ABSTRACT

Patterns in Nutrient Enrichment and Decomposition of Grass Litter in Kenai Peninsula Headwater Streams, Alaska

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The percent cover of alder in catchments drives dissolved inorganic nitrogen availability in southcentral Alaska headwater streams. I manipulated the availability of N and P to leaf packs of bluejoint grass litter using nutrient diffusion samplers (NDS) to experimentally test for nutrient limitation of litter breakdown, nutrient content, and bacterial biomass in three headwater streams differing in the percent cover of alder in their catchments. I found that litter breakdown rates, nutrient content, and bacterial biomass significantly increased in response to nitrogen treatments, and to a lesser degree, phosphorus treatments in the stream lacking alder in its catchment. Streams in catchments with even a low cover percentage of alder did not exhibit nitrogen limitation but exhibited phosphorus limitation even though all the streams in this study had relatively high phosphate levels.

Patterns in Nutrient Enrichment and Decomposition of Grass Litter in Kenai Peninsula Headwater Streams, Alaska

by

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A Thesis

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# CHAPTER ONE

## Introduction

Allochthonous sources provide the primary energy input to most headwater streams (Fisher and Likens 1973; Vannote et al. 1980). Decomposition of plant litter and the concomitant assimilation of detrital energy are driven by microbial communities (Suberkropp and Klug 1976) and macroinvertebrate shredders (Anderson and Sedell 1979; Hieber and Gessner 2002). Availability of nutrients in the water column and the nutrient content of leaf litter strongly influences decomposition rates (Grattan II and Suberkropp 2001; Greenwood et al. 2006). The establishment of microbial communities on the leaf litter increases the nutrient content of leaf litter. This interaction is an important trophic link in small headwater streams (Wallace et al. 1997).

Plant litter tends to be nutrient poor, particularly in areas where the bulk of litter comes from autumn leaves whose nutrients are reabsorbed by the parent plant before senescence (Hoch et al. 2003). Microbes, crucial to the decomposition process, have higher nitrogen (N) and phosphorus (P) content than the leaf litter they colonize and require an additional source of nutrients (Gulis et al. 2006b; Romaní et al. 2006). Given the nutritional deficiency found in most leaf litter, microbes have the ability to utilize nutrients in the water column to overcome the limited nutritional value in leaf litter (Greenwood et al. 2006; Gulis and Suberkropp 2003a; Webster et al. 2009). Numerous studies have found that increasing the availability of N or P in the water column can increase the decomposition rate and increase the biomass of microbes on leaf litter (Greenwood et al. 2006; Gulis and Suberkropp 2003b; Gulis et al. 2006a; Rosemond et

al. 2008). Decomposition of allochthonous materials also depends on variables aside from the nutrient value of the water column and leaf litter, such as the percent lignin in leaf matter.

Bluejoint grass (*Calamagrostis canadensis*) is an important source of terrestrial organic matter for small headwater streams on the Kenai Peninsula in Alaska. Grass litter is often viewed as unimportant in aquatic food webs because it is low in nutrients and high in lignin, making it difficult for microbes to degrade (Menninger and Palmer 2007; Webster and Benfield 1986; Gessner et al. 2007). However, Dekar et al. (2012) found that salmonid diets were primarily composed of nutrients originating from allochthonous sources (i.e. bluejoint grass). In addition, alder (*Alunus spp.*) cover in headwater stream catchments is a major controller of N availability in the region (Compton et al., 2003; Shaftel et al. 2010).

Alder roots are host to nitrogen-fixing bacteria. Recent studies of headwater streams in the Kenai Peninsula (Shaftel et al. 2011) showed that bluejoint grass litter decomposed faster and had higher N and P content in streams enriched in nitrate-N by alder. While the increases in nitrate-N due to alder may be small in watersheds with little alder coverage, even minimal increases in nitrate-N content have been shown to meet microbial demand (Ferreira et al. 2006). Understanding the mechanisms behind the energy transfer from decomposing grass litter to juvenile salmonids in these systems is an important step towards developing better management practices for economically important salmonid species.

A recent study on the Kenai Peninsula (Shaftel et al. 2011) analyzed at decomposition of bluejoint grass litter in headwater streams across an alder gradient. The

results of this study indicated that bluejoint grass litter decomposed at a faster rate and had higher N and P content in streams with high percentages of alder when compared to streams with a low percentage of alder in the catchment. This lead the authors to the conclusion that alder is a driving force in the breakdown rate of bluejoint grass litter in headwater streams of the Kenai Peninsula.

In order to confirm the observational results found by Shaftel et al. (2011) that indicated the importance of alder to stream decomposition processes on the Kenai Peninsula I chose to do a nutrient enrichment experiment. To do this I utilized NDS as a way to manipulate stream nutrient content on individual packs of grass leaf litter as a way to determine if nutrient availability limits the breakdown of allochthonous materials in my study streams. Examining the effect of increased nutrient levels on leaf decomposition provides insight into the complexities of nutrient limitation in headwater streams. I also examined the influence of nutrient enrichment on bacterial biomass. Specifically, the study was designed to determine whether (1) nitrogen or phosphorus were limiting nutrients in these headwater streams, (2) if nutrient enrichment alters breakdown rates of bluejoint grass litter, and (3) if bacterial biomass increased due to nutrient treatments and length of deployment time.

I predicted that nitrogen would not be limiting in streams with alder cover present in their catchment, but would be limiting in streams with no alder cover. Phosphorus would not be limiting in any of the streams due to the naturally high levels of phosphate in the streams. In streams with alder cover in their catchment there would be no effect of nutrient addition on breakdown rate, but breakdown rate would increase significantly in all nitrogen nutrient treatments in the stream with no alder cover. Finally, I predicted

bacterial biomass would not be affected by the nutrient treatments in alder cover streams, but would increase over time with nitrogen treatments in the no alder streams.

# CHAPTER TWO

## Methods

### *Study Area*

This study took place in headwater streams that are part of a comprehensive monitoring and evaluation project of headwater streams on the Kenai Peninsula. The geomorphic setting, climate, vegetation and environmental factors of the Kenai Peninsula are described in detail in Shaftel et al. (2011), Walker et al. (2012), and Dekar et al. (2012).

For this project, three stream catchments were selected on the Kenai Peninsula (Figure 1). Two of these streams, Anchor 1203 (ANC-1203) and Anchor 5 (ANC-5), were previously used in the grass litter breakdown study done by Shaftel et al. (2011) while the final site, Stariski 171 (STAR-171), was not. These streams are first order tributaries of major rivers of the region. ANC-1203 and ANC-5 are tributaries to the Anchor River while STAR-171 is a tributary to the Stariski River. These catchments were selected to represent a gradient of alder cover: high alder (ANC-5, 26.67%), low alder (ANC-1203, 10.47%), and no alder (STAR-171, 0%), and because grass litter breakdown was estimated at two of these sites by Shaftel et al. (2011). The addition of STAR-171 to the selected sites allowed the examination of breakdown processes in a stream without any alder derived inputs, an aspect that the Shaftel et al. (2011) study lacked. Catchment area, stream discharge, and velocity among streams were similar (Table 1). All three streams had the same predominant substrate types, but in varying

proportions. The high alder site had a sand/gravel substrate with limited cobble and fine organic matter. The low alder site had predominantly cobble substrate with sand and fine organic matter in depositional areas. The no alder site had a cobble and gravel riffle habitat with fine organic matter in the pools.

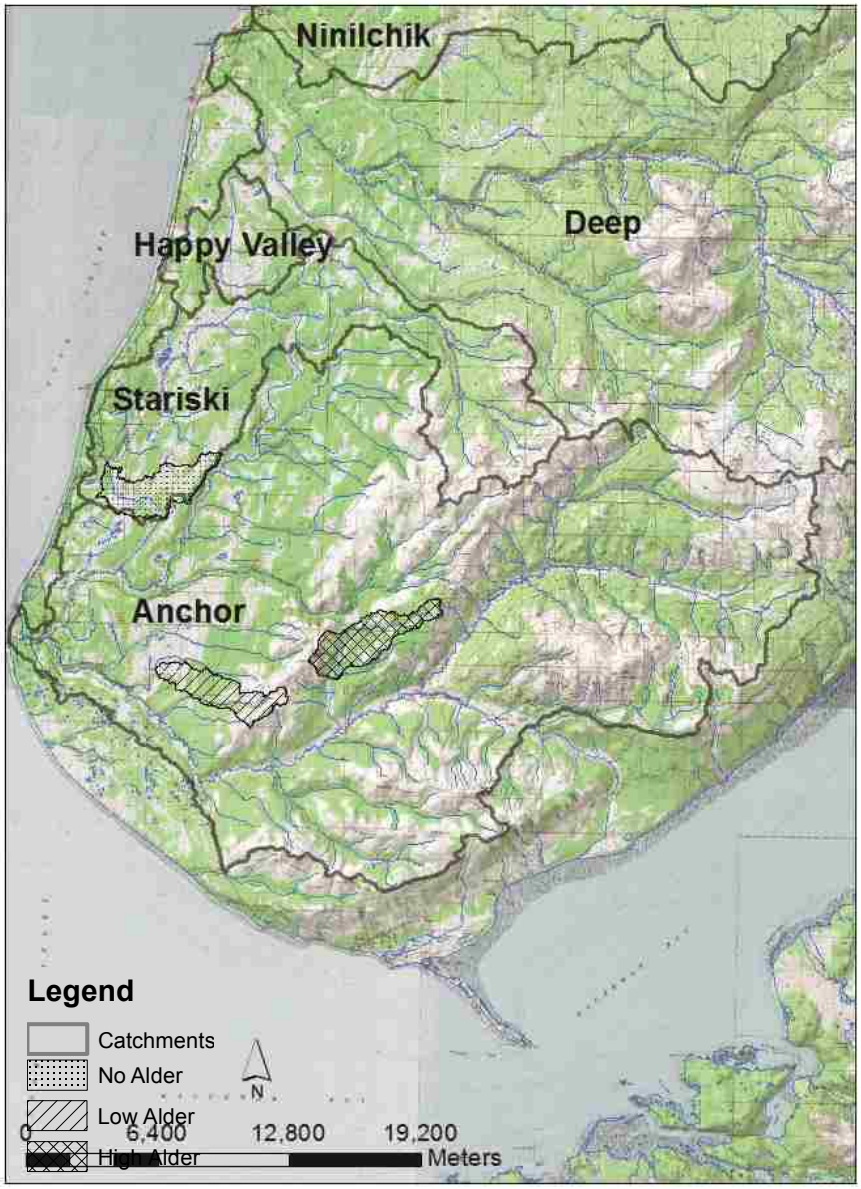


Figure 1. Map of the Kenai Peninsula of Alaska. The three headwater stream catchments are highlighted.

Table 1. Stream water chemistry and watershed characteristics for 3 headwater streams in the Kenai Peninsula, Alaska. Stream water chemistry (n=7) and physical variables (n=4) are means (SE) for samples taken over the course of the leaf decomposition experiment. $NO_x$-N = $NO_3$ + $NO_2$, TN = total N, TP = total P, DOC = dissolved organic C, DIN = dissolved inorganic N, DON = dissolved organic nitrogen, TDN = total dissolved nitrogen.

| Stream number | 5 | 1203 | 171 |
|---|---|---|---|
| Watershed | Anchor | Anchor | Stariski |
| Location (lat $^{o}$N, long $^{o}$W) | 151.42, 59.45 | 59.78, 151.55 | 59.84, 151.78 |
| Catchment area ($km^2$) | 7.9 | 3.3 | 4.1 |
| Percent alder cover | 26.67 | 10.47 | 0 |
| Discharge ($m^3$/s) | 0.03 *(0.003)* | 0.02 *(0.003)* | 0.03 *(0.005)* |
| Temperature ($^{o}$C) | 5.5 *(0.02)* | 8.8 *(0.02)* | 10.6 *(0.02)* |
| Degree Days | 521 | 495 | 756 |
| Conductivity (µS/cm) | 65.6 *(1.6)* | 64.8 *(2.3)* | 88.7 *(4.2)* |
| Dissolved Oxygen (%) | 108.6 *(4.2)* | 106.1 *(4.0)* | 92.8 *(3.8)* |
| pH | 7.3 *(0.1)* | 7.5 *(0.1)* | 6.8 *(0.1)* |
| $PO_4$-P (µg/L) | 29.5 *(2.8)* | 53.4 *(4.7)* | 27.8 *(1.9)* |
| TP (µg/L) | 35.7 *(3.9)* | 65.4 *(7.2)* | 86.2 *(16.8)* |
| DOC (mg/L) | 4.2 *(0.2)* | 3.2 *(0.6)* | 9.1 *(0.3)* |
| $NH_4$-N (µg/L) | 3.6 *(0.6)* | 4.5 *(1.5)* | 3 *(0)* |
| $NO_x$-N (µg/L) | 1402.8 *(72.3)* | 393.6 *(24.8)* | 6.2 *(2.5)* |
| TDN (µg/L) | 1466 *(76.7)* | 477.4 *(16.1)* | 232 *(27.3)* |
| TN (µg/L) | 1528 *(82.6)* | 536.9 *(20.4)* | 281.2 *(43.6)* |
| DON (µg/L) | 62.7 *(17.1)* | 79.3 *(20.3)* | 222.8 *(28.8)* |
| DIN (µg/L) | 1406.3 *(72.5)* | 398.0 *(24.5)* | 9.2 *(2.5)* |

### *Water Chemistry*

Water nutrient samples were taken in duplicate upon deployment and at each retrieval date along with instantaneous pH, conductivity, temperature, and dissolved oxygen measured with a YSI 556 Multiprobe System (Yellow Springs Instruments, Yellow Springs, Ohio). Nutrient samples were immediately placed in a cooler and kept on ice for transport to the lab. Samples were analyzed using Standard Methods for Water and Wastewater (American Public Health Association 2005) for total nitrogen (TN – persulfate digestion), ammonium (modified berthiolate process), nitrate and nitrite (cadmium reduction), total phosphorus (TP - persulfate digestion), and soluble reactive phosphorus (SRP; ascorbic acid two reagent method) on a flow-injection auto-analyzer (Lachat QuikChem 8500 and Series 520 XYZ Autosampler). Samples for dissolved organic carbon (DOC) were run on a Shimadzu TOC 5-5- analyzer.

### *Grass Packs*

Bluejoint grass litter was collected from standing dead tussocks at the no alder site at the end of May 2011. Litter was stored in the open air for less than 5 days and then separated into leaf and stem groups. The air-dried grass was weighed into leaf packs consisting of 2.5 g of grass leaf and 2.5 g of grass stem (5 g dry weight, Shaftel et al. 2011). Before handling the brittle leaf packs again, they were moistened in deionized water to reduce handling loss by submerging them in DI water for 5 minutes. Leaf packs were attached to NDS by zip tying the bundles of grass litter evenly around the diffuser end of the NDS (15.2 cm long 1.27 cm PVC pipe with 40 holes drilled through it; Figure 2). At each sampling site, diffusion tubes were attached to nutrient reservoirs filled with tap water enriched with one of four nutrient treatments: 5M nitrogen (N), 1M phosphorus

(P), 5M nitrogen and 1M phosphorus (NP), or a blank containing stream water (control, C). Sodium nitrate was used to make the 5M nitrogen solution. Sodium phosphate was used to make the 1M phosphorus solution. Nutrient solutions were kept in nutrient reservoirs by a glass fritted disk with 10-15 μm porosity held in place at the end of the PVC nutrient reservoir tube with silicone.

NDS were deployed in the stream by securing them to a 1 m by 1 m square of metal fencing (racks) held to the bottom of the stream by rocks and rebar. Breakdown

Figure 2. Photograph of the nutrient diffusion system constructed for this study with grass leaves attached on the left and just the diffusion tube on the right, the longer PVC pipe acted as the reservoir for the nutrient enriched water.

rates at the study sites were hypothesized to be influenced more by nitrogen limitation than phosphorous limitation, especially at no alder sites (Shaftel et al. 2011). Therefore, control and P treatments were placed upstream of N and NP treatments to prevent any influence of N on control and P treatments. Five racks of leaf packs were deployed at STAR-171 on May 25, ANC-5 on May 26, and ANC-1203 on May 27, 2011. Individual racks were placed at the transition from riffle to pool at a depth of 10-30 cm and velocities ranging from 0.2-0.4 m/s following Shaftel et al. (2011). Racks were separated by a minimum of 10 m within a 100 m longitudinal segment of each stream. Each rack contained 12 leaf packs (4 nutrient treatments x 3 replicates), for a total of 60 leaf packs

per stream (Figure 3) with replicates within racks intended for three different retrieval dates.

Two racks at each site were equipped with a Hobo Temp Logger set to record temperature at 15 minute intervals. Six leaf packs were deployed and immediately retrieved to account for handling loss on the first day of deployment. Oven dried mass(65$^{o}$C, 48 hours) of the six handling loss leaf packs was averaged and subtracted from the starting weight to account for handling loss on all leaf packs. Leaf packs were retrieved 26, 53, and 70 days after deployment.

Nutrient stock solutions (5M nitrogen, 1M phosphorus, and 5M nitrogen + 1M phosphorus) were replaced in each respective NDS that were not being retrieved on days 26 and 53. Nutrient diffusion from NDS systems was tested in lab experiments. The lab experiments found that nutrients were continuing to diffuse at a steady rate after 14 days of deployment in 5$^{o}$C water (pers. comm. R.S. King). Each diffusion unit, with litter attached, was removed from the nutrient reservoir upon retrieval, placed in a quart Ziploc® bag, and put on ice for transport back to the laboratory. Leaf packs were rinsed through two sieves, 1 mm and 250 μm, to remove debris and macroinvertebrates in the laboratory. Leaf material left in the 1 mm sieve was oven dried at 65$^{o}$C for at least 48 hours and weighed for oven dried mass. The oven-dried material was ground using a coffee grinder, then sub-sampled for further homogenization in a Mini Beadbeater™. After homogenization was completed, the material was sub-sampled again, placed in aluminum trays, dried for at least 24 hours at 65$^{o}$C, placed in a desiccator to cool, and weighed for initial dry mass. The sub-samples were then ignited at 550$^{o}$C for 1 hour and weighed again to determine ash free dry mass (AFDM). AFDM removes inorganic

material from the weight of the leaf pack leaving only the weight of organic material, so any sand or silt that was not washed from the leaves does not factor into the calculation of breakdown rates. AFDM % was calculated by subtracting the AFDM from the initial dry mass and then dividing by initial dry mass. The AFDM % was multiplied by the total final dry mass of each sample to find the AFDM weight. Another sub-sample (5-10 mg) of leaf material was weighed into tin capsules to determine carbon (% C) and nitrogen (% N) content using a ThermoQuest Flash EATM 1112 elemental analyzer. An additional sub-sample (8-10 mg) of litter was digested and analyzed for total phosphorus (% P) using a Lachat QuikChem 8500 flow-injection autoanalyzer using the molybdate colorimetric method.

*Bacterial Biomass*

Bacterial biomass was determined by the direct count method using DAPI (4',6-diamidino-2-phenylindole) as a fluorescent stain. DAPI binds to bacterial DNA and fluoresces under UV light. Porter and Feig (1980) compared DAPI and acridine orange staining methods and found the use of DAPI to improve visualization and counting of <1µm bacteria. Immediately after leaf packs were retrieved two 12 mm sections of grass leaf and two 12 mm sections of grass stem were placed in a plastic vial containing 5ml of bacteria free 10 % buffered formalin. Samples were stored at 2$^{o}$C. DAPI staining was done following the protocol outlined in Weyers and Suberkropp (1996). The combined grass sections were sonicated using a 550 Fisher Scientific sonic dismembrator at 30 – 40 watts at two-minute intervals for a total of four minutes to remove bacteria from the substrate. Between sonication times, samples were kept on ice. After vortexing for 20 seconds, 0.5 ml aliquots of sample were taken and placed into a vacuum filtering

manifold fitted with black polycarbonate filters (0.2 µm pore size) on top of a membrane backing filter (0.45 µm pore size) for staining. All staining and filtering was done under red light to minimize loss of fluorescence. 20 µl of 0.1 mg/ml DAPI solution was added to the solution in the vacuum filtering manifold and filtered through after a one-hour incubation period. The solution was filtered until dry at a vacuum pressure not exceeding 160 mm Hg. The filter was then placed on a microscope slide kept in the dark for

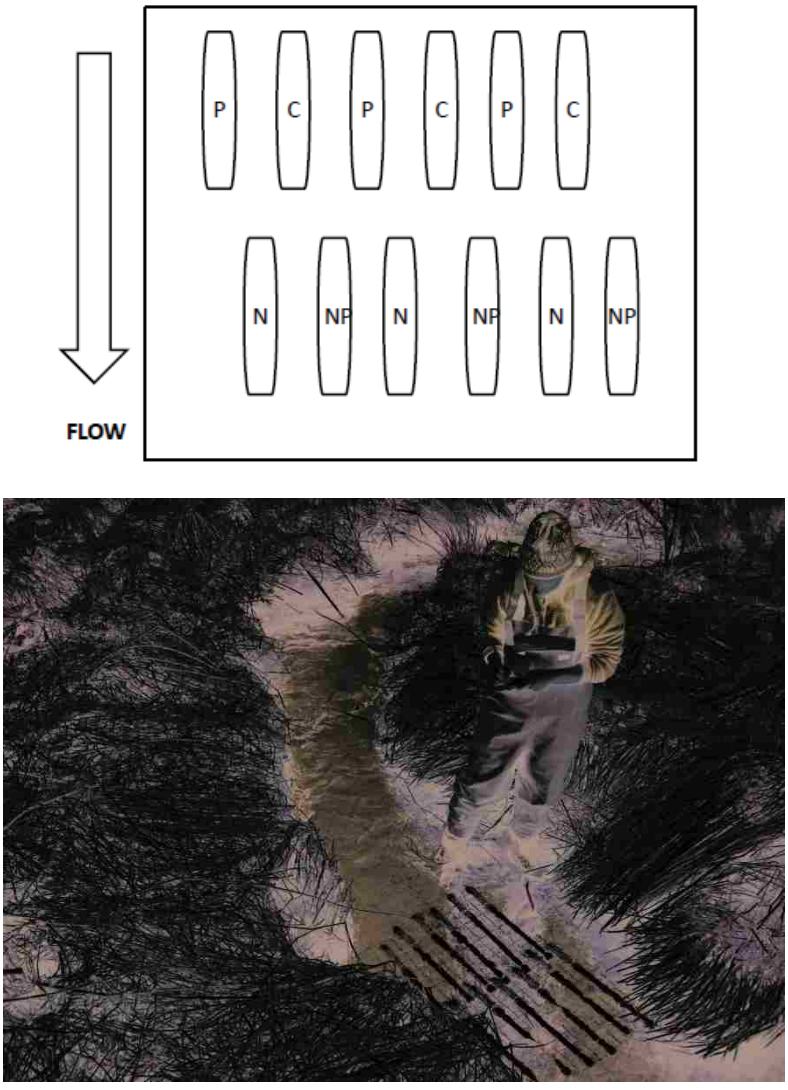


Figure 3. Diagram of the placement of NDS on the 1 m by 1 m wire fence used to deploy leaf packs in the streams and photograph of a rack deployed at stream 1203.

enumeration on an epifluorescence microscope using 1000X magnification. Bacterial cells were counted until ≥ 500 cells filter$^{-1}$ were counted or a maximum of 10 microscopic fields was reached using Image Pro Plus v.3. Bacteria were also measured for volume calculations. To calculate cell volume from length and width measurements the formula $V = (\pi/4)\ W^2\ (L\text{-}W/3)$ was used (Weyers and Suberkropp, 1996). Cell volume was converted to biomass using a conversion factor of 0.35g C/cm$^3$ (Bratbak 1993).

*Data Analysis*

An exponential decay equation, $M_t = [M_i]e^{(-kt)}$ , where Mt is the mass at time t, $M_i$ is the initial mass, and k is the exponential decay coefficient, was used to estimate breakdown rate following Benfield (2006). Breakdown rates were estimated from leaf packs collected from the same rack over time using initial handling-corrected AFDM (day 0) and AFDM estimates from days 26, 53, and 70. Rates were expressed per degree day following Shaftel et al. (2011). Degree day was calculated as the sum of the average daily temperature in each stream over the course of the 70 day deployment period (Benfield 2006).

Analysis of Variance (ANOVA) was used to analyze breakdown rate, C:P, C:N, and bacterial biomass following Zuur et al. (2009) using the R 2.15.1 statistical package (R Development Core Team 2012). The effect of nutrient treatments on each of the four response variables was estimated within each of the three streams separately (high alder, low alder, and no alder) because (1) hypotheses were limited to the experimental effects within streams relative to the controls and (2) stream types (high, low, and no alder) were unreplicated, thus statistical comparisons among sites were not meaningful. Breakdown

rate (*k*) was analyzed using a one-way ANOVA with nutrient as a fixed effect (4 levels: C, N, P, NP). Litter carbon to nitrogen (C:N) and phosphorus (C:P) ratios and bacterial biomass were analyzed using a nested repeated measures ANOVA with nutrient as a fixed effect nested within date (Zuur et al. 2009). Mean comparisons between the control and each level of nutrient were estimated using a posteriori contrasts when nutrient (*k*) or nutrient within day (C:N, C:P, bacterial biomass) were deemed significant by ANOVA. The p-value was set at a significance level of 0.05 for all tests.

# CHAPTER THREE

## Results

### *Water Chemistry*

Dissolved inorganic nitrogen (DIN) values remained relatively constant in all three streams throughout the study period (Figure 4). The high alder site had the highest mean DIN (1406 µg/L) while the no alder site had the lowest mean DIN (9 µg/L). Phosphate-P ($PO_4$-P) concentrations averaged 27.8 µg/L to 53 µg/L among the three streams over the 70-day study period (Table 1). $PO_4$-P levels showed a downward trend after 34 days in the high and no alder streams, but remained much higher at the low alder stream (Figure 4).

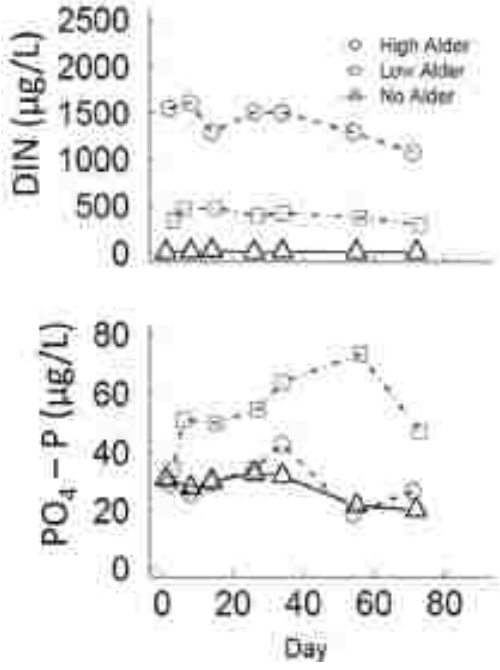


Figure 4. Water chemistry variables from the three headwater streams (n=7). High alder = ANC-5, Low alder = ANC-1203, No alder = STAR-171.

Mean stream temperatures ranged between 5.5 and 10.6$^{o}$C across the three sites (Table 1). The low alder site had the least number of degree days (495) while the no alder site had the largest number of degree days (756). Dissolved organic carbon (DOC) for the high and low alder sites ranged between 2 and 5 mg/L. DOC was highest at the no alder site with a mean of 9 mg/L. Stream discharge was similar between all three streams, with mean discharge values ranging from 0.02 to 0.04 $m^3/s$.

*Grass Litter Breakdown*

Nutrient treatments significantly affected breakdown rates at both the high and no alder sites but not in the low alder site (Figure 5). The P and NP treatments increased breakdown rates at the high alder site (ANOVA, $F_{3, 16}$=5.783 P = 0.0071 ). Litter breakdown at the low alder site trended toward higher rates in response to N, P, and NP treatments, but was not different from the control treatment (ANOVA, $F_{3,16}$ = 1.933, P = 0.165). All three nutrient treatments increased breakdown rates at the no alder site in comparison to the control (ANOVA, $F_{3,16}$ =5.167, P= 0.0109; Figure 5).

*Nutrient Ratios*

Litter C:N ratios responded differently to nutrient treatments among sites (Figure 6). C:N ratios at the high alder site did not significantly differ between the four treatment types (ANOVA, $F_{9,47}$ = 1.552, P = 0.158; Figure 6), however the C:N ratios did significantly decrease over the 70 day deployment period compared to the initial C:N (ANOVA, $F_{2,47}$ = 20.027,P ≤ 0.0001). Conversely, C:P ratios at the high alder site were significantly lower than the control treatment in both the P and NP treatments on day 53 and 70 (ANOVA, $F_{9,47}$ = 4.837, P = 0.0001; Figure 7). The high alder site also showed a

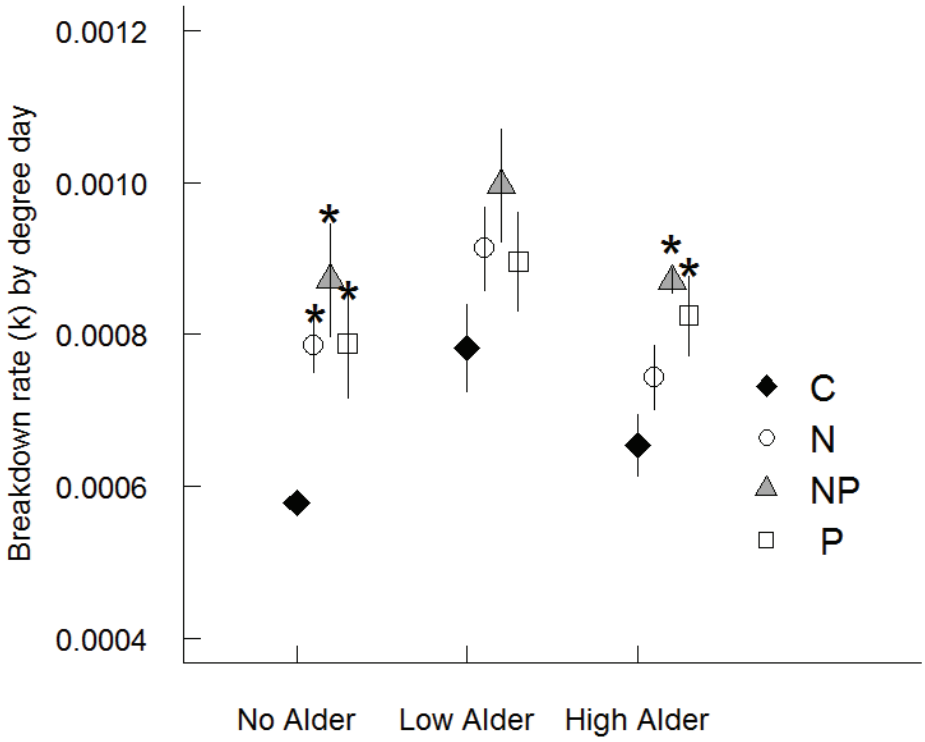


Figure 5. Mean breakdown rate for each nutrient treatment over the course of the 70 day deployment period (± 1 SE, n= 5). C = control, N= nitrogen , NP = nitrogen and phosphorus, P = phosphorus. * indicates significantly different from the control.

significant decrease in C:P ratio over the 70 day deployment period compared to initial leaf C:P ratio (ANOVA, $F_{2,47} = 5.022$, P = 0.0106). C:N ratios at the low alder site did not differ among nutrient treatments (ANOVA, $F_{9,46} = 1.538$, P = 0.163), or over the 70 day deployment period (ANOVA, $F_{2,46} = 2.046$, P = 0.141). At the low alder site C:P ratios differed between the control treatment and both the NP and P nutrient treatments on day 53 and 70 (ANOVA, $F_{9,46} = 2.736$, P = 0.0120; Figure 7). The low alder site C:P ratios also showed a significant decline over the 70 day deployment period, regardless of nutrient treatment (ANOVA, $F_{2,46} = 4.583$, P = 0.0153).

Both C:N and C:P ratios significantly responded to nutrient treatments at the no alder site. Litter C:N ratios were significantly lower for N and NP treatments compared to the control treatment on day 53; by day 70 C:N ratios were lower for all three nutrient

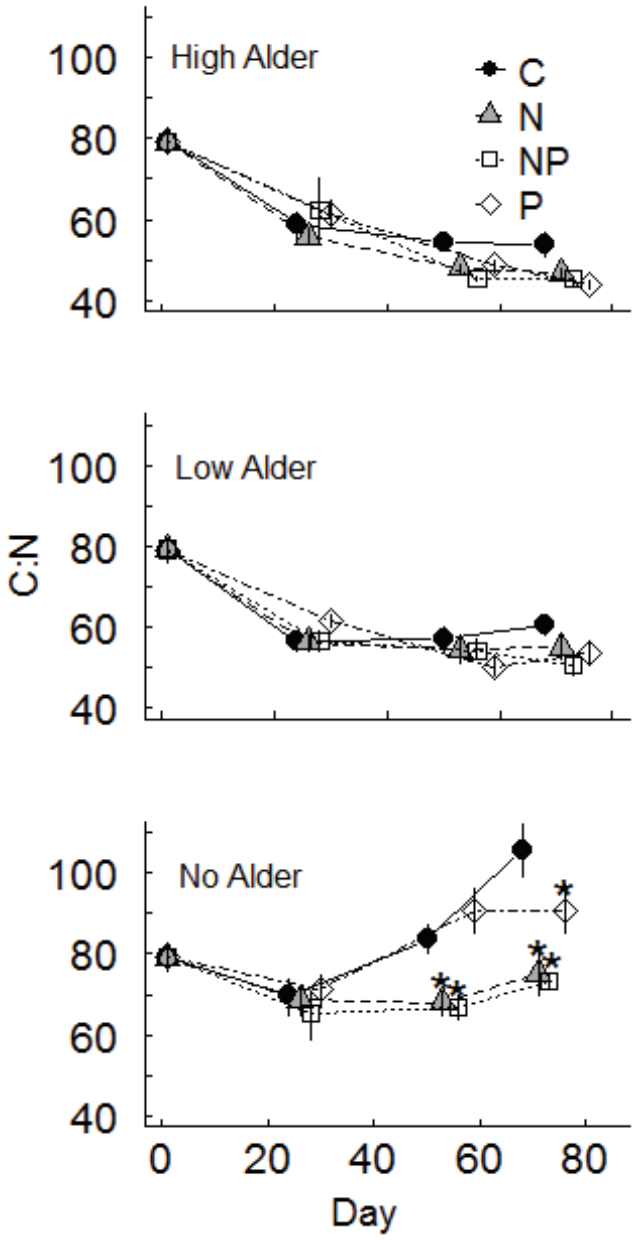


Figure 6. Mean molar ratio of carbon to nitrogen found in leaf material at time of retrieval (±1 SE, n=5) in each of the four treatment types. * indicates significantly different from control.

treatments compared to the control treatment (ANOVA, $F_{9,47} = 5.977$, $P \leq 0.0001$; Figure 6). Litter C:P ratios also were significantly lower compared to the control treatment for the P and NP treatments on days 53 and 70 at the no alder site (ANOVA, $F_{9,47} = 9.15$, $P \leq 0.0001$; Figure 7). Contrary to patterns

at the high and low alder streams, C:N and C:P ratios for control treatments significantly increased over time (C:N ANOVA, $F_{2,47} = 13.947$, $P \leq 0.0001$; C:P ANOVA, $F_{2,47} = 12.25$, $P \leq 0.0001$).

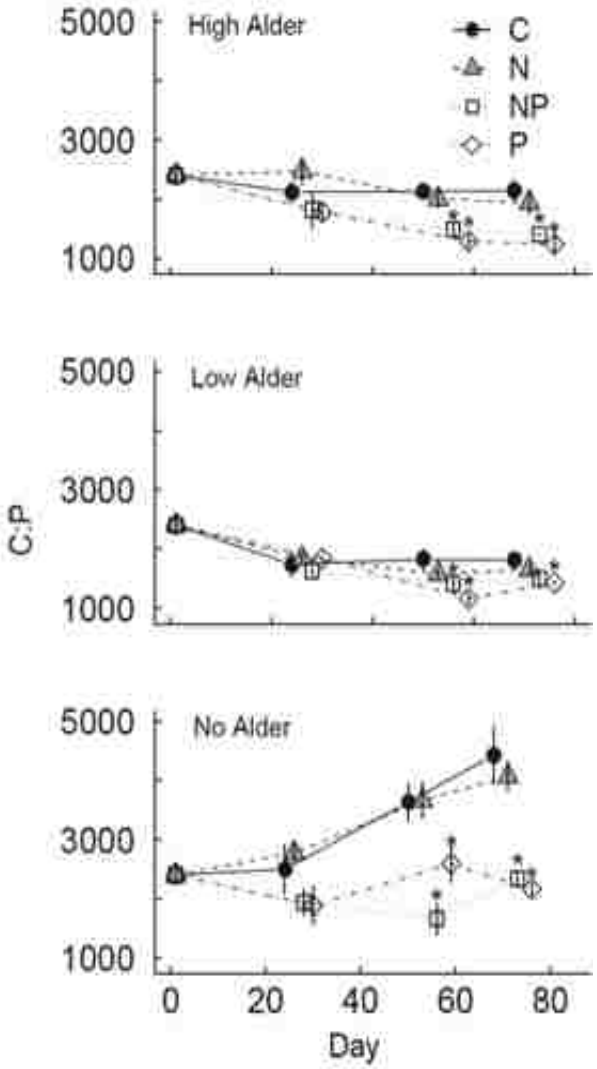


Figure 7. Mean molar ratio of carbon to phosphorus found in leaf material at time of retrieval (±1 SE, n=5) in each of the four treatment types. * indicates significantly different from control

*Bacterial Biomass*

Bacterial biomass did not significantly differ between day 26 and day 70 for any of the nutrient treatments compared to the control treatment at both high and low alder sites (Figure 8). At the no alder site, day 70 showed a significant increase in bacterial

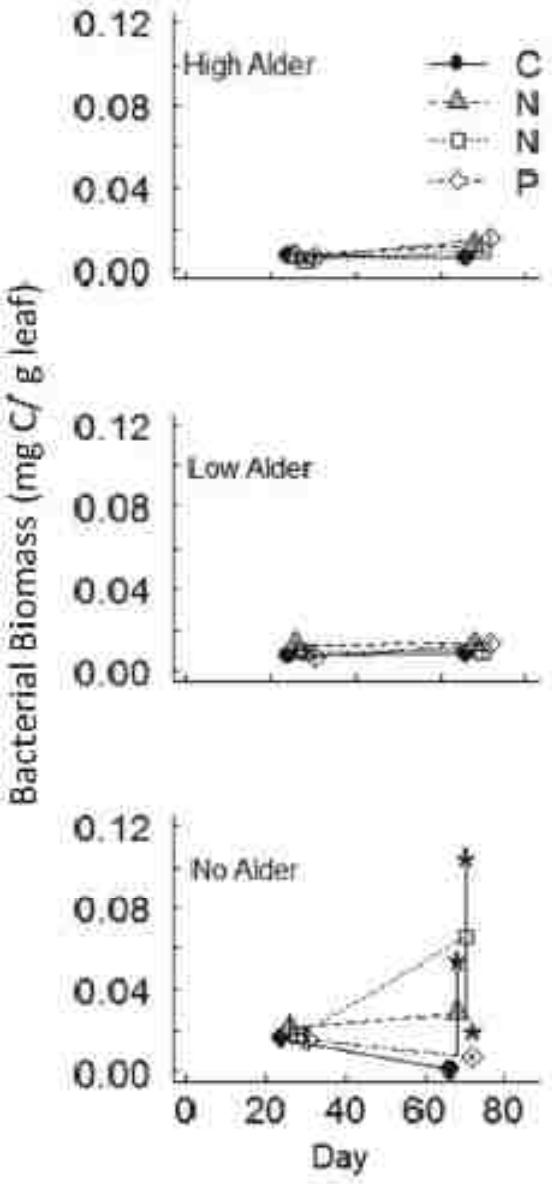


Figure 8. Mean bacterial biomass per gram of leaf material at time of retrieval (±1 SE, n=5) in each of the four treatment types at day 28 and day 70. * indicates significantly different from control.

biomass compared to day 26 , caused by the increase of bacterial biomass on N and NP nutrient treatments (ANOVA, $F_{1,30}$, P = 0.0117). All three treatments (N, NP, P) at the no alder site had significantly higher bacterial biomass compared the control treatment by day 70 (ANOVA, $F_{6,30}$, P = 0.0061).

# CHAPTER FOUR

## Discussion

Controlled nutrient enrichments of aquatic systems can provide important experimental evidence about the complex nutrient demands and limitations within decomposition processes of stream systems. Data from the present study indicate that alder derived N inputs to stream systems on the Kenai Peninsula drive breakdown rates and nutrient quality in leaf litter. In addition, this study provides evidence that nitrogen is not limiting in catchments where alder is present but is limiting in catchments with no alder cover, directly supporting my hypotheses. The results of this study strengthen our understanding of the importance of alder to headwater streams and juvenile salmon habitat in Alaska, and corroborate previous observational studies (Shaftel et al. 2011) with experimental data.

### *Grass Litter Breakdown*

Previous nutrient enrichment studies have shown mixed results, with some studies indicating no change in breakdown rate and nutrient quality of litter when enriched (Royer and Minshall 2001) while other studies showed significant increases in breakdown rate and/or nutrient quality (Abelho and Graça 2006; Greenwood et al. 2006; Robinson and Gessner 2000). In the present study, the effects of nutrient enrichment on leaf litter breakdown varied with alder cover. Breakdown rates at both high and low alder sites behaved as predicted. The breakdown rates of nitrogen treatments did not

differ significantly from the control due to alder driven high nitrate-N levels found in the catchments containing alder. At the no alder site, however, all three treatments had significantly increased breakdown rates with respect to the control, indicating N and P limitation within the no alder catchment as hypothesized.

### *Nutrient Ratios*

C:N ratio did not differ due to nutrient treatments at the high and low alder sites, but did differ significantly at the no alder site when treated with nitrogen providing further support that alder derived nitrate-N plays a key role in the decomposition process on the Kenai Peninsula. The C:P ratio at the high and low alder sites showed a significant decrease over time, indicating no P limitation. As the experiment progressed, however, P and NP treatment C:P ratios were significantly lower than the control. This would indicate slight P limitation. The limitation may be due to the high nitrate-N levels driving demand for phosphorus beyond what the naturally high concentrations in the two alder cover streams could support. Alternatively, this pattern may be an artifact of the nutrient enrichment design. The nutrient diffusion system may increase the availability of nutrients to grass litter in deep layers of the leaf pack where microbes would otherwise have limited contact with the water column and therefore be limited by nutrient availability. The no alder site also showed the only increase in sample C:P and C:N ratio in the control and N or P treatment respectively, indicating nutrient limitation in both N and P at the site. While our assumptions of uniformly high levels of phosphorous are supported by water nutrient levels, the leaf pack data from the no alder site indicates that the water column phosphorous did not meet demand for decomposing leaf litter. The no alder system has a higher percentage of wetland in the catchment, resulting in a different

dominant substrate type (mud-silt compared to sand-cobble) than the other sites. P limitation at the no alder site could potentially be attributed to the binding of phosphates to the sediment since DO levels were always high (Correll 1999)

### *Bacterial Biomass*

The differences between alder and no alder sites are further accentuated by bacterial biomass data. There were no significant increases in bacterial biomass over time or due to nutrient enrichment at both the high and low alder sites. These sites had fewer degree days, around 500 compared to around 700 degree days at the no alder site. Colder stream temperatures decrease metabolic rates of bacteria, slowing down the reproductive process and the rate at which bacterial biomass accumulates. The only significant difference in bacterial biomass by day and nutrient treatment was at the no alder site. The increased availability of nutrients around the leaf packs at the no alder site spurred bacterial growth to levels otherwise unachievable in the nutrient poor system. The bacteria clearly exhibited both N and P limitation increasing significantly in N, NP, and P treatments compared to the control treatment. The higher NP bacterial biomass compared to all other treatment types indicates dual nutrient limitation at the no alder site. The no alder site also had more than double the amount of DOC in the water column compared to both the high and low alder sites. The high availability of DOC at the no alder site may be able to support a greater bacterial biomass than the high and low alder sites where DOC levels were relatively lower.

### *Conclusion*

In this study, catchments containing at least 10% alder cover appear to have adequate nutrient levels within their stream systems. Catchments with no alder cover

were nutrient limited in both N and P. Phosphate limitation was observed in this study, but further exploration is needed to shed light onto why phosphorus limitation is appearing in streams with naturally high phosphate levels. Expansion of this study to include replicates of alder cover would improve generalizations about nutrient conditions in headwater stream catchments. Dekar et al. (2012) traced the energy pathway from grass to juvenile salmonids, but in headwater streams with no alder cover, that pathway could be hampered due to nutrient limitation. Further experimental nutrient manipulations in headwater streams may help to refine our understanding of the relationships between alder cover, nutrient dynamics, and habitat of juvenile salmonids of the Kenai Peninsula.

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