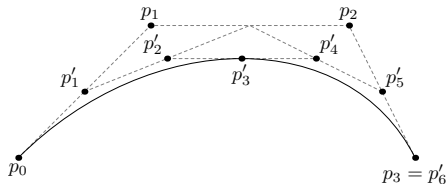


Figure 3.3: Subdiv. graph

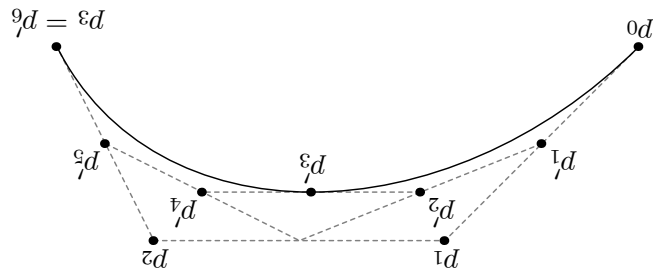


Figure 3.4: A Larger Figure, Flipped Upside Down

Next, we will explore the use of the `out.extra` chunk option, which can be used to shrink or expand an image loaded from a file by specifying "`scale=` ". Here we use the mathematical graph stored in the “subdivision.pdf” file. Here is a reference to this image: Figure 3.3. Note that `echo=FALSE` is specified so that the **R** code is hidden in the document.

More Figure Stuff

Lastly, we will explore how to rotate and enlarge figures using the `out.extra` chunk option. (Currently this only works in the PDF version of the book.) As another example, here is a reference: Figure 3.4.

3.3 Footnotes and Endnotes

You might want to footnote something.¹ The footnote will be in a smaller font and placed appropriately. Endnotes work in much the same way.

¹footnote text

3.4 Cross-referencing chapters and sections

The [bookdown documentation](#) is an excellent source for learning how to cross-reference in a bookdown project such as a huskydown document. Here we only cover the most common uses for a typical thesis. If you want something more complex or fancy, please refer to the bookdown documentation and seek help from the developers of that package.

By default, all of your chapter and section headers will get an auto-generated ID label. For example, e.g., `# Chapter 1` will have an auto-generated ID `chapter-1`. Note that the ID label is all lower case, and has no spaces. If you have any kind of punctuation in your header, such as a colon (:), it will not appear in the ID label. Then in your text you can reference chapter one in your Rmd file like this: ‘as discussed in Chapter `\@ref(chapter-1)`’, which will print as ‘as discussed in Chapter 1’

We strongly recommend that you to manually assign ID labels to your chapter header to make it easy to cross-reference. For example, at the top of the Rmd file for this chapter, you can see:

```
# Tables, Graphics, References, and Labels {#ref-labels}
```

The `{#ref-labels}` part of this header is the ID label. It doesn’t show in the output, but is there for us to use for easy cross-referencing, because it can be short, and we don’t need to change it elsewhere our document when we update the chapter header. We can use this custom ID label in our Rmd document like this: ‘as discussed in Chapter `\@ref(ref-labels)`’, which will print as ‘as discussed in Chapter [3](#)’. If you need to show custom text instead of the chapter number, you use this syntax in your Rmd document: `see [my chapter about labels]({#ref-labels}) for more details` which will appear as ‘see [my chapter about labels](#) for more details’

To cross-reference a specific section in the same chapter, we recommend adding a custom ID label to the section header, and using that to cross-reference. For example, earlier in this chapter we have a section on tables and in the Rmd file we see `## Tables`

`{#tables}`. We can cross-reference that in the text like this ‘as discussed in the section on `[tables]({#tables})`’ which will appear as ‘as discussed in the above section on [tables](#)’

To cross-reference a section in a different chapter we can use the ID label from that section directly. For example, we can write in our Rmd document `as discussed in the section on [R code chunks]({#r-chunks}) in Chapter \@ref(rmd-basics)` which will appear as ‘as discussed in the section on [R code chunks](#) in Chapter ??’.

If you prefer to cross-reference by the section number, we can use custom ID labels in our Rmd document. For example, to refer to a section in our first chapter, we can write in the Rmd document: `as discussed in section \@ref(r-chunks) in Chapter \@ref(rmd-basics)`. This will appear with section and chapter numbers like so: as ‘as discussed in section ?? in Chapter ??’.

3.5 Bibliographies

Of course you will need to cite things, and you will probably accumulate an armful of sources. There are a variety of tools available for creating a bibliography database (stored with the `.bib` extension). In addition to BibTeX suggested below, you may want to consider using the free and easy-to-use tool called Zotero. Some Zotero documentation is at <http://libguides.reed.edu/citation/zotero>. In addition, a tutorial is available from Middlebury College at <http://sites.middlebury.edu/zoteromiddlebury/>.

R Markdown uses *pandoc* (<http://pandoc.org/>) to build its bibliographies. One nice caveat of this is that you won’t have to do a second compile to load in references as standard LaTeX requires. To cite references in your thesis (after creating your bibliography database), place the reference name inside square brackets and precede it by the “at” symbol. For example, here’s a reference to a book about worrying: `(???)`. This `Molina1994` entry appears in a file called `thesis.bib` in the `bib` folder. This bibliography database file was created by a program called BibTeX. You can call this file something else if you like (look at the YAML header in the main `.Rmd` file) and, by default, is to placed in the `bib` folder.

For more information about BibTeX and bibliographies, see (<http://web.reed.edu/cis/help/latex/index.html>)². There are three pages on this topic: *bibtex* (which talks about using BibTeX, at <http://web.reed.edu/cis/help/latex/bibtex.html>), *bibtexstyles* (about how to find and use the bibliography style that best suits your needs, at <http://web.reed.edu/cis/help/latex/bibtexstyles.html>) and *bibman* (which covers how to make and maintain a bibliography by hand, without BibTeX, at <http://web.reed.edu/cis/help/latex/bibman.html>). The last page will not be useful unless you have only a few sources.

If you look at the YAML header at the top of the main .Rmd file you can see that we can specify the style of the bibliography by referencing the appropriate csl file. You can download a variety of different style files at <https://www.zotero.org/styles>. Make sure to download the file into the csl folder.

Tips for Bibliographies

- Like with thesis formatting, the sooner you start compiling your bibliography for something as large as thesis, the better.
- The cite key (a citation’s label) needs to be unique from the other entries.
- When you have more than one author or editor, you need to separate each author’s name by the word “and” e.g. `Author = {Noble, Sam and Youngberg, Jessica},.`
- Bibliographies made using BibTeX (whether manually or using a manager) accept LaTeX markup, so you can italicize and add symbols as necessary.
- To force capitalization in an article title or where all lowercase is generally used, bracket the capital letter in curly braces.

²(???)

3.6 *Anything else?*

If you'd like to see examples of other things in this template, please [contact us](mailto:bmarwick@uw.edu) (email bmarwick@uw.edu) with your suggestions. We love to see people using *R Markdown* for their theses, and are happy to help.

CONCLUSION

If we don't want Conclusion to have a chapter number next to it, we can add the `{-}` attribute.

More info

And here's some other random info: the first paragraph after a chapter title or section head *shouldn't be* indented, because indents are to tell the reader that you're starting a new paragraph. Since that's obvious after a chapter or section title, proper typesetting doesn't add an indent there.

Appendix A

THE FIRST APPENDIX

This first appendix includes all of the R chunks of code that were hidden throughout the document (using the `include = FALSE` chunk tag) to help with readability and/or setup.

In the main Rmd file

```
# This chunk ensures that the huskydown package is
# installed and loaded. This huskydown package includes
# the template files for the thesis.
if(!require(devtools))
  install.packages("devtools", repos = "http://cran.rstudio.com")
if(!require(huskydown))
  devtools::install_github("benmarwick/huskydown")
library(huskydown)
```

In Chapter 3:

```
# This chunk ensures that the huskydown package is
# installed and loaded. This huskydown package includes
# the template files for the thesis and also two functions
# used for labeling and referencing
if(!require(devtools))
  install.packages("devtools", repos = "http://cran.rstudio.com")
if(!require(dplyr))
```

```
  install.packages("dplyr", repos = "http://cran.rstudio.com")
if(!require(ggplot2))
  install.packages("ggplot2", repos = "http://cran.rstudio.com")
if(!require(ggplot2))
  install.packages("bookdown", repos = "http://cran.rstudio.com")
if(!require(huskydown)){
  library(devtools)
  devtools::install_github("benmarwick/huskydown")
}
library(huskydown)
flights <- read.csv("data/flights.csv")
```

Appendix B

THE SECOND APPENDIX, FOR FUN

COLOPHON

This document is set in **EB Garamond**, **Source Code Pro** and **Lato**. The body text is set at 11pt with *lmr*.

It was written in R Markdown and \LaTeX , and rendered into PDF using **huskydown** and **bookdown**.

This document was typeset using the XeTeX typesetting system, and the **University of Washington Thesis class** class created by Jim Fox. Under the hood, the **University of Washington Thesis LaTeX template** is used to ensure that documents conform precisely to submission standards. Other elements of the document formatting source code have been taken from the **Latex**, **Knitr**, and **RMarkdown templates for UC Berkeley's graduate thesis**, and **Dissertate: a LaTeX dissertation template to support the production and typesetting of a PhD dissertation at Harvard, Princeton, and NYU**

The source files for this thesis, along with all the data files, have been organised into an R package, **xxx**, which is available at <https://github.com/xxx/xxx>. A hard copy of the thesis can be found in the University of Washington library.

This version of the thesis was generated on 2021-05-23 14:37:02. The repository is currently at this commit:

The computational environment that was used to generate this version is as follows:

```
- Session info -----
setting  value
version  R version 4.0.2 (2020-06-22)
os       macOS Catalina 10.15.6
```

```

system    x86_64, darwin17.0
ui         X11
language  (EN)
collate   en_US.UTF-8
ctype     en_US.UTF-8
tz        America/Los_Angeles
date      2021-05-23

```

- Packages -----

package	* version	date	lib	source
assertthat	0.2.1	2019-03-21	[1]	CRAN (R 4.0.0)
backports	1.1.6	2020-04-05	[1]	CRAN (R 4.0.0)
bookdown	0.22.3	2021-05-22	[1]	Github (rstudio/bookdown@aa75b5f)
callr	3.7.0	2021-04-20	[1]	CRAN (R 4.0.2)
cli	2.5.0	2021-04-26	[1]	CRAN (R 4.0.2)
colorspace	1.4-1	2019-03-18	[1]	CRAN (R 4.0.0)
crayon	1.3.4	2017-09-16	[1]	CRAN (R 4.0.0)
desc	1.2.0	2018-05-01	[1]	CRAN (R 4.0.0)
devtools	* 2.3.1	2020-07-21	[1]	CRAN (R 4.0.2)
digest	0.6.27	2020-10-24	[1]	CRAN (R 4.0.2)
dplyr	* 1.0.2	2020-08-18	[1]	CRAN (R 4.0.2)
ellipsis	0.3.0	2019-09-20	[1]	CRAN (R 4.0.0)
evaluate	0.14	2019-05-28	[1]	CRAN (R 4.0.0)
farver	2.0.3	2020-01-16	[1]	CRAN (R 4.0.0)
fs	1.5.0	2020-07-31	[1]	CRAN (R 4.0.2)
generics	0.0.2	2018-11-29	[1]	CRAN (R 4.0.0)
ggplot2	* 3.3.0	2020-03-05	[1]	CRAN (R 4.0.0)
git2r	0.27.1	2020-05-03	[1]	CRAN (R 4.0.2)

glue	1.4.2	2020-08-27	[1]	CRAN	(R 4.0.2)
gtable	0.3.0	2019-03-25	[1]	CRAN	(R 4.0.0)
highr	0.9	2021-04-16	[1]	CRAN	(R 4.0.2)
htmltools	0.5.1.1	2021-01-22	[1]	CRAN	(R 4.0.2)
httr	1.4.2	2020-07-20	[1]	CRAN	(R 4.0.2)
huskydown	* 0.0.5	2021-05-16	[1]	Github	(benmarwick/huskydown@addb48e)
kableExtra	1.3.4	2021-02-20	[1]	CRAN	(R 4.0.2)
knitr	1.33	2021-04-24	[1]	CRAN	(R 4.0.2)
labeling	0.3	2014-08-23	[1]	CRAN	(R 4.0.0)
lifecycle	0.2.0	2020-03-06	[1]	CRAN	(R 4.0.0)
magrittr	2.0.1	2020-11-17	[1]	CRAN	(R 4.0.2)
memoise	1.1.0	2017-04-21	[1]	CRAN	(R 4.0.2)
munsell	0.5.0	2018-06-12	[1]	CRAN	(R 4.0.0)
pillar	1.4.3	2019-12-20	[1]	CRAN	(R 4.0.0)
pkgbuild	1.0.6	2019-10-09	[1]	CRAN	(R 4.0.0)
pkgconfig	2.0.3	2019-09-22	[1]	CRAN	(R 4.0.0)
pkgload	1.0.2	2018-10-29	[1]	CRAN	(R 4.0.0)
png	0.1-7	2013-12-03	[1]	CRAN	(R 4.0.2)
prettyunits	1.1.1	2020-01-24	[1]	CRAN	(R 4.0.0)
processx	3.5.2	2021-04-30	[1]	CRAN	(R 4.0.2)
ps	1.6.0	2021-02-28	[1]	CRAN	(R 4.0.2)
purrr	0.3.4	2020-04-17	[1]	CRAN	(R 4.0.0)
R6	2.4.1	2019-11-12	[1]	CRAN	(R 4.0.0)
remotes	2.2.0	2020-07-21	[1]	CRAN	(R 4.0.2)
rlang	0.4.11	2021-04-30	[1]	CRAN	(R 4.0.2)
rmarkdown	2.8	2021-05-07	[1]	CRAN	(R 4.0.2)
rprojroot	1.3-2	2018-01-03	[1]	CRAN	(R 4.0.0)
rstudioapi	0.11	2020-02-07	[1]	CRAN	(R 4.0.0)

rvest	0.3.6	2020-07-25	[1]	CRAN	(R 4.0.2)
scales	1.1.0	2019-11-18	[1]	CRAN	(R 4.0.0)
sessioninfo	1.1.1	2018-11-05	[1]	CRAN	(R 4.0.2)
stringi	1.6.2	2021-05-17	[1]	CRAN	(R 4.0.2)
stringr	1.4.0	2019-02-10	[1]	CRAN	(R 4.0.0)
svglite	2.0.0	2021-02-20	[1]	CRAN	(R 4.0.2)
systemfonts	1.0.2	2021-05-11	[1]	CRAN	(R 4.0.2)
testthat	3.0.2	2021-02-14	[1]	CRAN	(R 4.0.2)
tibble	3.0.1	2020-04-20	[1]	CRAN	(R 4.0.0)
tidyselect	1.1.0	2020-05-11	[1]	CRAN	(R 4.0.0)
usethis	* 1.6.1	2020-04-29	[1]	CRAN	(R 4.0.2)
vctrs	0.3.4	2020-08-29	[1]	CRAN	(R 4.0.2)
viridisLite	0.3.0	2018-02-01	[1]	CRAN	(R 4.0.0)
webshot	0.5.2	2019-11-22	[1]	CRAN	(R 4.0.0)
withr	2.4.2	2021-04-18	[1]	CRAN	(R 4.0.2)
xfun	0.23	2021-05-15	[1]	CRAN	(R 4.0.2)
xml2	1.3.2	2020-04-23	[1]	CRAN	(R 4.0.2)
yaml	2.2.1	2020-02-01	[1]	CRAN	(R 4.0.0)

[1] /Library/Frameworks/R.framework/Versions/4.0/Resources/library

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