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Effects of Western Spruce Budworm Herbivory on Forest Soils and Litter Decomposition in Central Washington

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EFFECTS OF WESTERN SPRUCE BUDWORM HERBIVORY ON FOREST SOILS AND LITTER DECOMPOSITION IN CENTRAL WASHINGTON

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Master of Science

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by

Izak Roland Neziri

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CENTRAL WASHINGTON UNIVERSITY

Graduate Studies

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Dean of Graduate Studies

ABSTRACT

EFFECTS OF WESTERN SPRUCE BUDWORM HERBIVORY ON FOREST SOILS AND LITTER DECOMPOSITION IN CENTRAL WASHINGTON

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Disturbances by herbivores can drive nutrient cycling in forest ecosystems by adding frass, carcasses, and molts to the forest floor which are broken down into nitrogen, phosphorous, and other elements to be recycled into biomass. Western spruce budworms are defoliators native to the central Cascades and their herbivory could increase the decomposition rate of forest materials by adding essential nutrients and/or by increasing light and rainfall penetration to the forest floor by thinning the forest canopy during outbreaks. Budworm defoliation events are expected to increase in severity as the climate warms, potentially altering forest ecosystem function. The purpose of this study was to measure how budworms influence nutrient availability in forest throughfall, decomposition rate on the floor, and soil nutrient concentrations by sampling 4 sites with low budworm activity and 4 sites with high budworm activity from September 2015 through November 2016. Budworms appeared to accelerate ammonium and nitrate loss from the canopy via throughfall during the spring 2016 feeding season, yet those forms of nitrogen did not increase concurrently in soils suggesting rapid immobilization of nitrogen. In contrast, budworms did not influence throughfall phosphorous yet soils in budworm sites had significantly higher phosphorus concentrations, possibly due to frass

addition or dead adults. Decomposition rates were unexpectedly faster in the low budworm sites in the Teanaway suggesting that the nitrogen losses and/or canopy changes by budworms were not strong enough to influence forest floor organic matter cycling. Overall, my study shows that budworms can influence nitrogen movement from the canopy to soils during feeding, but much of that nitrogen appears to be immobilized in soils. Other environmental factors must control litter decomposition rates. Due to the short term nature of this study, I cannot conclude whether or not the ecological effect of budworms changes during the course of an outbreak; however, if budworm activity increases due to continued fire suppression and climate change, budworms could accelerate nitrogen losses from the canopy which could be lost to downstream ecosystems with a large or prolonged outbreak.

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CHAPTER I

INTRODUCTION

Forests make-up approximately one-third of the Earth's terrestrial surface (Likens et al. 1970). In these ecosystems, a large portion of the nutrients are generally retained within that system (Qualls et al. 2002). Nutrients enter the system by dry deposition from the atmosphere, and from precipitation (Potter et al. 1991), and the general flow of these nutrients within a forest ecosystem includes precipitation washing out both the dry deposition and its own contents through the canopy as throughfall, while leaching nutrients from vegetation to soil, and nutrient uptake by plants that converts nutrients into biomass.

When these systems are subject to disturbance, the cycling of forest nutrients is subject to changes and losses (Vitousek et al. 1979). Common disturbances include clear cut logging, fire, and insect outbreaks. For example, in 1970, a famous experiment in the Hubbard Brook Experimental Forest in New Hampshire found that experimental clear cutting dramatically increased annual runoff and loss of nitrate and other ions to downstream ecosystems (Likens et al. 1970). The central Cascades are prone to drought and regular fires that can decrease biodiversity and alter species distribution and forest dynamics (Clarke et al. 2016). Initially, fires can increase concentrations of ammonium (NH_4^+) and decrease PO_4^{3-} in soil in the short term, but a study in a Florida ecosystem showed that in less than 100 days post fire, available nutrients in the soil were reduced by half (Schafer and Mack 2010), suggesting that fires also contribute to nutrient losses via runoff or by wind. Further, in a study of prescribed fires in a French Mediterranean forest, N, P, K, and Ca were all shown to decrease (Gillon and Rapp 1989). Similar results were found in a Spanish Mediterranean forest, where nutrient loss was seen in the

form of volatilization (Pausas and Vallejo 1999). Herbivory by forest insects can also increase loss of P and N to downstream ecosystems (Hunter 2001; Metcalfe et al. 2016). While all of these studies point to disturbance as a mechanism of nutrient loss from forests to downstream aquatic ecosystems, the effect of insect herbivores has not been studied in the western United States, where, as global temperatures rise, an uptick in forest insect activity is expected.

The process of defoliation is an important part of forest ecosystem health and function. When forests become too thick, defoliators such as herbivorous insects act as a negative feedback loop by killing trees and reducing the population to lower levels (Poirier 2017; Murdock et al. 2012). Defoliation also drives material cycling in forests through consumption of the canopy and excretion as frass which returns nutrients as organic matter to soils. Although defoliators are a natural part of forest material cycling, the activity of these insects is increasing due to fire suppression and climate change (Abatzolou and Williams 2016). As a result, the rate and severity of insect outbreaks has increased dramatically over the last century in the western United States (Senf et al. 2016).

For centuries, frequent, low intensity fires, some naturally caused and some ignited for landscape management by indigenous people, shaped the structure of coniferous forests across the United States (Klenner et al. 2008), creating, for example, ponderosa pine forests with grasses and shrubs growing between widely spaced trees. Under these historic fire regimes, insect pests were maintained via two avenues. First, frequent, low intensity fires increased distance between trees making it challenging for insects to disperse and decreasing the rate at which defoliators damaged the forest

(McRae et al. 2001). Secondly, fires killed pests directly (Roques et al. 2020). Since the 1930s, intense fire suppression throughout the American West has led to thicker forests with increased canopy cover (Keane et al. 2002), and insect pests have increased with the thickening tree stands. Moreover, decreases in winter severity due to climate change have reduced the frequency of cold winters that can also kill insect pests (Murdock et al. 2012). Therefore, a multi-decadal history of fire suppression, coupled with summer drought stress and warmer winters due to climate change, has generated conditions that encourage sustained insect outbreaks and disease in the forest (Keane et al. 2002), and these insect outbreaks are expected to intensify as climate change progresses (Flower et al. 2014; Harvey et al. 2018).

Notable outbreak insects in the PNW are Douglas fir tussock moths, tent caterpillars, and western spruce budworms. A major defoliator of the coniferous forests of Central Washington, as well as western North America in general (Senf et al. 2016), is the western spruce budworm (WSB) (*Choristoneura freemani).* These are a native lepidopteran that range from Southern British Columbia to Arizona and New Mexico (Fellin and Dewey 1982). These insects emerge during budburst around mid-May to feed on the new growth of short needle conifers, specifically Douglas fir (*Pseudotsuga menziesii*) and grand fir (*Abies grandis*), which have benefitted from fire suppression, but also spruce (*Picea* spp.) trees (Fellin and Dewey 1982), until late June or early July. They then pupate and emerge as adults, taking flight around mid to late July for oviposition. Larvae then emerge the following year in mid-May to repeat their life cycle. A multi-century reconstruction of budworm outbreaks using dendrological records in Central Oregon to Western Montana showed that budworm outbreaks were tied to

climate and drought, with the end of droughts usually being tied to outbreaks (Flower 2014). In a more natural fire regime that maintained an open forest structure, WSB outbreaks would occur about once every decade and last for an average of 12 years (Flower 2014). The study also suggested that it is possible that changes in forest composition and current land use practices may influence outbreaks, but this was not directly studied. Outbreaks are predicted to increase as climate and drought become more prevalent. Although this study was not done directly in Washington State, Central Oregon is very similar climate wise and has a similar forest structure. In recent years, thicker forests from fire suppression and increased drought stress from climate change have created conditions that encourage more frequent, widespread, and longer WSB outbreaks (Willis et al. 2008; Lovett et al. 2006). This shift in forest structure and herbivore behavior has the potential to change internal forest ecosystem nutrient dynamics with implications for nutrient loss to nearby stream ecosystems that would alter forest-stream ecological connectivity.

Under conditions without active defoliation, leaf litter would fall to the forest floor and be broken down by microbes over time, gradually releasing nutrients into the soil. However actively defoliating WSB are likely to alter the nutrient cycle in forest soils (Schlesinger et al. 2015). For example, the large amount of frass that these defoliators excrete falls to the forest floor and has the potential to increase nutrient availability in soils after rainfall leaches nutrients to soils, due to the complex carbohydrates of the tree needles being broken down by herbivores through digestion, making them available for the forest nutrient cycle (Tukey 1966). In particular, nitrogen availability may increase (Griffin and Turner 2012), and via various transformations into

ammonium (NH_4^+) or nitrate (NO_3^-), the increased nitrogen can meet a variety of fates. For example, ammonium or nitrate can be taken up by plants or immobilized by bacteria or fungi, remaining in the ecosystem as organic N. Organic N in frass can be mineralized as ammonium via decomposition or through excretion processes, whereupon it can be converted to nitrate via nitrification which is subject to leaching losses to downstream ecosystems (Lewis and Likens. 2006). Leaching losses of nitrate are due to the cation exchange capacity in soils. Because soils are negatively charged like nitrate, the ability for nitrate export potential is high. High nitrification and nitrate export after a bark beetles outbreak was found in a previous study (Griffin and Turner 2012). Furthermore, defoliation by WSB has the potential to allow more light and rainfall to reach the forest floor, increasing microbial activity via temperature and moisture increases and leading to a quicker break down in litter via decomposition (Chapman et al. 2013).

Any time an ecosystem experiences a major disturbance, there is an overall change in ecosystem dynamics, leading to implications for both wildlife and for human concerns (Pecl et al. 2017). However, little research has been done on how WSB herbivory disturbs the forest nutrient cycle, so it is uncertain how the predicted increases in their outbreaks might alter ecosystem dynamics in the Pacific Northwest and other western coniferous forests. To better understand how WSB defoliation affects internal forest nutrient pathways, I studied a WSB outbreak in the east slope of the Cascades in Central Washington with an aim of understanding how WSB herbivory affected throughfall nutrient composition, leaf litter decomposition rate, soil chemistry, and soil nitrogen transformations. I hypothesized that WSB activity would accelerate the movement of nutrients from the canopy to soils which would: 1) increase throughfall

nutrient concentration (NH_4^+, NO_3^-, DOC, and PO_4^{3-}), 2) increase litter decomposition rate, 3) increase soil nutrient concentrations (NH_4^+, NO_3^-, and PO_4^{3-}), and 4) increase net nitrification in soils. I also hypothesized that canopy opening via defoliation would alter soil temperature and moisture patterns by allowing more radiation to reach the forest floor, as well as more precipitation, with implication for decomposition.

This study took place during the last two years of a WSB outbreak and will add to the literature of how WSB outbreaks effect soil nutrient dynamics. Most studies of WSB have been concerned with their effect on tree defense response, tree mortality, and insect outbreak distribution. Many studies have been done on Douglas fir tussock moths, and the eastern spruce budworm, a cousin of the western spruce budworm, but little research has been done on WSB and soils.

CHAPTER II

METHODS

Study Area

This study took place in the eastern Cascades in central Washington state (Figure 1). In the rain shadow of the Cascades, summers (May-September) are relatively dry, with seasonal drought (sometimes no precipitation during the summer months) and temperatures ranging from 15°C-25°C whereas 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 2018) with most falling as snow between November-February. Because of the distinct seasonal patterns in precipitation and temperature, eastern Cascades forests are characterized by drought tolerant trees such as Douglas fir (*Pseudotsuga menziesii)*, grand fir (*Abies grandis)*, ponderosa pine (*Pinus ponderosa*), western larch (*Larix occidentalis*) and at higher elevations, lodgepole pine (*Pinus contorta*).

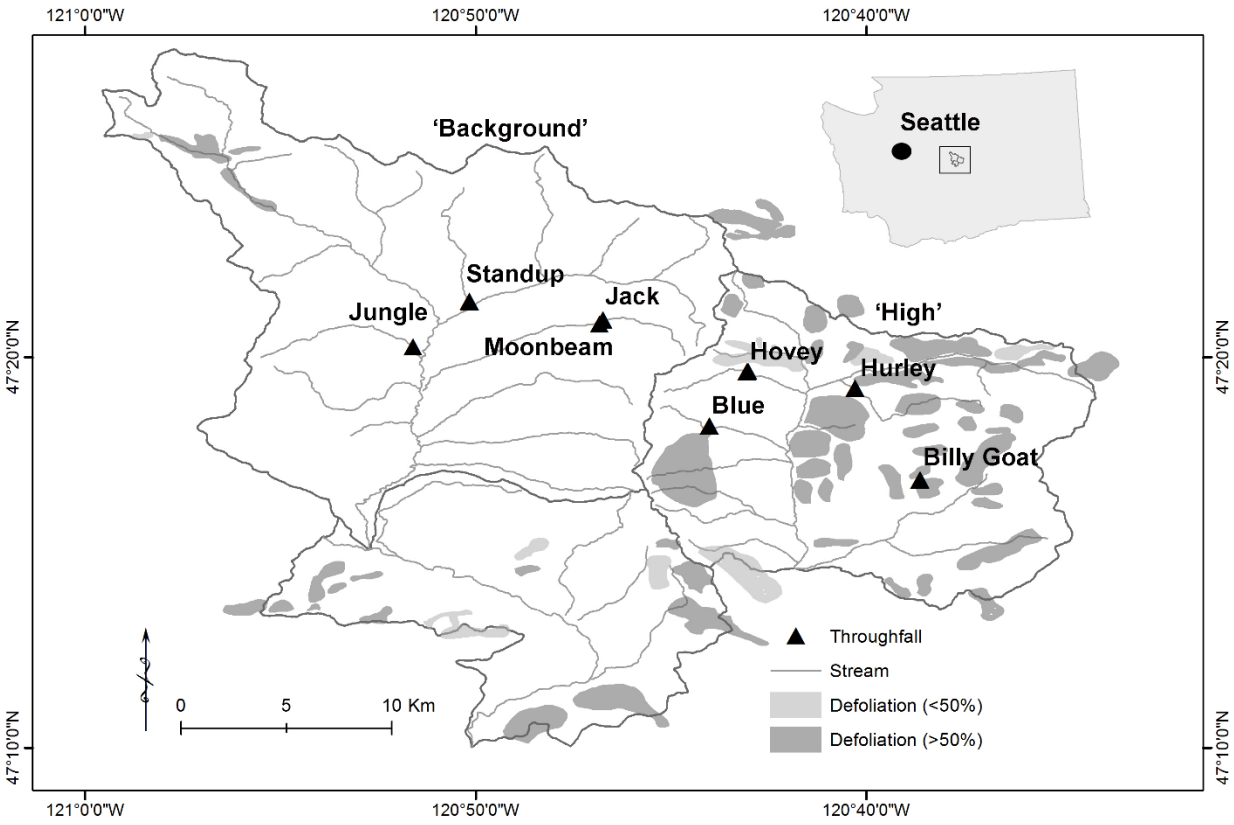

Figure 1: Site locations with budworm activity level derived from United States Forest Service aerial detection surveys (https://www.fs.fed.us/foresthealth/applied-sciences/mapping-reporting/gis-spatial-analysis/detection-surveys.shtml) flown in 2015 (Arango et al. 2019).

Land use in this study area has changed over the last few centuries, as European settlers spread westward in late 1800s. Prior to that time, indigenous peoples of the area burned forests and prairies in the Pacific Northwest to encourage growth of desirable edible plants, although distinguishing this from natural fires is difficult with no pre-human data to support the claim (Hessburg and Agee 2003). As Euro-American settlers arrived, logging removed some of the largest and oldest trees, mainly ponderosa pine, leaving behind a more homogeneous forest that was susceptible to intense fires (Hessburg and Agee 2003). In addition to logging, Europeans brought horses, cattle, and sheep that have caused a large decrease in native grasses due to overgrazing (Walsh et al. 2018).

Mining drastically increased in the area in the 1850s and with population and commerce increasing, railroads became a necessity for the area, leading to more logging to make room for roads and railways (Hessburg and Agee 2003).

I used a nested study design with repeated sampling through time to investigate how WSB herbivory influenced throughfall composition, litter decomposition, and soil nutrient concentrations. I established 4 study sites each within low and high WSB herbivory level stands (n = 8 study sites; Figure 1), and at each study site I established three replicate plots approximately 15 m from each other from upstream to downstream (n = 24 total sample plots). The low budworm sites were located in the Teanaway Community Forest in Washington state, approximately 40 miles northeast of Central Washington University (Figure 1) near the following creeks: Stand Up Creek where sites were on a slope with light tree cover; Jungle Creek where sites were often disturbed by free range cattle; Jack Creek where sites were under moderately heavy tree cover; and Moonbeam Creek where sites were also under moderately heavy tree cover. Elevations for these sites ranged from 824 to 973 m a.s.l. 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 (Figure 1). These study sites were located near the following creeks: Cougar Creek where sites were on a slope; Hurley Creek 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 where sites were under moderately heavy tree cover; and Blue Creek where sites were also further away from the stream due to difficulty of access. Elevations for these sites ranged from 978 to 1055 m a.s.l. Although each

individual site varied based on microclimatic factors, sites were exposed to similar temperature and precipitation patterns based on similar elevation and being within roughly 20 km of each other.

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 late September 2016, roughly every 6 weeks with a break from sampling when snow pack precluded site access. At each sample event, I collected decomposition bags to calculate one decomposition rate for each plot over the course of the study. Throughfall water chemistry was collected on an event basis when accumulated precipitation allowed (> 100 mL). I measured net nitrification at each site twice; the first measurement aggregated net soil nitrogen dynamics between summer 2015 and fall 2015, and the second aggregated net soil nitrogen dynamics between fall 2015 and spring 2016. Thus, my study design included measurements taken before, during, and after, one complete WSB life cycle, during the last year of a multi-year outbreak.

Throughfall

A throughfall collector was installed under the canopy of a randomly selected tree near each sample plot (n = 24). Each throughfall collector consisted of a funnel (20 cm diameter) that drained through tygon tubing into a 4-L acid-washed collection jug (Michalzik and Stadler 2005). The tubing was protected by feeding it through a PVC pipe pounded into the ground with a hole in the side so the tubing could leave the PVC and 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, polywool

was placed at the base of the funnel, and the opening of the jug was sealed with parafilm which also kept the tubing in place.

Upon rainfall, water entered the funnel and traveled through the tubing into the jug until I retrieved it within 48 h of the rain stopping. Upon collection, the total sample volume was recorded as the sample was transferred to an acid washed HDPE bottle and returned to the lab for filtration using a 1.0 μm glass fiber filter. Samples were frozen until later water chemistry analysis (described below). 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 a low budworm study site and two in a high budworm study site.

After deploying collectors on 25 Jun 2015 and collecting 4 samples in 2015, throughfall and rainfall collectors were taken down 8 November 2015 to prevent damage due to snowpack, and they were redeployed 23 April 2016 just after snowmelt to begin sampling again. All collectors were taken down on 19 September 2016 after collecting 6 samples in 2016.

Frass and Litter Measurements

To measure organic matter movement from the canopy to the forest floor, I collected frass and litterfall at each site. One funnel (0.25 m^2 diameter) made of tarp and garden hose connected to a one-liter Nalgene bottle was set up under one tree at each sample plot (n = 24). These were sampled regularly during budworm feeding and less frequently after feeding. The samples were dried, sorted by frass versus litter, weighed in the laboratory, and converted to a daily litter or frassfall rate (mg frass/m^2d or mg litter/m^2d). Frass collectors were taken down in 5 November 2015 to prevent damage

during winter snow accumulation, and they were reinstalled in 23 April 2016. While studies have shown the peak litter fall for Douglas firs is between October and December (Reukema 1964; Weiskittel and Maguire 2007) even if I was able to protect the samplers from snow damage, there was no way to access the sites during the snow season. Therefore, these litterfall estimates are lower than actual litterfall. 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. However, budworm activity was confirmed by visual observation of the webbing they create at the tips of conifer tress, as well as finding the worms themselves.

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 plots. Ten bags at each contained an air-dried, mixed conifer needle sample of Douglas fir, grand fir, and ponderosa pine in a 2:1:1 ratio to represent the most abundant species in the study area. The other ten bags at each replicate plot contained sugar maple (*Acer saccharum*) leaves which are non-native to the area but commonly used in decomposition studies for comparison across biomes (Bärlocher 2005).

Within each litter bag, I placed ~3-5 g of air-dried needles or leaves (Benfield 1996), recorded the needle mass, and 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 13-month deployment.

Mesh bags with needles or leaves were subsequently placed into plastic mesh 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 8 September 2015, the mesh bags were deployed and strung together on an approximately 6 m nylon 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. To determine mass loss per bag during deployment and extraction, I deployed twenty bags, ten deciduous and ten coniferous, and retrieved them immediately. Mass loss per bag was averaged and applied to all bags extracted throughout the study, with separate calculations for conifer and deciduous leaves.

Bags were collected 7 times beginning 11 October 2015 and ending 6 November 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, one conifer bag and one maple bag was randomly collected from each plot 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). Upon retrieval decomposition bags were hung on a clothesline in paper bags (Genung et al. 2013) and air dried in the lab to constant mass (Schweitzer 2005). 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 rate (k) was calculated as:

$$Rate\ (k)\ =\ -slope = \ln\left(\frac{percent\ mass\ remaining}{number\ of\ days\ deployed}\right)$$

Equation 1: The rate of decomposition where k is the slope.

Soil Collection and Processing

Upon each collection of decomposition bags, I also used a thermocouple to measure temperature at three soil depths: 2 cm, 10 cm, and 20 cm, corresponding 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 when I collected litter bags. Soil cores were stored on ice for return to the laboratory whereupon the core from each plot was homogenized in a Ziploc bag. Soils were immediately analyzed for moisture content and percent organic matter and then frozen for later analysis of ammonium, nitrate, and inorganic P using methods detailed below.

Moisture Content and Percent Organic Matter:

Homogenized soil was sieved at 2 mm and a subsample was placed into an ashed aluminum pan and weighed immediately for initial field mass. Pans were then placed in a drying oven at 60ºC until constant mass (approximately 24-48 hours), cooled to room temperature, and weighed to obtain dry mass (DM). The difference between initial field mass and dry mass was used to calculate percent moisture (Jarrett 1983).

$$Percent\ Moisture = \frac{Mass_{Initial\ field} - Mass_{dry}}{Mass_{Initial\ field}}\ x\ 100$$

Equation 2: The determination of moisture content in soil samples.

Then dried soil samples were ashed 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 ashed mass, and the difference between dry mass and ashed mass (ash free dry mass) was used to calculate percent organic matter.

$$Percent\ Organic\ Matter = \frac{Mass_{dry} - Mass_{ash}}{Mass_{dry}}\ x\ 100$$

Equation 3: The determination of how much organic matter each soil sample contained.

Net changes in the soil inorganic N pool

To measure changes in the soil inorganic N pool at each site, I also deployed 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) 10 cm deep after initial soil samples were taken. Bags were deployed in September 2015 and removed in May 2016 to measure changes over winter, and fresh bags were deployed in May 2016 November 2016 to measure changes during the growing season.

Net changes in the inorganic N pool were calculated as:

$$Net\ changes\ in\ inorganic\ N = \frac{(Final\ Soil\ N + Resin\ Bag\ N - Initial\ Soil\ N)}{Incubation\ Time}$$

Equation 4: Calculation of net changes in soil inorganic nitrogen (N) pool. Net increase in NH_4^+ indicates net mineralization, net increase in NO_3^- indicates net nitrification, and net decrease in either ion indicates immobilization of that ion (Griffin and Turner 2012).

A 2M KCl extraction method (Keeney and Nelson 1983) was used to extract inorganic nitrogen from each soil sample and from the ion exchange resins. Five grams of air-dried soil were added to 37.5 mL 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 glass fiber filter and stored in the freezer until analysis.

The Bray P1 method was used to extract phosphorus from each soil sample (Bray and Kurtz 1945). One gram of air-dried soil was added to 10 mL of the Bray P1 extractant solution (30 mLs 1 N NH_4F to 50 mL 0.5 HCl) and shaken at 100 rpm for 15 minutes on a shaking table and 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.

Chemical Analyses for Throughfall and Soil

Samples were analyzed for NO_3^-+NO_2^- (hereafter referred to as NO_3^-) using the cadmium reduction method (U.S. Environmental Protection Agency (EPA) 1993) and NH_4^+ using the phenate method (Solórzano 1969) on a Seal AQ1 Discrete Analyzer (Seal AQ1, Seal Analytical; Mequon, Wisconsin, USA). Samples were analyzed for PO_4^{3-} using the ascorbic acid method (Murphy and Riley 1962) on a Seal AQ1 Discrete

Analyzer (Seal AQ1, Seal Analytical; Mequon, Wisconsin, USA). After acidifying the samples to a pH of less than 2 to remove inorganic carbon, DOC samples were measured using infrared methods (American Public Health Association (APHA) 1995) on a total organic carbon analyzer (TOC-L Total Organic Carbon Analyzer, Shimadzu, Kyoto, Japan)

Statistical Analysis

All data was analyzed in R version 3.6.1 (R Core Team 2019). Throughfall composition was analyzed using (R Core Team 2019) to see how patterns varied by budworm herbivory level and time. Frass and litterfall was compared by budworm level and time using a generalized least squares (GLS) model, and leaf decomposition was analyzed using a linear model (LM) with leaf type and location as interacting factors. I used 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, soil nutrients, and net nitrification/mineralization through time. LM models were also used to regress decomposition rates of both deciduous and conifers leaf litter on total N in throughfall and total water falling at each plot. To optimize models, I compared alternate model structures with an interaction between impact factors and sample event and models with a nested design (Zuur et al. 2009). Additional models were constructed with weighted variances to improve model residuals. Models were compared using the anova command in R and the model with the lowest AIC score was selected. To evaluate the assumptions of the model, I plotted the residuals using a Q-Q Normal Plot and normalized when applicable. For LME models that yielded significant results, estimated marginal means (EMMs) analysis was used as a post hoc test to

determine which sample events differed significantly. EMMs are calculated and presented based on the model predictions which include any nesting terms and/or weighted variance terms. I used EMMs for my plots rather than raw data because EMMs used with semi continuous (Smith et all 2017) multivariate analyses gives equal weight to all predictors in the model, thus providing a more accurate analysis of how each predictor interacts within the model (https://cran.r-project.org/web/packages/emmeans/vignettes/basics.html. accessed 12 August 2020). All statistical tests were evaluated against $\alpha = 0.05$.

CHAPTER III

RESULTS

Throughfall Chemistry

Concentrations of throughfall ammonium differed in low and high budworm stands (LME, $p = 0.015$) and by sample event (LME, $p < 0.001$) throughout the course of the study (Figure 2A). There was a significant interaction (LME, $p < 0.001$) whereby on four dates (11 Sep 15, 21 Jun 16, 13 Jul 16, and 21 Jul 16) throughfall NH_4^+ was higher in high budworm stands, but on 4 Jun 16, it was higher in low budworm stands. Generally speaking, in times where budworms were inactive (11 Oct 15, 29 Oct 15, 8 Nov 15, 9 Sep 16), there was no difference in throughfall NH_4^+ concentration between high and low budworm sites. Throughfall nitrate differed by sample event (LME, $p < 0.001$) but not budworm activity level throughout the course of the study (Figure 2B). There was a significant interaction (LME, $p < 0.001$) whereby the low budworm stands had higher concentration NO_3^- on 8 May 16, but the high budworm stands had higher concentration on 13 Jul 16 and 21 Jul 16, which were generally during or after peak budworm herbivory. There was a general trend of increasing concentration of throughfall

ammonium and nitrate during the time of WSB budworm activity between 8 May 16 and 13 Jul 16 (Figure 2, indicated by red boxes).

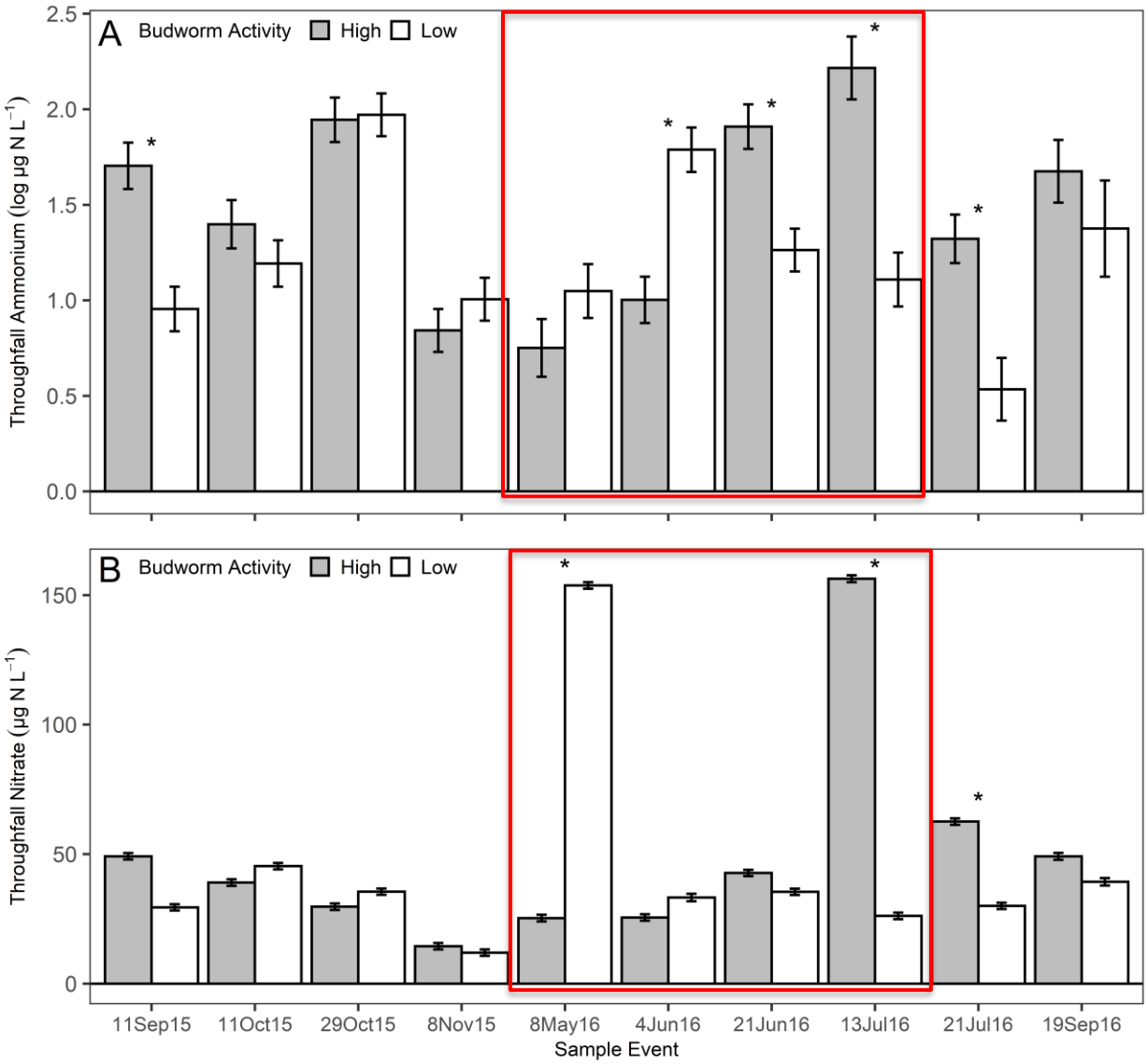

Figure 2: Estimated marginal means (EMM) of (A) throughfall ammonium (NH_4^+) concentrations (Budworm Impact: $p = 0.012$; Sample Event: $p < 0.0001$, and Interaction: $p < 0.0001$) and (B) throughfall nitrate (NO_3^-) concentrations (Budworm Impact: $p = 0.63$; Sample Event: $p < 0.0001$, and Interaction: $p < 0.0001$) in low and high budworm stands by sample date. Significant interactions are noted with an asterisk. Bars represent estimated marginal means, not raw data. Error bars represent standard error of estimated marginal means.

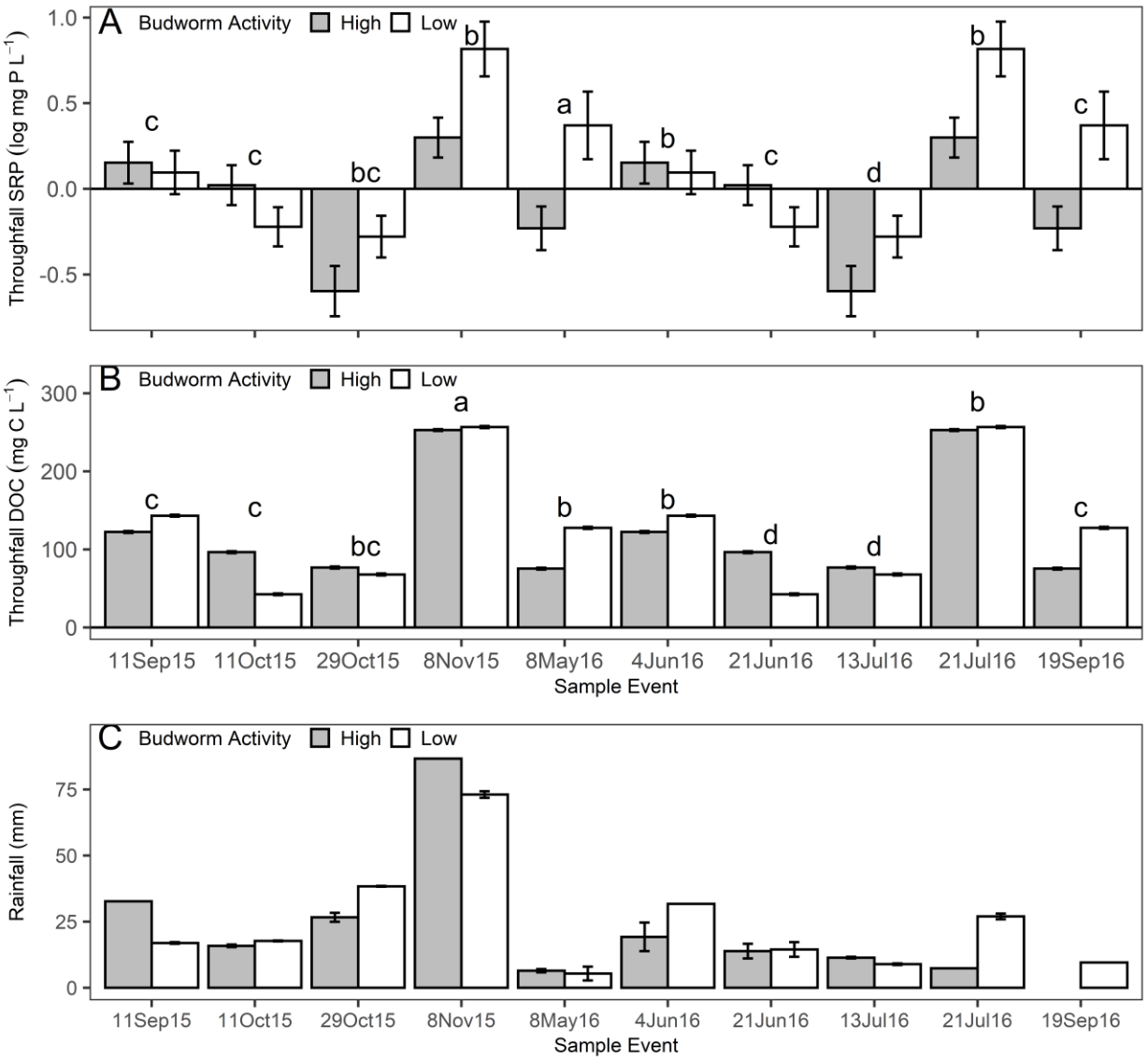

Figure 3: Estimated marginal means (EMM) of (A) throughfall soluble reactive phosphorous (SRP) concentration (Budworm Impact: $p = 0.43$; Sample Event: $p < 0.0001$) and (B) dissolved organic carbon (DOC) concentration (Budworm Impact: $p = 0.26$; Sample Event: $p < 0.0001$), and (C) rainfall in mm in low and high budworm stands by sample date. Significant differences among sample events are noted with letters. Error bars (A and B) represent standard error of estimated marginal means and (C) standard error of the mean. Samples without error bars only had one data point for that sample event.

Throughfall SRP concentration differed by sample event (LME, $p < 0.001$) throughout the study with highest concentrations on two dates (8 Nov 15 and 21 Jul 16). However, SRP concentration did not differ between high and low budworm sites (LME, $p = 0.43$) (Figure 3 A). Throughfall DOC concentration also differed by sample event (LME, $p < 0.001$) with 8 Nov 15 having the highest concentration. Like SRP, DOC did not differ between high and low budworm sites (LME, $p = 0.26$) (Figure 3B). The biggest pulses of SRP and DOC from the canopy appeared on the same dates (8 Nov 15 and 21 Jul 16), which also coincided with one large and one moderate rainfall event respectively.

Frass and Litter Deposition

Litterfall and frassfall significantly interacted between budworm impact and time (GLS, $p = 0.04$). In high impact sites, frass content was greater than in low impact sites during peak herbivory times. Once the budworm feeding season ended, frass input for high impact and low impact sites was virtually the same. Low budworm site collectors contained more leaf litter than high impact sights during the cooler months where the highest amount of litter fell during the October sampling dates.

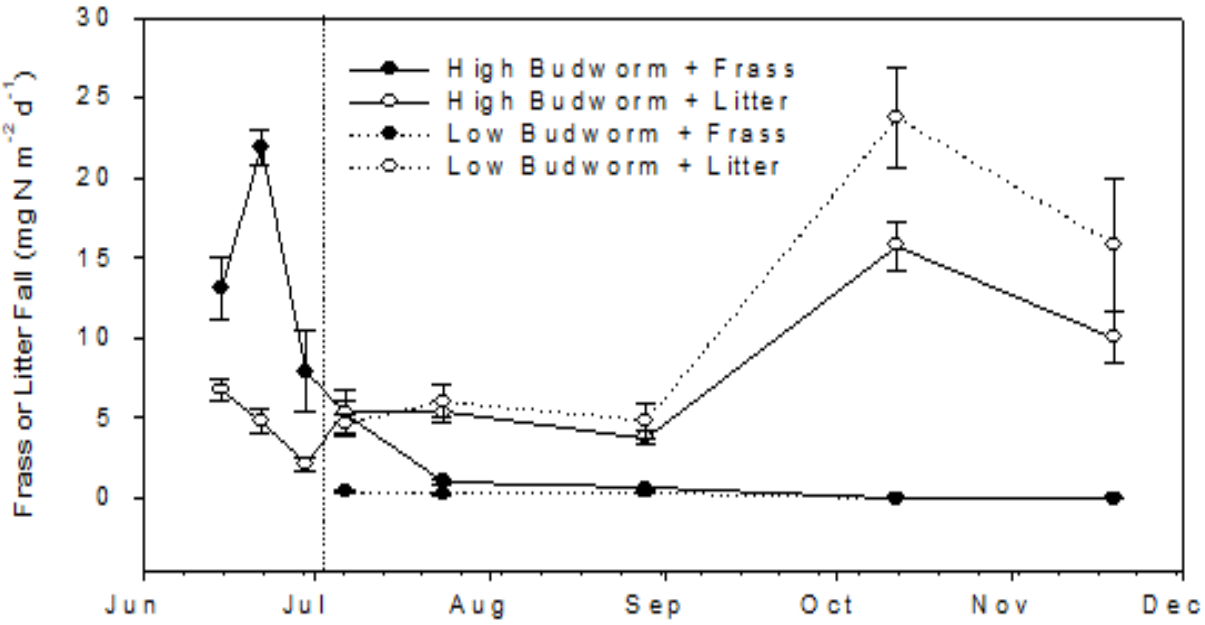

Figure 4: The deposition of frass and litter in high impact and low impact sites with vertical dashed line indicating end approximate end of budworm feeding. Analysis comparing high and low budworm interactions took place after the dashed line as well. Error bars represent the standard error of the mean.

Decomposition Rates

The decomposition rate of coniferous and deciduous leaf litter did not vary by leaf type ($p = 0.68$); however, decomposition was faster in low budworm sites for both leaf litter types ($p = 0.0024$, LME; Figure 5). The total mass of dissolved inorganic nitrogen (DIN) deposited by throughfall was negatively associated with the deciduous decomposition rate ($R^2 = 0.15$, $p = 0.033$; Figure 6) but not the coniferous decomposition rate ($p = 0.13$), and the decomposition rate for both leaf types was unrelated to total rainfall sampled. Because I only measured DIN and precipitation while samplers were deployed, these values do not represent actual totals of DIN or precipitation.

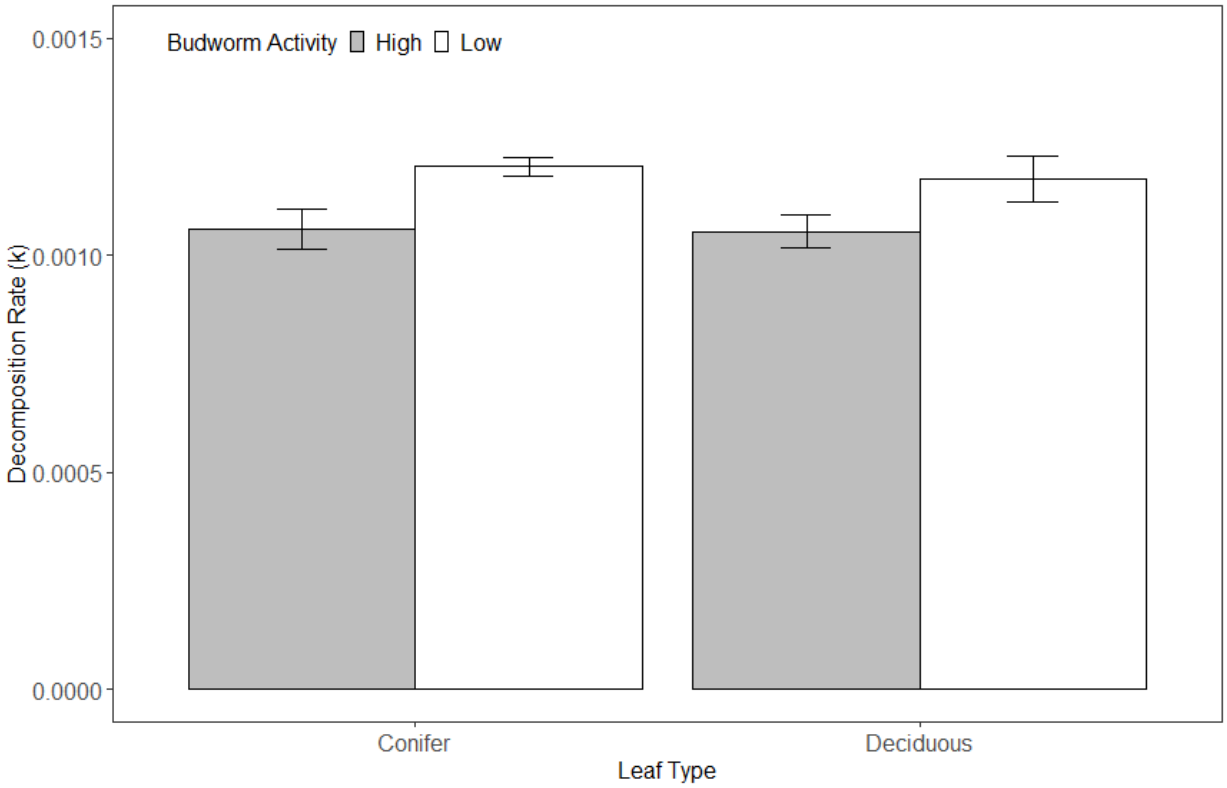

Figure 5: Decomposition rates (*k*) of deciduous and coniferous leaf litter in high and low budworm sites (Budworm Impact: p = 0.0024; Leaf Type: p = 0.68; and Interaction: p = 0.79).

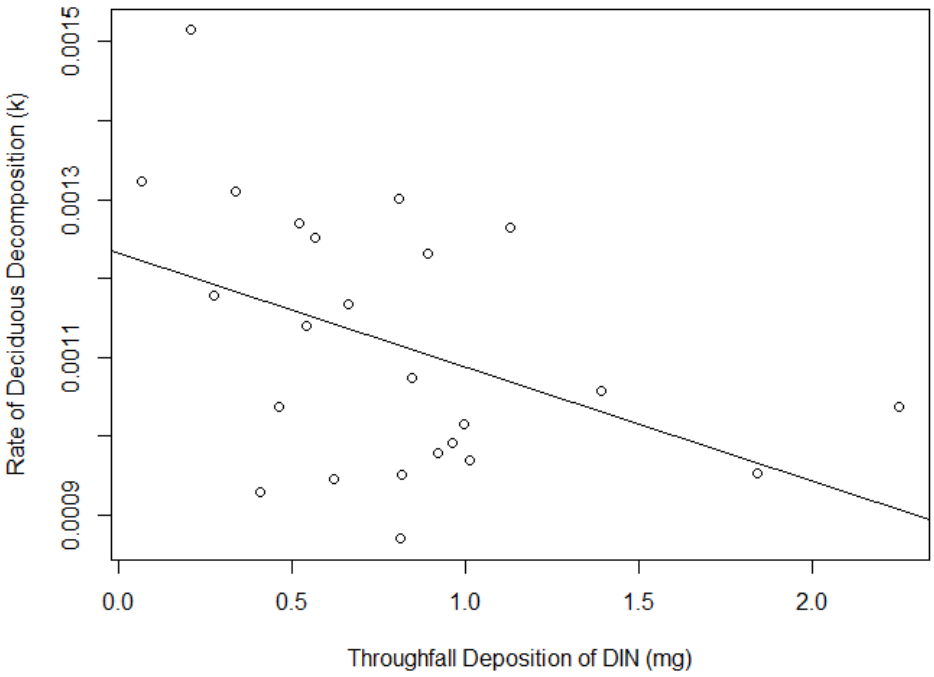

Figure 6: Regression analysis of throughfall DIN and deciduous decomposition rate (k).

Soil Chemistry

Soil ammonium concentrations differed by sample date (LME, $p < 0.001$) with higher concentrations on 8 May 16 and 6 Nov 16. These were times when budworms were generally not active, however, there was no difference between high and low budworm site ($p = 0.33$, LME). These times also coincided with the beginning of and the end of the growing season, respectively. Although soil nitrate did not differ between high and low budworm sites ($p = 0.76$, LME), it did differ by sample event ($p < 0.0001$, LME) with a significant interaction between sample event and budworm ($p = 0.003$, LME). In the interaction, high budworm sites had higher soil NO_3^- concentration than low budworm sites on 6 Nov 16 whereas as low budworm sites had higher NO_3^- on 4 Aug 16.

Usually soil NH_4^+ was 60 times higher than soil NO_3^-. Soil SRP was significantly higher in high impact sites ($p = 0.047$, LME) but did not differ by sample event ($p = 0.91$). Changes in the soil N pool indicated net nitrification and net mineralization, ($p < 0.001$, LME) in all sites, but neither net nitrification ($p = 0.33$, LME) nor net mineralization ($p = 0.66$, LME) differed by budworm activity level. Net mineralization significantly interacted with time ($p = 0.03$) whereby low budworm sites had higher rates of mineralization over the winter. Net nitrification was highest across sites in fall ($p < 0.0001$).

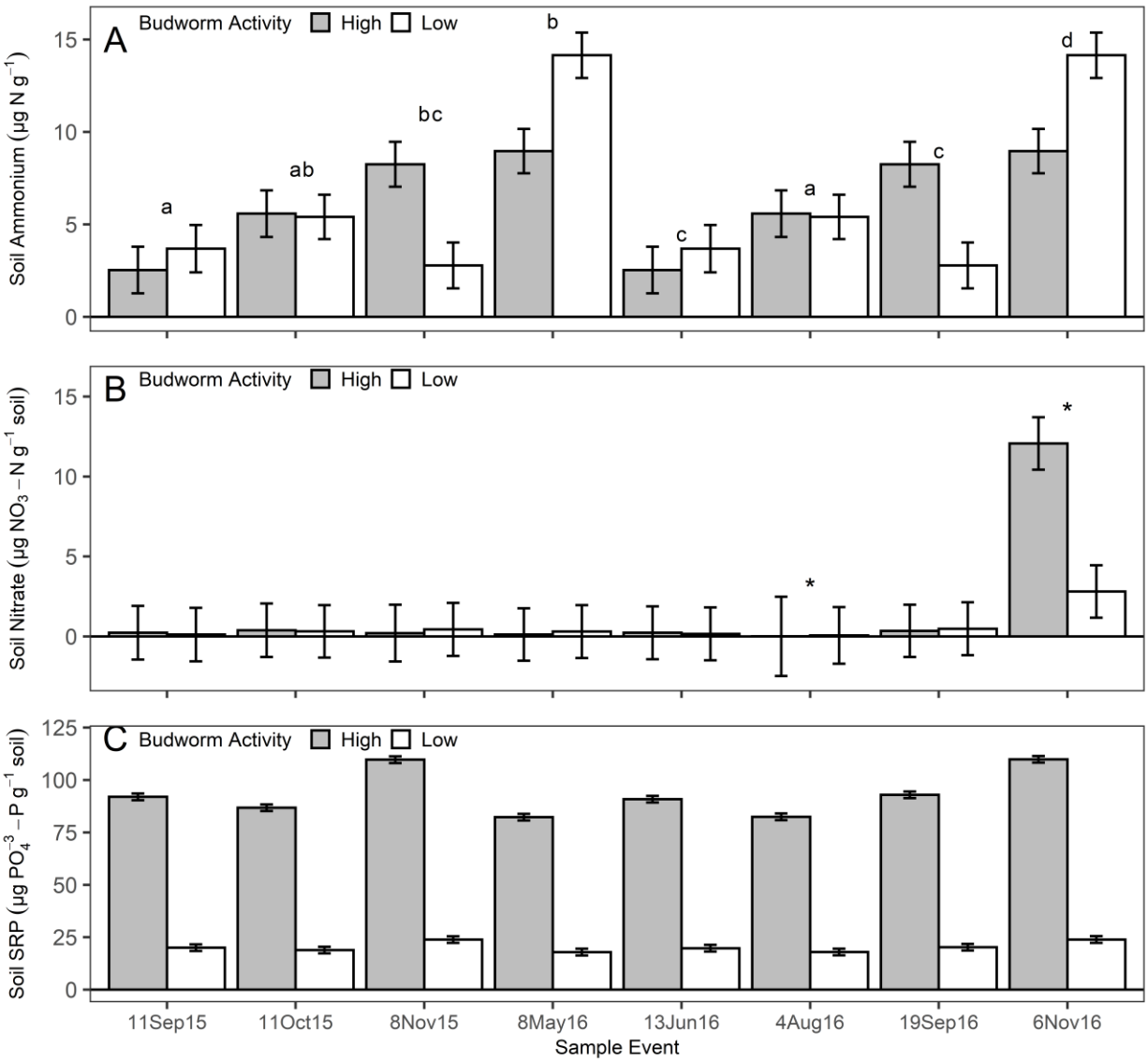

Figure 7: Estimated marginal means (EMM) of soil (A) ammonium (NH_4^+) (Budworm Impact: p = 0.33; Sample Event: p < 0.0001), (B) nitrate (NO_3^-) (Budworm Impact: p = 0.76; Sample Event: p < 0.0001; and Interaction: p = 0.0030), and (C) soluble reactive phosphorous (SRP) (Budworm Impact: p = 0.047; Sample Event: p = 0.91) and concentrations in low and high budworm stands by sample date. Significant sample events are noted with letters and significant interactions are noted with an asterisk. Error bars represent standard error of estimated marginal means.

Soil moisture varied among sample events (p < 0.001, LME) and was greater during the sample events 11 Oct 15, 8 Nov 15, 4 Aug 16, and 19 Sep 16, but there was no difference between high and low budworm sites (p = 0.86, LME) (Figure 8A). Soil

organic matter did not differ between high and low budworm sites (p = 0.49, LME) or among sample dates (p = 0.70, LME) (Figure 8B).

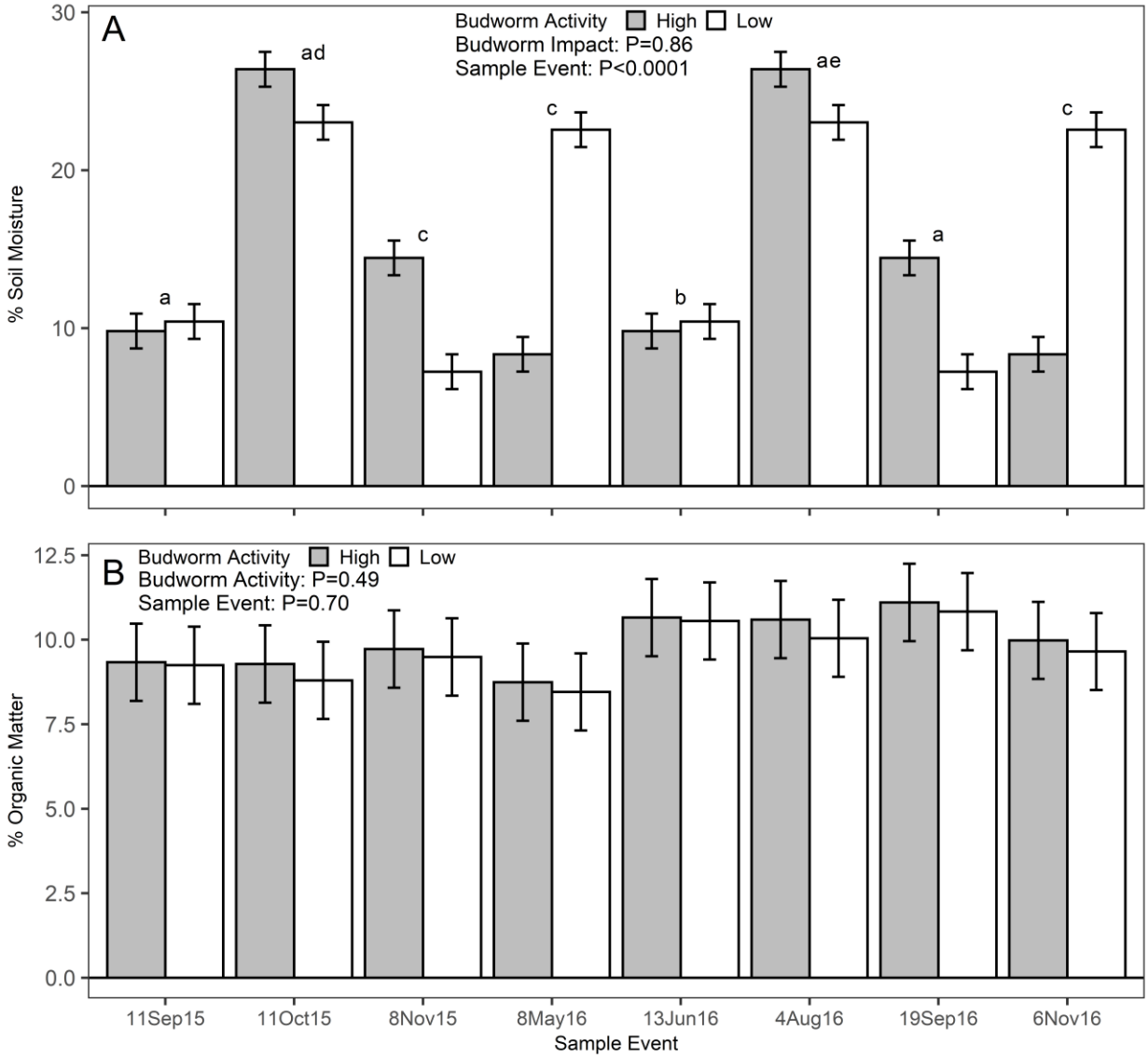

Figure 8: Estimated marginal means (EMM) of (A) soil moisture (Budworm Impact: p = 0.86; Sample Event: p < 0.0001) and (B) soil organic matter (Budworm Impact: p = 0.49; Sample Event: p = 0.70) in low and high budworm stands by sample date. Significantly different sample events are noted with letters. Error bars represent standard error of estimated marginal means.

Soil temperature followed the expected pattern of increasing during spring and summer months and decreasing during winter and fall months (data not shown), and soil temperature was strongly correlated with air temperature ($R^2 = 0.78$, $p < 0.0001$, linear regression). Budworm herbivory level did not influence soil temperature. As expected, temperature increased and decreased more rapidly at shallow compared to deeper depths, and soil temperature differences among dates were less variable in the deepest measurement at 10 cm (data not shown).

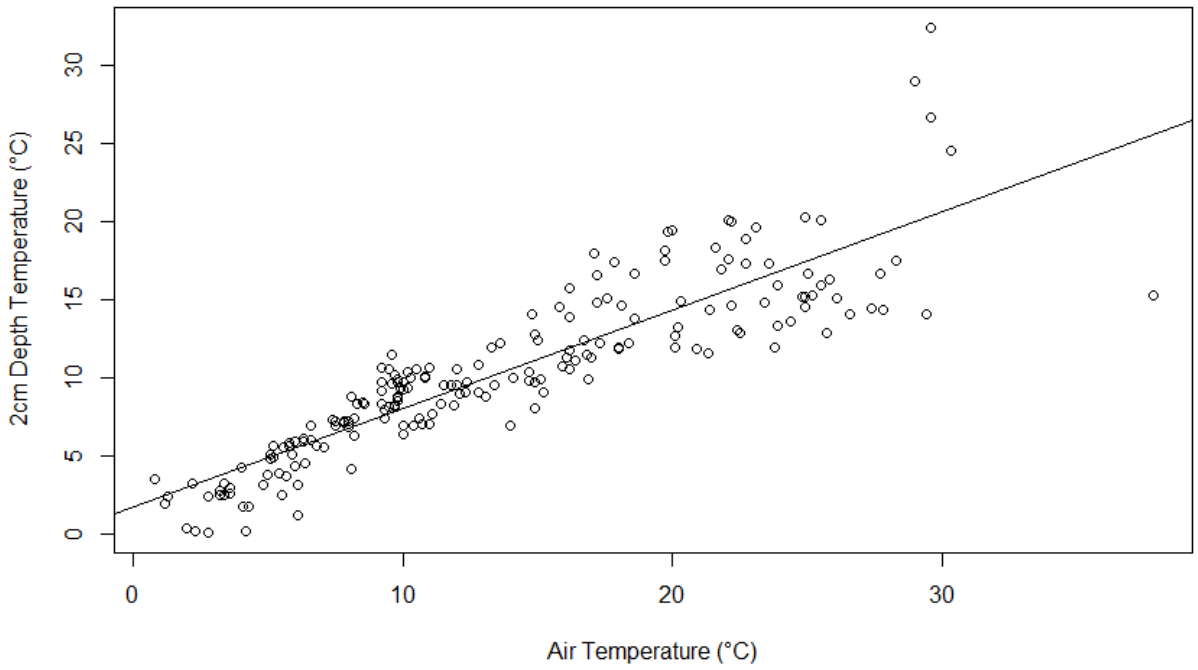

Figure 9: A regression analysis comparing soil temperature at 2 cm depth and air temperature ($p < 0.0001$, R^2 of 0.78).

CHAPTER IV

DISCUSSION

In this study, I investigated how WSB herbivory affected throughfall chemistry, leaf litter decomposition, and soil chemistry in the eastern Cascades of central Washington. Although budworm herbivory increased N loss from the canopy via throughfall, especially for NH_4^+, WSB did not affect throughfall SRP and DOC. Instead, higher concentrations of SRP and DOC in throughfall were seen in one heavy and one moderate rainfall event, suggesting hydrologic control. Budworm herbivory increased organic matter loss from the canopy as frass in summer in comparison to low budworm sites, which lost more organic matter as litter in fall. Unexpectedly, decomposition rates were faster in low budworm sites compared to high budworm sites for non-native deciduous litter and for native coniferous litter. Decomposition of deciduous litter was additionally negatively influenced by total N deposited by throughfall. Seasonality was the main driver of differences in soil moisture, soil temperature, and soil ammonium whereas budworm herbivory and seasonality interacted in soil nitrate concentrations. Soil phosphorus concentrations were clearly higher in high budworm sites compared to low budworm sites. Although all sites experience net mineralization and net nitrification in soils, unexpectedly, budworm activity did not influence net nitrification rate.

Throughfall Nitrogen

I hypothesized that budworms would increase the amount of ammonium and nitrate in throughfall, and I observed an interaction between WSB activity and sample date for throughfall ammonium. On three of four sample dates, 21 Jun 16, 13 Jul 16, and 21 Jul 16, I observed higher concentration of ammonium coinciding with budworm

feeding or immediately after feeding. During pest outbreaks in Scots pine forests, N fluxes from throughfall increased during defoliation events, and then decreased in the fall in the absence of defoliation, supporting my findings for WSB (Grüning et al. 2017). Feeding season is tied to growing season, and this can be seen from the 8 May 2016 sample time to the 13 July 2016 sample date. As the season changes from late spring to mid summer, there is an increase in both herbivory and NH_4^+ concentrations. As the season changes from mid summer to early fall, herbivory decreases and so does NH_4^+ concentrations as seen from 13 July 2016 to 21 July 2016. On a fourth date, 11 Sept 2015, I also observed higher ammonium concentrations in high budworm stands, but this date was well after budworm feeding in 2015. It is possible that ammonium generated by budworm feeding was stored in the canopy and washed out during the first major rain event in months that happened on 10 Sept 2015. Although these findings are consistent with defoliation accelerating ammonium loss from the canopy to forest soils, right as budworm feeding was beginning in 2016, a 4 Jun 16 throughfall showed the opposite pattern whereby low budworm sites had a higher ammonium concentration. Aphids and lepidopteran larvae have been shown to increase DOC concentrations and decrease NH_4^+ and NO_3^- concentrations in throughfall as they consume foliage (Stadler et al. 2001), so it is possible that the initial phases of larval feeding released DOC from plant tissues that stimulated ammonium uptake (Kirchman et al. 1990).

Similar to patterns in throughfall ammonium, budworm activity interacted with sample date to affect throughfall nitrate concentrations. Again, I saw that from 8 May 2016 to 13 July 2016 there is an increase in NO_3^- as feeding and growing season progress, and then a decrease in NO_3^- as feeding season begins to end. It appears that the general

trend of canopy N is that it increases from late spring to mid summer, coinciding with feeding and growing season, then decreases from mid summer to early fall as herbivory slows and the temperature cools. This general pattern suggests that budworm herbivory and growing season are related to canopy N. Interestingly, 13 Jul 16 and 21 Jul 16 have higher nitrate in high budworm stands that coincided with higher ammonium, which suggests canopy nitrification, similar to coniferous throughfall in Adirondack Mountains of New York (Chen et al. 1983). More recent studies have challenged the idea of canopy nitrification being such a large factor. For example, leaching of partially consumed or damaged leaves in the canopy from mature trees, which become less hydrophobic as they age, allows for more anions and cations to be released in water droplets during rain and wind events (Tukey 1966; Reynolds et al. 2000; Hunter et al. 2001). An experiment on the effect of nitrogen fertilizer on a mature spruce-hemlock forest in Maine also suggests that canopy nitrification was not responsible for nitrate increases in throughfall, instead suggesting that it was due to nitrate in dry deposition washed off by rain events (Gaige et al. 2007). Although I did not collect the data required to discriminate between these alternate mechanisms of NO_3^- loss from the canopy, the data I did collect suggest that WSB herbivory is associated with NO_3^- loss from the canopy to forest floor.

Throughfall ammonium and nitrate generally increased concentration from 1.5x to 2x as herbivory intensified relative to the low herbivory stands (Figure 2), consistent with a sustained and increasing budworm effect during their active feeding activity. Both winter moths (*Operophtera brumata*) and the mottled umber moth (*Eranis defoliaria*) have been shown to increase canopy N during herbivory outbreaks in oak forests in Germany with evidence that herbivores affect the canopy much more than they do soils

(LeMellec et al. 2011). The generalized increase in throughfall inorganic N coinciding with the summer growing season could have implications for plant uptake during that time. An experiment using Gala apples showed that photosynthesis rates increase after defoliating events leading to new growth, and although this study only measured carbohydrates, plants also need nitrogen to grow. If photosynthesis rates increase after defoliation, then nitrogen consumption must also increase (Zhou and Quebedeaux 2003). Therefore, the increased N in throughfall during the budworm feeding period may benefit the growth of understory plants, or it could contribute to continued leaf growth in the defoliated trees after WSB feeding ends with pupation.

Throughfall SRP and DOC

The observed pattern of throughfall SRP does not support my hypothesis of increased SRP in high WSB sites, but SRP concentrations did differ by sample event. On 8 Nov 15 there was a large rainfall event and on 21 July 16 there was a smaller but very heavy rainfall event, and on those days, I saw higher concentrations of SRP. I expected that phosphorus levels would be higher in heavily impacted areas due to an increase in frass input and increased SRP from leaching of partially consumed leaves as seen in multiple herbivore ecosystem interactions (Hunter et al. 2001). However, not all studies have shown that herbivory leads to increased nutrient fluxes. In a study in western Oregon on Douglas fir trees, throughfall data suggests that precipitation plays a much bigger role in throughfall nutrients than defoliation, consistent with my findings (Schowalter. 1999). Another study showed that the low level consumption of red maple trees and black locust trees in the southern Appalachians by canopy arthropods may not significantly alter throughfall concentrations of PO_4^{3-}, but the authors of that study

attributed imprecise method design for a potential reason for not being able to detect small changes (Seastedt et al. 1983). Therefore, SRP fluxes from the canopy to soils appear to be more strongly influenced by hydrology than by herbivory in my budworm sites.

Like with throughfall SRP, there were also significant sample event differences for throughfall DOC but no differences for budworm impact. Again, I hypothesized higher DOC concentrations in high budworm impact sites as many studies have shown that insect herbivory leads to increased throughfall DOC and N (LeMellec et al. 2011; Kindlmann and Stadler 2004; Stadler and Michalzik 2001). Given the relatively consistent observation that herbivory increased DOC fluxes from the canopy, it is unclear why this pattern was not observed for WSB. It could be due to my concentrations being 5x to 10x lower than those found by LeMellec (2011). However, the samples dates with highest DOC coincided with the highest SRP concentrations, again suggesting hydrologic control over DOC delivery to forest floors in this study system. Another possibility is that while my data may not show that WSB increased throughfall DOC, they could still be adding carbon to the forest floor via frass (LeMellec et al 2009). During a pine lappet (*Dendrolimus pini L.)* outbreak in a scots pine forest, herbivory was shown to increase DOC in throughfall, but C:N ratios were much higher in frass than in needle biomass. TOC was also higher in throughfall than DOC (LeMellec et al. 2009), and although I did not measure frass DOC, I can speculate that budworms may still have an effect on carbon entering the forest floor, just not directly in the form of throughfall DOC.

Frassfall and Litterfall

Peak litter fall during the months that I sampled (June to November) occurred for both high and low budworm areas during late fall, but litter fall was greater for low budworm sites compared to high budworm sites. In contrast, peak frass fall for those times was greater in high budworm sites during the summer budworm feeding season. In the southern Appalachians native herbivores cause peak frass input between the months of June and August with temporal differences attributed to the elevation gradient and correspondingly different growing seasons (Hunter et al. 2003). Moreover, increased frass deposition during the growing season was positively correlated with throughfall nitrogen inputs (Hunter et al. 2003), consistent with the ammonium and nitrate throughfall patterns I observed. Because frass has fewer complex organic molecules that need to be broken down, it is readily susceptible to leaching (Hunter et al. 2003) allowing for quick microbial immobilization of ammonium, and the possibility of nitrate export to local watersheds. The consistent findings of higher throughfall ammonium and nitrate and more frass deposition during feeding strongly suggest that frass is a mechanism for inorganic nitrogen delivery to forest soils.

Leaf Litter Decomposition

I had hypothesized that decomposition would occur at a faster rate in high herbivory areas, as herbivory would decrease forest canopy to allow more water to reach the forest floor, and herbivory would increase nitrogen to stimulate bacterial growth (Lovett et al. 1995). My results showed the opposite effect whereby litter in high budworm sites decomposed at a slower rate than litter in low budworm sites. Interestingly cottonwood leaf litter decomposition in response to galling aphids

(*Pemphigus betae*) was 34-40% slower than non-galled leaf litter (Schweitzer et al. 2005) suggesting that leaf litter from defoliated trees might have reduced quality. Although I used standardized leaf packs across study sites, perhaps leachates from defoliated trees slowed decomposition, however I cannot test that hypothesis because I only measured DOC concentration and not DOC quality. Fungi are less able to contribute to decomposition in N-rich environments (Diepen et al. 2017), suggesting that as more N enters the soil from throughfall, decomposition rates could decrease, which could also decrease decomposition in high budworm sites. This was further supported by my regression analysis (Figure 6). As total DIN deposition increased, the rate of decomposition decreased. I had hypothesized that microclimate differences associated with herbivory (i.e., open canopy allowing more light and/or water to reach the forest floor) would influence decomposition, ultimately but others have argued that climate has a small effect on late stage decomposition so the rate of decay should not increase (Berg and Meentemeyer 2002). A study compared the Hubbard Brookes Experimental forest to a forest in North Carolina, and found that the rate of sugar maple decomposition had a k value of -0.25, about twice as fast as mine, but that decomposition could vary based on N concentrations in the soil, in leaf biomass, lignin in leaf biomass, and/or lignin to N ratios, making it hard to attribute decomposition rates to N alone (Melillo et al. 1982). Overall, my data support the idea that there is a limited role for WSB to play in litter decomposition, possibly due to the dry summers which limit water availability and slow decomposition rates.

Soil Moisture and Organic Matter

I hypothesized that budworms would affect soil moisture, but I found no evidence for this. As defoliation by budworms occurred, the canopy could have had more openings for moisture to reach the forest floor during rain events, thus increasing soil moisture during wet periods and decreasing soil moisture during warmer dry periods. While I did see an association between large rainfall events and higher soil moisture, for example on 11 Oct 15 and 4-Aug-16, in high impact sites and on 8-May-16 and 6-Nov-16 in low impact sites, there was no consistent difference between high and low impact sites. It is possible that even in high herbivory sites, most herbivory occurs in the new growth on the fringe of the trees, so herbivory might not open the canopy significantly until it becomes heavy enough to kill trees. Alternatively, in this mountainous environments, nearby sites might have had enough differences in microclimate to exceed any variability caused by budworms.

I hypothesized that more frass input would lead to higher soil organic matter content. Despite seeing higher frass deposition in high budworm sites, I did not observe any differences in soil organic matter content between high and low budworm sites. Due to the possibly lower carbon quality of consumed leaf litter (Schweitzer et al. 2005), it is possible that frass had not decomposed enough to enter the soil organic matter pool in my relatively short study. Alternatively, herbivores might not have a consistent effect on soil organic matter. For example, grasslands worldwide show that soil organic carbon can increase, decrease, or stay the same in the presence of herbivory by grazers, over a wide range of temperatures, precipitation levels, and elevation, but bulk density either

increases or stays the same in response to herbivory (Piñeiro et al. 2010). Regardless of the mechanism, budworm herbivory does not appear to influence soil organic matter.

Soil Nutrients

I hypothesized that soil nutrients would increase in the presence of budworms, but this did not occur for ammonium despite higher throughfall ammonium concentrations. The lack of consistency between throughfall and soil ammonium concentrations suggests very high rates of immobilization of ammonium via soil bacterial production or understory plant uptake. There was, however, a significant sample event effect indicating that ammonium concentrations differed through time. Highest concentrations of soil ammonium occurred on 8 May 2016 and 6 Nov 16, which are before and after the growing season, respectively. Higher soil ammonium during these times may represent reduced plant uptake from soil ammonium pools and/or net mineralization. My resin bags indicated net mineralization especially over winter when high soil moisture could support microbial activity, but that it was not significantly affected by budworms, which is consistent with the observed patterns in bulk soil ammonium. A study in Puerto Rico found similar results whereby increases in soil NH_4^+ were not related to herbivory (Fonte 2003).

Soil nitrate concentrations interacted between sample event and budworm herbivory, but concentrations were relatively low throughout the study. Nitrate is taken up at similar rates during growing season (Nadelhoffer et al. 1984), which could explain the low concentrations for most of the sample dates. Similar to ammonium, there did not seem to be a concordance between throughfall and soil nitrate concentration. For example, on 8 May 2016 and 13 June 2016 pulses of throughfall nitrate were not seen in

the soil, suggesting rapid microbial immobilization or plant uptake into biomass. I did observe two pulses of nitrate in soils, one during the growing season (August 2016) and one right before winter after the growing season (November 2016). There was a large rainfall event just prior to both sampling events, and rainfall has been shown to leach nitrates into the soil solution (Wang et al. 2010) with potential for runoff to streams (Wang 2020). Like soil ammonium concentration patterns, soil nitrate suggested rapid immobilization of N leached from the canopy, which implies a relatively tight link between canopy N losses via WSB followed by soil and/or plant retention of canopy-leached N. Soil resin bags indicated more net nitrification between spring and fall across all sites regardless of budworm activity, suggesting that herbivory played a limited role in nitrification, unlike other studies that showed a significant link (Hunter et al. 2003), consistent with what appears to be rapid immobilization.

SRP concentrations in soils were higher in high budworm sites, supporting my hypothesis. SRP was higher in high budworm sites at all sample dates, a trend that has also been seen in tropical forests experiencing herbivory (Metcalfe et al. 2013). Because SRP throughfall concentration did not differ by herbivory level in my study, it suggests that WSB in highly impacted areas are adding more phosphorous to soils in particulate forms such as frass, molts, dead adults, or damaged leaf litter than can be taken up by soil microbes as seen in other systems (Metcalfe et al. 2013). A study with potted Douglas fir seedlings found that in soils containing high levels of weathered basalt, WSB increased soil P concentrations (Kolb et al. 1999), suggesting that budworms can increase soil P in systems that are not limited by P. The central Cascade region is high in weathered basalt, which coupled with apparently rapid immobilization of inorganic N, would suggest that

high budworm sites are not P limited. In systems not limited by P, excess P has the potential to be leached into the nearby streams during rain or snow melt, and excessive P leaching can lead to eutrophic downstream systems. Finally, while evidence suggests a role for WSB to influence soil P concentration, because my study sites are not interspersed between the Swauk and Teanaway drainages due to where budworms were active, I cannot dismiss the possibility that Swauk soils generally have higher P than Teanaway soils in the absence of budworms.

Limitations

All samples were taken during the months of late April to early November because of site inaccessibility during winter. This leaves four and half months of the year out of my sampling scheme. As snowmelt beings to occur in the spring and microbial metabolism starts to pick up, there is the potential for N-transformations and runoff that are not shown in this study, which could change interpretations of how budworms affect soils chemistry and the potential for export to streams. Litterfall results could also be interpreted differently because there is a large amount of litter that falls to the forest floor during the winter months (Reukema 1964; Weiskittel and Maguire 2007) that I was not able to measure.

In addition to seasonal limitations, because soil order and soil horizons affect nutrient-soil interactions, lack of soil characterization makes it difficult to draw concrete conclusions about my results. For example, soils with high clay content have the ability to bind positively charged ions such as NH_4^+, so knowing the clay content could help interpret my results. Soil texture and soil composition are also likely to support different soil microbes and fungi, which would have different effects on soil nutrients. Even by

characterizing soil by order, I would have been able to draw a few more general conclusions about nutrient interactions and how they could be influenced by budworm herbivory. The depth at which I took my soil samples could also have influenced my results. While my resin bags did show net nitrification, a study in an oak forest in Georgia cited potential error with resin bags, stating that shallow depth could have missed nitrification. Because my samples were only 10 cm deep (Frost and Hunter 2004), there may have been more nitrification occurring in deeper soil layers than I measured.

Although I ran regression analyses for soil and air temperature, the temperature measurements I had were spot measurements, and therefore provide very little information on what is occurring in both the soil and the air throughout the day and throughout the seasons. I should have deployed temperature loggers and left them throughout the duration of the study, as well as air temperature monitors so that I could capture information about the difference between high and low temperatures on a diel basis at each site. I cannot use Forest Service data for air temperature because of site differences, which could lead to even more inaccurate conclusions.

I gathered all of my samples at the end of the WSB outbreak in the Swuak Drainage, and it is possible that I saw a lessened effect from WSB due to missing the peak outbreak event. It is also possible that my background locations in the Teanaway had a residual effect of a WSB outbreak three years prior in that area. A study looking at tree ring growth in response to defoliation events has been show that there is a lag time between an outbreak effect on tree growth (Swetnam et al. 1995), and so it is possible that nutrient concentrations would have a lag time response as well.

Future Studies

Future studies could expand on the nutrients measured to include organic N and P, to help support the findings in this study that only looked at inorganic N and P. Other ions could also be recorded to help characterize the soil and to look at potential accumulation of PO_4^{3-} as it can bind to positively charged ions including K, Ca, Mg, and Fe. Looking at these ions can also provide information on export potential.

In additional to looking at nutrients, a study to look at the invertebrate, fungal and microbial communities in the forest soil to help support missing aspects of this study, such as what happens to the inorganic nutrients. It would give more insight as to whether they are being incorporated into those communities or being exported into stream systems, yielding greater insight into the nuanced effects of WSB herbivory on forest ecosystems.

Doing a manipulated frass leaching experiment in addition to this field study model would more clearly outline whether or not budworm frass contributes significant nutrient deposition to the forest floor, or if budworm frass has little to no effect on soil chemistry. By adding frass and leaching it, as well as having no frass controls in both high impact and low impact sites, there would be more concrete evidence of the direct effect budworms are having while avoiding the interspersion problem of this study.

Finally, future studies should include sampling at the beginning, during the peak, and at the end of an outbreak and should use specialized equipment to measure over the winter to provide more evidence as to whether or not these native herbivores have a significant effect on Central Washington terrestrial forest systems.

Conclusion

This study thoroughly investigated the effects of WSB on throughfall and soil nutrients, and their implications in both forest soil health and stream ecosystem health. I hypothesized that budworms would increase throughfall nutrients, and budworms had an effect during the growing and feeding season and this supported my hypothesis of increased throughfall N as seen in the interaction between these native defoliators and the times that I sampled also affected throughfall N, as well as soil NO_3^-. Budworms did not have an effect on throughfall SRP, DOC, or soil moisture, but sample date did. I also hypothesized that budworm defoliation would increase soil temperature by allowing more light in, but this was not seen. This could be because while more radiation made its way to the forest floor during the light hours, during the dark hours of the day, radiation escaped at a faster rate, thus not being retained, and ultimately not affecting soil temperature. I hypothesized that budworms would increase the rate of decomposition, and there was a significant relationship between the rate of decomposition and WSB, but the effect was the opposite of what I hypothesized; in the presence of WSB, the rate of decomposition was slower than in areas not affected by the herbivore. My last hypothesis was that budworms would increase soil nutrients. Soil NO_3^- had a significant interaction, supporting my hypothesis. Soil moisture and NH_4^+ were affected by sample date, and while soil SRP had a significant output for budworms, I cannot attribute it to budworm interaction because of lack of interspersed sites.

Unfortunately, I sampled the last two years of a major WSB outbreak, and due to a decline in severity, it is possible that I was not able to capture the full effect of WSB on

the forest ecosystem that I studied. Moreover, while I studied the budworm outbreak in Swauk drainage, in prior years budworms were active in the Teanaway (my low budworm sites) so there might have been lingering budworm effects despite no active defoliation. Based on my data, I can conclude that WSB have the potential to affect N levels in throughfall, and as outbreak severity is expected to increase due to human driven climate change, there is the possibility that these insects will have even greater effects on the N cycle within the forest increasing the possibility that they could affect forest streams as well. Potential changes in forest ecosystem dynamics in response to budworms could lead to the need for management practices. Traditional logging practices in the area of cutting only the biggest and oldest trees in the area and leaving a homogeneous age group of trees as discussed earlier could be changed. By logging trees of different sizes and ages, as well as selectively logging instead of clear cutting, a more open stand, heterogeneous forest could provide protection from future outbreaks, or minimize outbreak severity (Pettit et al. 2020).

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