**Methods**

Study Area

This study took place in the Eastern Cascades in Washington State. Summers (May-September) are relatively dry, with seasonal drought and temperatures ranging from 15°C-25°C, and winters (October-April) are wet with temperatures ranging from -5°C-11°C. The average precipitation for the area is 720 mm (Northwest River Forecast Center, NOAA) with most falling during the winter and spring months. Because of the distinct seasonal patterns, eastern Cascades forests are characterized by a mix of Douglas fir (*Pseudotsuga menziesii)*, grand fir (*Abies grandis)*, ponderosa pine (*Pinus ponderosa*), western larch (*Larix occidentalis*) and at higher elevations, lodgepole pine (*Pinus contorta*).

I used a nested study design with repeated sampling through time to investigate how budworm herbivory influenced throughfall composition, litter decomposition, and soil nutrient dynamics. Within each budworm herbivory level (low versus high), I established study sites along 4 different streams (n=8). At each stream I established three replicate plots approximately 15 m from each other from upstream to downstream. At each replicate plot, I measured throughfall water chemistry, litter decomposition rates, and soil chemistry 8 times between early September 2015 and early November 2016. Frass collectors were also deployed at each site to measure budworm activity over the course of the study.

The low budworm sites for this study were located in the Teanaway Community Forest in Washington State, approximately 40 miles north east of Central Washington University on public land. These sites were located near creeks called: Stand Up Creek (903 m above sea level) where sites where on a slope with light tree cover, Jungle Creek (824 meters above sea level) where sites were often disturbed by free range cattle, Jack Creek (963 meters above sea level) where sites were under moderately heavy tree cover, and Moonbeam Creek (973 meters above sea level) where sites were also under moderately heavy tree cover. The high budworm sites were located in the Swauk drainage in the Okanogan-Wenatchee National Forest in Washington State approximately 45 miles north of Central Washington University and east of the low budworm sites, also on public land. The creeks near these sites were called: Cougar Creek (984 meters above sea level) where sites were on a slope, Hurley Creek (978 meters above sea level) where sites were located further away from the stream in comparison to other sites due to the stream being less accessible down the bank, Hovey Creek (1050 meters above sea level) where sites were under moderately heavy tree cover, and Blue Creek (1055 meters above sea level) where sites were also further away from the stream do to where the stream was in comparison to tree cover.



Figure 1: Site locations with activity level shown in relation to major city.

Although each individual site varied based on microclimatic factors, sites were exposed to similar temperature and precipitation patterns.

Throughfall

Throughfall collectors were installed under the tree canopy close to each decomposition site. Two rainfall collectors were set up in an area with no canopy cover in the low budworm activity sites and two in the high impacted sites as controls. During rain events, water came through the canopy into a 20-cm diameter polyethylene funnel with polywool filtering out any litter that happened to fall into the apparatus. The funnel was attached to Tygon tubing and was held in place with parafilm. Water traveled through the tubing into an acid washed 4 L polyethylene container. Water was then transferred to a 1 L acid washed Nalgene bottle and was taken to the lab, filtered through a 1.0 μm fiberglass filter using vacuum filtration. The volume of each collector was also measured in order to calculate mg of nutrient per L of water. Water samples were frozen until analysis of solutes at a later date. Throughfall collectors were taken down November 8, 2015 just before snowpack and redeployed in April 2016 just after snowmelt due to lack of accessibility to sites and to prevent damage to the apparatus.

Frass and Litter Measurements

Frass and litter fall were also collected at each site to quantify and differentiate organic matter inputs to the forest floor. Funnels (0.25 m2 diameter) made of tarp and garden hose connected to a one-liter Nalgene bottle were placed on the ground under trees affected by budworms. Polyethylene funnels collected frass and litter, and samples were collected approximately monthly until November 2015, just before snowpack. The samples were dried, sorted by frass versus litter, and weighed in the laboratory. Weights were then converted to a daily litter or frassfall rate by mgs of frass/m2d or litter/m2d. Frass collectors were taken down in November 2015 due to lack of site accessibility and to prevent damage and were reinstalled in April 2016. Unfortunately, due to frequent rains in the spring months of 2016, samples decomposed before they could be collected and measured, so no data are available for the second half of the study.

Decomposition

At each replicate plot I deployed twenty 20x20cm mesh litter bags (García-Palacios et al., 2016) with a top sieve size of 2 mm (Genung et al, 2013) and a bottom sieve size of 0.5 mm (Schweitzer et al, 2005) to reduce the loss of its contents while still allowing small detritivores to enter the bags with a total of 480 bags across all sites. Ten bags at each site contained a mixed conifer needle sample of Douglas fir (*Pseudotsuga menziesii)*, grand fir (*Abies grandis*), and ponderosa pine (*Pinus ponderosa*), to represent the most abundant species in the study area. To make this mixed sample, I harvested needle material in an approximate ratio of 1-part ponderosa pine, two parts Douglas fir, and one-part grand fir from the forest and air dried the needles until they reached constant weight. The other ten bags at each replicate plot contained sugar maple (*Acer saccharum*) leaves which are non-native to the area but are commonly used in decomposition studies for comparison across biomes.

Within each litter bag, I placed ~3-5 grams of air dried needles (Benfield, 1996) after recording the needle mass, and I added an aluminum tag with a unique ID. Bags were assembled by stapling the two sieve sizes together and by reinforcing them with super glue at the corners. The bags stayed intact throughout the 14-month deployment. Mesh bags with leaves were subsequently placed into red peanut bags (mesh size XX Clay will get) to further protect them during deployment and to simplify sample collection, and each individual bag was placed into a Ziploc for transport to the field.

On September 8, 2015, the mesh bags were deployed and strung together on an approximately 6 m fishing line held in place by a 0.6 m piece of rebar driven into the ground on either side. The rebar anchors and fishing line prevented bags from being moved by the wind, displaced by hillslope runoff, or moved by animals. A coin flip determined which bags (conifers or deciduous maple) were placed upstream and downstream at each site. Handling loss was applied to the mass of the material by deploying twenty bags, ten deciduous and ten coniferous and extracting them immediately to determine mass loss per bag during deployment and extraction. Mass loss per bag was averaged and applied to all bags extracted throughout the study. This was done separately for conifer and deciduous leaves.

Bags were collected 7 times beginning October 11, 2015 and ending November 6, 2016 in approximately 1-2-month intervals with a 5-month break during winter snowpack (December 2015 to April 2016) when the sites were inaccessible. During each retrieval from the field, one conifer bag and one maple bag were collected from each plot for a total of 48 bags per sampling time. Bags were randomly chosen for pickup and returned to the lab in a Ziploc bag to prevent additional leaf mass loss. On the final collection day, all remaining bags were collected from the sites (n=8 per plot and leaf type). Decomposition bags were air dried in the lab to constant mass (Schweitzer, 2005) in paper bags (Genung et al. 2013) hung on a clothesline. After air drying, each bag was sorted to remove any noticeable debris that had become incorporated in the sample (Chapman et al. 2013). Because of natural loss of conifer needles from the canopy, it was difficult to determine what was originally in the bag and what had fallen into it, so the mass of conifer needles accumulated in the maple decomposition bags was sorted and used as a correction factor for the mass of conifer needles that entered the conifer bags. Decomposition was calculated as: Rate (k) = slope = [ln(% mass remaining) / # days deployed].

*Soil Analyses*

During each collection of decomposition bags, I also used a thermocouple to measure temperature at three soil depths: 2 cm, 10 cm, 20 cm. These corresponded approximately to the O horizon, the top of the A horizon, and within the A horizon respectively. A soil core of ~10 cm depth was also collected from each replicate plot at each stream site each time I collected litter bags. Soil cores were stored on ice for return to the laboratory whereupon each core was homogenized in a Ziploc bag. Soils were immediately analyzed for moisture content and percent organic matter, and soils were frozen for later analysis of ammonia, nitrate, inorganic P, and using methods detailed below.

*Moisture Content and Percent Organic Matter:*

Soil was sieved at 2 mm and a subsample was placed into an ashed aluminum pan and weighed immediately for field mass. Pans were then placed in a drying oven at 60ºC until constant mass, cooled to room temperature, and weighed to obtain dry mass (DM). The difference between field mass and dry mass was used to calculate percent moisture. Then dried soil samples were placed in a muffle furnace at 500ºC for 48 h. to combust all organic compounds. After ashing, samples were cooled to room temperature, rehydrated with Milli-Q water to rehydrate clays and colloids containing water molecules, and then placed again into a drying oven until constant mass. Pans were cooled to room temperature and reweighed to obtain ash-free dry mass, with the difference between dry mass and ash-free dry mass used to calculate percent organic matter.

*Phosphorous Analysis*

The Bray P1 method was used to extract phosphorus from each soil sample (Hamilton, 1997; Patton and Kryskalla, 2003). One gram of air dried soil was added to 10 mLs of the Bray P1 extractant solution (30 mL 1 N NH4F to 50 mL 0.5 HCl) and shaken on a shaking table at 100 rpm for 15 minutes then centrifuged at 10,000 g () The sample was then filtered with a syringe through a 1.0 µm glass fiber filter and stored in the freezer until analysis. Samples were analyzed for inorganic phosphorous using the Bray P1 method on a Seal AQ1 Discrete Analyzer (Seal AQ1, Seal Analytical; Mequon, Wisconsin, USA) with EPA equivalent methods.

*Nitrogen Analyses*

A 2M KCl extraction method was used to extract inorganic nitrogen from each soil sample. Five grams of air-dried soil were added to 37.5 mLs of 2M KCl and shaken at 100 rpm for 2 hours on a shaker table and then centrifuged at 10,000 g. The sample was then filtered with a syringe through a 1.0 µm fiberglass filter and stored in the freezer until analysis. Samples were analyzed for NO3-+NO2- (hereafter referred to as NO3-) using the cadmium reduction and NH4+ using the phenate method on a Seal AQ1 Discrete Analyzer (Seal AQ1, Seal Analytical; Mequon, Wisconsin, USA) using EPA equivalent methods.

Each site also contained a resin bag made of bleached nylons (to prevent color leaching that may affect results) filled with 20 g of ion exchange resin (IONAC NM-60 mixed bed exchange resin, strong acid/strong base; sulfonated alkyl quaternary ammonium polystyrene; J.T. Baker #JT4631-1) that was deployed 10 cms deep while initial soil samples were taken to measure changes in the DIN pool in soils throughout the deployment, and were also extracted using 100 mLs of 2M KCl and were analyzed for NO3- and NH4+ to look at net nitrification and mineralization. This rate was calculated as [(final soil N + resin bag N) – initial soil N/incubation time] (Griffin and Turner, 2012) on a Seal AQ1 Discrete Analyzer using EPA equivalent methods. Bags were deployed in September 2015 and extracted in November 2015. Bags were replaced during this time and were extracted again in April 2016. Net nitrification and mineralization allowed me to make inferences on microbial activity in the soil.

**Statistical Analysis**

All data was analyzed in RStudio version 3.6.2 at an alpha level of 0.05. Throughfall was compared using XXXXX. Frass and Litterfall was compared using a Generalized Least Squares model. Decomposition was analyzed with a linear model (LM) with leaf type and location as factors as well as looking at the interaction between high impact and low impacted sites. A two-sample t-test to compare the two treatments; coniferous litter vs deciduous. I used generalized least squares (GLS) models and linear mixed effects (LME) models (Senf et al. 2016) to compare the variances within each of the 8 sites and each of the 3 replications within to compare percent soil moisture, percent organic matter, NO3-, NH4+, SRP, N:P ratio, total inorganic N, and net nitrification/mineralization. Data was normalized when possible. Estimated marginal means (EMMS) was used as a post hoc test on data that yielded significant results to determine at which sample events they were significant.

When selecting models, I compared ones with both an interaction between impact factors and sample event and ones with a nested design. I plotted the residuals using a Q-Q Normal Plot and normalized when applicable. Additional models were constructed with weighted variances to help reduce residual patterns. Models were compared using the anova command in R and the model with the lowest AIC score was selected.