**II**

**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, https://www. ncdc.noaa.gov,accessed 7 September2018) 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 frassfall and litterfall, soil chemistry, soil organic matter and moisture content, and soil temperature 8 times between early September 2015 and early November 2016. Throughfall water chemistry was collected when accumulated precipitation allowed (> 100 mL). At each sample event, I collected decomposition bags to calculate one decomposition rate for each plot 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 northeast of Central Washington University on public land (Figure X). These study sites were located near the following creeks: Stand Up Creek (903 m a.s.l.) where sites where on a slope with light tree cover, Jungle Creek (824 m a.s.l.) where sites were often disturbed by free range cattle, Jack Creek (963 m a.s.l.) where sites were under moderately heavy tree cover, and Moonbeam Creek (973 m a.s.l. 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 (Figure X). These study sites were located near the following creeks: Cougar Creek (984 m a.s.l.) where sites were on a slope, Hurley Creek (978 m a.s.l.) where sites were located further away from the stream in comparison to other sites due to the stream being less accessible in a confined valley, Hovey Creek (1050 m a.s.l.) where sites were under moderately heavy tree cover, and Blue Creek (1055 m a.s.l.) where sites were also further away from the stream due to where the stream was in comparison to tree coverFigure 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

At each plot (n=24) a throughfall collector was installed under the canopy of a randomly selected tree close to each decomposition site. Each throughfall collector consisted of a funnel (20 mm diameter) that drained through tygon tubing into a 4-L acid-washed collection jug. To protect the tubing, I fed it through a PVC pipe, pounded into the ground with hole in the side so the tubing could enter the collection jug. The PVC pipe was stabilized by wiring it to a piece of rebar pounded into the ground. To prevent material from entering the collection jug, the opening was sealed with parafilm to keep the tubing in place, and polywool at the base of filter prevented litter from entering the jug from the funnel.

Upon rainfall, water entered the funnel and traveled through the tubing into the jug until I retrieved within 48 h of the rain stopping. Upon collection, the total sample volume was recorded as the sample was transferred to an acid washed nalgene bottle and returned to the lab for filtration using a 1.0 μm glass fiber filter. Samples were frozen until later water chemistry analysis. In order to differentiate nutrients in bulk rainfall compared to throughfall that had percolated through the canopy, a total of four rainfall collectors were set up in areas with no canopy cover, two in the low budworm study sites and two in the high budworm study sites

Throughfall and rainfall collectors were taken down November 8, 2015 just before snowpack due to lack of accessibility to sites and to prevent damage to the apparatus, and they were redeployed April 23, 2016 just after snowmelt to begin sampling again. All collectors were taken down on November 5, 2016

Frass and Litter Measurements

To ensure a qualitative difference between low and high budworm herbivory, frass was collected at each site. Funnels (0.25 m2 diameter) made of tarp and garden hose connected to a one-liter Nalgene bottle were set up under trees at each site. These were sampled regularly during budworm feeding and less frequently after feeding. 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 mg frass/m2d or mg litter/m2d. Frass collectors were taken down in November 5, 2015 due to lack of site accessibility and to prevent damage, and they were reinstalled in April 23, 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.

Litter 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 content loss while still allowing small detritivores to enter the bags. I deployed a total of 480 bags across all sites. Ten bags at each site contained a mixed conifer needle sample of Douglas fir, grand fir, and ponderosa pine, 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 1 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 (Webster and Benfield 69) (Graça et al, 2005) .

Within each litter bag, I placed ~3-5 grams of air dried needles or leaves (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 needles or leaves were subsequently placed into red peanut bags (mesh size ~ 3.1 mm) 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 nylon parachute cord held in place by 0.6 m pieces of rebar driven into the ground on either side. The rebar anchors and parachute cord 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 sites were inaccessible. During each retrieval from the field, one conifer bag and one maple bag were randomly 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=4 per leaf type at each plot). 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:

*Soil Analyses*

Upon 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 matter. 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.

*Net changes in the soil inorganic N pool*

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 cm deep when initial soil samples were taken. Bags were deployed in September 2015 and extracted in November 2015. Bags were replaced during this time and were extracted again in April 2016. These resin bags were used to measure changes in the DIN pool in soils throughout the deployment, so I could calculate net changes in the inorganic N pool. As with soil samples, resin bags were extracted using 100 mL of 2M KCl and were analyzed for NO3- and NH4+ on a Seal AQ1 Discrete Analyzer using methods described below. Net changes in the inorganic N pool were calculated as (Griffin and Turner, 2012) Net nitrification was indicated by … and net mineralization was indicated by … etc.

*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.

*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 mLs 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 ascorbic acid method (Murphy and Riley, 1962)on a Seal AQ1 Discrete Analyzer (Seal AQ1, Seal Analytical; Mequon, Wisconsin, USA) with EPA equivalent methods.

**Statistical Analysis**

All data was analyzed in RStudio version 3.6.2. Throughfall was analyzed using XXX (package). . Frass and litterfall was compared using a generalized least squares (GLS) model (package). 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 GLS models and linear mixed effects (LME) models (Senf et al. 2016) to see how budworm herbivory level (low versus high) influenced percent soil moisture, percent organic matter, temperature, NO3-, NH4+, SRP, N:P ratio, total inorganic N, and net nitrification/mineralization through time and by site. Data was normalized when residuals did not meet the assumptions of the test. For GLS and LME models that yielded significant results, estimated marginal means (EMMS) analysis (package) was used as a post hoc test on data to determine which sample events differed significantly. All statistical tests had were run with a = 0.05.

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.