EFFECTS OF THE WESTERN SPRUCE BUDWORM ON NITROGEN CYCLING IN CENTRAL WASHINGTON

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

A Thesis

Presented to

The Graduate Faculty

Central Washington University

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

Biological Sciences

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

by

Izak Roland Neziri

July 2020

CENTRAL WASHINGTON UNIVERSITY

Graduate Studies

We hereby approve the thesis of

Izak Roland Neziri

Candidate for the degree of Master of Science

APPROVED FOR THE GRADUATE FACULTY

\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Dr. Clay Arango, Committee Chair

\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Dr. Karl Lillquist

\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Dr. Paul James

\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Dean of Graduate Studies

ABSTRACT

EFFECTS OF THE WESTERN SPRUCE BUDWORM ON NITROGEN CYCLING IN CENTRAL WASHINGTON

by

Izak Roland Neziri

January 2020

ACKNOWLEDGMENTS

I would like to thank Dr. Clay Arango for taking me on as a graduate student under his budget and for helping me being my project related to the NSF EAGER Grant and his lab assistants for helping me with my research. I would also like to thank my committee members Dr. Karl Lillquist, and Dr. Paul James for helping me with my proposal and for offering advice on ways that I can improve my research. I also could not have done this without the people who helped me collect and process samples during the course of this project so a special thank you to Julia Bramstedt, Michael Dallas, Natalie Levesque, Antonia Stewart, and Sarah Trikha for all of your time and effort in helping me complete my thesis project.

TABLE OF CONTENTS

Chapter Page

I INTRODUCTION 1

First-Order Subheading 3

First-Order Subheading 4

First-Order Subheading 6

II METHODS 30

First-Order Subheading 30

First-Order Subheading 46

First-Order Subheading 55

III RESULTS 60

First-Order Subheading 60

First-Order Subheading 67

First-Order Subheading 75

IV DISCUSSION 78

First-Order Subheading 78

First-Order Subheading 81

First-Order Subheading 85

REFERENCES 91

LIST OF TABLES

Table Page

1 Title of table—tables may be titled using sentence style or headline

style capitalization, depending on the rules of your style guide 60

2 Title of table 61

3 Title of table 63

4 Title of table 64

5 Title of table 67

6 Title of table 69

7 Title of table 70

8 Title of table 72

9 Title of table 72

10 Title of table—some titles may wrap to more than one line, so use

this one as a model—multiple-line titles should be formatted in

inverted pyramid style (descending lines get shorter) 73

11 Title of table 74

LIST OF FIGURES

Figure Page

1 Title of figure—figures are captioned using sentence style

capitalization 1

2 Title of figure 4

3 Title of figure 60

4 Title of figure 63

5 Title of figure 65

6 Title of figure 66

7 Title of figure 72

8 Title of figure 73

10 Title of figure—some captions may wrap to more than one line, so use

this one as a model—multiple-line captions should be formatted in

inverted pyramid style (descending lines get shorter) 75

11 Title of figure 75

LIST OF EQUATIONS

EQUATION Page

1 Title of figure—figures are captioned using sentence style

capitalization 1

2 Title of figure 4

3 Title of figure 60

4 Title of figure 63

10 Title of figure—some captions may wrap to more than one line, so use

this one as a model—multiple-line captions should be formatted in

inverted pyramid style (descending lines get shorter) 75

11 Title of figure 75

**I**

**INTRODUCTION**

The process of defoliation is an important part of forest ecosystem health and function. Defoliators such as herbivorous insects act as a negative feedback loop for forests when they become too thick by killing trees and reducing the population to lower levels (CITATION). Defoliation is an important part of material cycling in forests by returning nutrients in organic matter to soils through consumption of the canopy and excretion as frass. Although defoliators are a natural part of forest material cycling, trends towards WHAT are being seen. This can be attributed to the rate at which insect outbreaks are occurring as well as outbreak severity—which has increased dramatically over the last century (Senf et al. 2016).

Since the 1930s, intense fire suppression throughout the West has led to thicker forests with increased canopy cover (Keane et al, 2002). High frequency, low intensity wildfires that formerly maintained an open forest stand occur less often, leading to increased incidence of forest insect pests. Historic fire regimes used to maintain insect pests via two avenues. First, frequent low intensity fires increased distance between trees making it challenging for insects to disperse. This decreased the rate at which (defoliators) damaged the forest. Secondly, fires killed pests directly. A multi-decadal history of fire suppression, coupled with summer drought stress due to climate change, has generated conditions that encourage sustained insect outbreaks and disease in the forest (Keane et al, 2002). As climate change progresses, theses insect outbreaks are expected to intensify (CITATION).

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)*—a native lepidopteran that ranges 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*). They are known to feed on a handful of other species as well (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. In a more natural fire regime that maintained an open forest structure, WSB outbreaks would occur about once every decade (CITATION find Flowers 2014? Paper..i think she cites 30 years). In recent years, thicker forests from fire suppression and increased drought stress from climate change have created conditions that encourage more frequent and widespread WSB outbreaks (Willis et al, 2008; Lovett et al, 2006). Furthermore, the cold weather that would have normally killed off pests in the past is occurring less often. This allows these pests to stay out longer, causing more damage to plants more often than they otherwise would (Griffin and Turner, 2012). This shift in forest structure and herbivore behavior has the potential to change forest ecosystem nutrient dynamics with implications for forest-stream connectivity. It has also been suggested that pest outbreaks can lead to increased fires due to the dead and dying trees they leave behind (Schlesinger et al, 2015), but new research has shown that this may not be the case, and in fact may have the opposite effect. These insects are defoliators as opposed to wood burrowers and therefore potentially have different effects on ecosystem dynamics.

This study examined some of the possible ecological effects of sustained WSB herbivory—including the rate of decomposition of mixed conifer needles to see whether or not that rate is increasing in areas highly impacted by WSB meaning that more nutrients would be added to the system. Under non WSB conditions, leaf litter would fall to the forest floor and be broken down by microbes over time, gradually releasing nutrients into the soil. Areas highly impacted by WSB have the potential to lead to increased nutrient availability in soils due to the large amounts of frass that these defoliators excrete that then falls to the forest floor. Once rainfall occurs, the leaching of frass frees up those nutrients, making them available for the forest system to use. From there, if nitrogen amounts are decreasing (net mineralization) then it can be inferred that nitrogen is taking the form of ammonium (NH4+) and is be taken up by plants and bacterial immobilization. If nitrogen levels are increasing (net nitrification) then it can be inferred that it is taking the form of nitrate (NO3-) that can then be exported to streams (Lewis and Likens, 2006). Defoliation by WSB also has the potential to increase microbial activity via the changing of an ecosystem’s chemistry through allowing more light and rainfall to reach the forest floor, in turn leading to a quicker break down in litter (Chapman et al, 2013). Pests, mixed with the current drought in the region are likely to alter the areas nutrient cycles on the forest floor as well as in soils (Schlesinger et al, 2015).

Any time an ecosystem experiences a major disturbance, there is an overall change in balance, leading to implications for both wildlife and for human concerns. It has been shown that in fish, removing even one key species in the food web can greatly alter an ecosystem's health (Taylor et al, 2006). If the WSB are altering the nitrogen and phosphorous cycles in soils, it is important to know how the process happens. Looking at total phosphorus, net nitrification/net mineralization, canopy damage, and decomposition rates will help to offer explanations as to the nature of the cycle change. This can show where there might be potential problems and may help lead to suggestions as to how we might be able to manage this pest outbreak. As outbreaks occur, there is a shift in biomass. Through knowing the degree of shift, we can then look at overall litter quality to provide more explanations of the effects of these pests (Genung et al, 2013).

Little research has been done on the western spruce budworm. Griffin and Turner (2012) did an extensive field study on *Dendroctonus pseudotsugae* (Douglas fir beetle) and *Dendroctonus ponderosae* (Mountain pine beetle) and found that herbivorous insect outbreaks cause noticeable changes to soil nitrogen cycling (2012). There is also no evidence that the new growth of conifers is occurring earlier or that it is lasting for longer in our region as it is in many flowering plants on the East Coast (Miller-Rushing and Primack, 2008).

To summarize, this study is important to local soil ecosystem dynamics. By looking at the rate of decomposition, it is possible to see if the rate of conifer leaf breakdown is influenced by herbivory and microbial activity Leading to the addition of supplemental nutrients to the soil. We can measure whether those soil nutrients are being taken up by plants or are accumulating with potential to enter the stream due to runoff to monitor changes in stream chemistry and the community food web. From that information we can look at whether those changes are significant and whether we should be concerned with the WSB outbreaks.

In order to belter understand how outbreak insects affect forest internal forest nutrient cycles, I studied how WSB feeding affected throughfall nutrient composition, leaf litter decomposition rate, soil chemistry, and net nitrification in the eastern Cascades of central Washington. In general, I hypothesized that WSB activity would: 1) increase throughfall nutrient concentration, 2) increase litter decomposition rate, 3) increase soil nutrient concentrations, and 4) increase net nitrification in soils.

**II**

**METHODS**

Study Area

This study took place in the eastern Cascades in central Washington state. In the rainshadow of the Cascades, summers (May-September) are relatively dry, with seasonal drought 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, NOAA, https://www. ncdc.noaa.gov,accessed 7 September 2018) with most falling as snow between month-month. 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*).

I used a nested study design with repeated sampling through time to investigate how budworm herbivory influenced throughfall composition, litter decomposition, and soil nutrient concentrations. I established 4 study sites each within low and high budworm herbivory level stands (n=8 study sites), 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). 1 1access difficulty

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, 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). Thus my study design included measurements taken before, during, and after, one complete WSB life cycle.

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

Throughfall

A throughfall collector was installed under the canopy of a randomly selected tree close to each sample plot (n=24). Each throughfall collector consisted of a funnel (20 mm diameter) that drained through tygon tubing into a 4-L acid-washed collection jug. 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 the low budworm study sites and two in the high budworm study sites.

After 4 samples in 2015, throughfall and rainfall collectors were taken down November 8, 2015 to prevent damage to the apparatus due to snowpack , and they were redeployed April 23, 2016 just after snowmelt to begin sampling again. All collectors were taken down on November 5, 2016 after collecting X sample 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 m2 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/m2d or mg litter/m2d). Frass collectors were taken down in November 5, 2015 to prevent damage during winter snow accumulation, 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 plots. Ten bags at each plot 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 are commonly used in decomposition studies for comparison across biomes (Webster and Benfield 1969; Graça et al, 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 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. 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 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 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 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 rate was calculated as:

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

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, 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 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 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 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.

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

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.

**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 November 2015, and fresh bags were deployed in November 2015 and removed in April 2016. Net changes in the inorganic N pool were calculated as:

**Equation 4:** Where N is the combination of ammonium and nitrate.

(Griffin and Turner, 2012) Net nitrification was indicated by … and net mineralization was indicated by … etc.

Soil Nutrient Chemistry 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 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. Samples were analyzed for NO3-+NO2- (hereafter referred to as NO3-) using the cadmium reduction method (CITATION) and NH4+ using the phenate method (CITATION) on a Seal AQ1 Discrete Analyzer (Seal AQ1, Seal Analytical; Mequon, Wisconsin, USA).

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 mL of the Bray P1 extractant solution (30 mLs 1 N NH4F 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. 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).

Statistical Analysis

All data was analyzed in R version 3.6.2 (CITATION). Throughfall was analyzed using XXX (package). Frass and litterfall was compared using a generalized least squares (GLS) model (package). Decomposition was analyzed using a linear model (LM) with leaf type and location as factors as well as looking at the interaction between impact and leaf type. 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, NO3-, NH4+, SRP, N:P ratio, and net nitrification/mineralization through time. To optimize models, I compared alternate model structures with an interaction between impact factors and sample event and models with a nested design (Zuur XXXX). 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 (package) was used as a post hoc test on data to determine which sample events differed significantly. All statistical tests were evaluated against  = 0.05.

**III**

**RESULTS**

Throughfall Chemistry

A screenshot of a cell phone

Description automatically generated

**Figure 2:** Estimated marginal means (EMM) of (A) throughfall ammonium concentrations and (B) throughfall nitrate (NO3-) concentrations in low and high budworm stands by sample date. Significant interactions are noted with an asterisk.

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 two-year study (Figure XA). 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 NH4+ 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 NH4+ concentration. Throughfall nitrate differed by sample event (LME, p<0.001) but not budworm activity level throughout the course of the two-year study (Figure XB). There was a significant interaction (LME, p<0.001) whereby the low budworm stands had higher concentration NO3- on 8 May 16, but the high budworm stands had higher concentration on 13 Jul 16, 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 3:** Estimated marginal means (EMM) of (A) throughfall soluble reactive phosphorous (SRP) concentration and (B) dissolved organic carbon (DOC) concentration in low and high budworm stands by sample date. Significant differences among sample events are noted with letters.

Throughfall SRP concentration differed by sample event (LME, p<0.001) throughout the course of the two-year study with highest concentration 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). 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). 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 the two rainfall events with the most water collected.

Decomposition Rates



**Figure 4:** Decomposition rates (-*k*) of deciduous and coniferous leaf litter in high and low budworm sites.

Decomposition 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 X). The mass of DIN deposited by throughfall was positively associated with the deciduous decomposition rate (R2=.15, p=0.033; Figure X) but not the coniferous decomposition rate (p=0.13), and the decomposition rate for both leaf types was unrelated to rsampled

![A close up of a map

Description automatically generated]()

**Figure 5:** Regression analysis of throughfall DIN and deciduous decomposition rate.

Soil Chemistry:



**Figure 6:** Estimated marginal means (EMM) of soil (A) ammonium (NH4+), (B) nitrate (NO3-), and (C) soluble reactive phosphorous (SRP) 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.

Soil ammonium concentrations differed by sample date (LME, p<0.001) with higher concentrations on 8 Nov 15 and 8 May 16 compared to 13 Jun 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 end of and the beginning 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 NO3- concentration than low budworm sites on 6 Nov 16 whereas as low budworm sites had higher NO3-on 4 Aug 16. Usually soil NH4+ was 60 times higher than soil NO3-. Soil SRP was significantly higher in high impact sites for every sample event (p=0.047, LME) but did not differ by sample event (p=0.91). Changes in the soil N pool indicated net nitrification (instead of net immobilization or net mineralization), but net nitrification did not differ by budworm impact (p=0.53, LME) despite the very high NO3- value on 6 Nov 16, suggesting an alternate source for that recorded NO3- spike.



Figure 7: Estimated marginal means (EMM) of (A) soil moisture and (B) soil organic matter in low and high budworm stands by sample date. Significantly different sample events are noted with letters.

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 \_ A). 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 \_ B). 

Figure 8: A regression analysis comparing soil temperature at 2 cm depth and air temperature (p<0.0001, R2 of 0.78).

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 (R2 = 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 deepest measurement at 10 cm (data not shown).

**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 seemed to positively influence N loss from the canopy, especially for NH4+, WSB did not seem to influence , but higher concentrations of these in throughfall were seen in two heavy rainfall events suggesting hydrologic control. Unexpectedly, decomposition rates were faster low budworm sites compared to high budworm sites for non-native deciduous litter and for native coniferous litter. Decomposition of deciduous litter was additionally positively 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. Unexpectedly, budworms did not influence net nitrification rate, but soil phosphorus concentrations were clearly higher in high compared to low budworm sites.

Throughfall Nitrogen

suggesting an N limited system.

This could be due to fluctuation of nitrification and mineralization throughout the duration of this study. These results are similar to soil nitrogen, as we also see a lot of variability in NH4 as well. In addition to the similarities between throughfall and soil NH4, NO3 concentrations were also comparable. I saw the same peaks in the nitrate throughfall as I did in the soil, and confirmed this using the ion exchange resin beads, again seeing two large pulses of nitrate entering the system. Although we see spikes in the data shape, the concentrations are still relatively low in comparison to SRP.

Throughfall SRP

which was not what I hypothesized. I would have expected that phosphorus levels would be higher in heavily impacted areas due to an increase in frass input.

Throughfall DOC

which in turn will eventually be exported to streams, which has the potential to increase stream metabolism.

Leaf Litter Decomposition

I had hypothesized that in high herbivory areas, decomposition would occur at a faster rate, as a decrease in forest canopy would allow more water to reach the forest floor, simulating fungal and bacterial growth (Source). It is possible that with less cover, greater amounts of light could reach the forest floor during the warmer months, drying out the forest floor, and slowing the rate of decay (Source).

Soil Nitrogen

This could be due to fluctuation of nitrification and mineralization throughout the duration of this study, and it suggests N limitation. More available NH4 would suggest there is more potential for nitrification.

Soil tends to have less nitrogen during the winter (<https://link.springer.com/article/10.1007/BF02183092>) but the data did not follow that pattern as seen in the late fall and early spring sampling events. I do not suspect that plants were taking up extra nitrogen during that time as production rates tend to be lower in the cooler months (<https://link.springer.com/article/10.1007/s00442-005-0044-1>). Since throughfall samples showed low NH4 input during these sampling events, I can only attribute the increase in soil NH4 to nitrogen-fixing microorganisms due to N limitation.

Soil SRP

There is potential for SRP to be washed into the nearby streams during rain events. Although SRP is important for productivity in stream ecosystems, an excess amount of SRP can lead to over productive systems, causing algae blooms, which will eventually lead to mass die off events and oxygen depletion. This also suggests that due to accumulating levels of soil SRP, it is not a limiting resource in the soil systems that I studied.

because this was not seen in the SRP samples from throughfall, it suggests that the WSB in highly impacted areas are adding more phosphorous than can be taken up by soil microbes

Future Studies

This study thoroughly investigated soil and throughfall nutrients, and their implications in both forest soil health and stream ecosystem health. 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.

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 us more insight as to whether they are being incorporated into those communities or being exported into stream systems, having different implications for the effects of WSB on forest ecosystems.

**REFERENCES**

Taylor, B. W. Flecker, A. S. Hall Jr., R. O. 2006. Loss of a Harvested Fish Species Disrupts Carbon Flow in a Diverse Tropical River. Science, 313, 833-836.

Miller-Rushing, A. J. Primack, R. B. 2008. Global Warming and Flowering Times in Thoreau’s Concord: Community Perspective. Ecology, 89(2), 332-341.

Zhao, T. Krokene, P. Hu, J. Christiansen, E. Bjorklund, N. Langstrom, B. Solheim, H. Borg-Karlson, A.K. (2011). Induced Terpene Accumulation in Norway Spruce Inhibits Bark Beetle Colonization in a Dose-Dependent Manner. Plos One, 6(10), 1-8.

Benfield, E. F. 1996. Leaf Breakdown in Stream Ecosystems. Methods in Stream Ecology, 579-589.

Willis, C. G. Ruhfel, B. Primack, R. B. Miller-Rushing, A. J. Davis, C. C. 2008. Phylogenetic patterns of species loss in Thoreau’s woods are driven by climate change. PNAS, 105(44), 17029-17033.

Smith, R. M. Kaushal, S. S. 2015. Carbon cycle of an urban watershed: exports, sources, and metabolism. Biogeochemistry, DOI 10.1007/s10533-015-0151-y.

Genung, M. A. Bailey, J. K. Schweitzer, J. A. 2013. The Afterlife of Interspecific Indirect Genetic Effects: Genotype Interactions Alter Litter Quality with Consequences for Decomposition and Nutrient Dynamics. Plos, DOI 10.1371/journal.pone.0053718.

Lewis, G.P. Likens, G. E. 2006. Changes in stream chemistry associated with insect defoliation in a Pennsylvania hemlock-hardwoods forest. Forest Ecology and Management, 238, 199-211.

Lovett, G. M. Canham, C. D. Arthur, M. A, Weathers, K. C. Fitzhuge, R. D. 2006. Forest Ecosystem Response to Exotic Pests and Pathogens in Eastern North America. BioScience, 56(5), 395-405.Bott, T. L. 1996. Primary Productivity and Community Respiration. Methods in Stream Ecology, 533-556.

Liu, L. Wang, X. Lajeunesse, M. J. Miao, G. Piao, S. Wan, S. Wu, Y. Wang, Z. Yand, S. Li, P. Deng, M. 2015. A cross-biome synthesis of soil respiration and its determinants under simulated precipitation changes. DOI 10.1111/gcb.13156.

Webster, J. R. Ehrman, T. P. 1996. Solute Dynamics. Methods in Stream Ecology, 145-160.

Hamilton, S. 1997. Analysis of nitrate in Michigan waters.

Hamiltion, S. 1997. Combined persulfate digestion for analysis of total nitrogen and phosphorous in Michigan waters.

Graça, Manuel A.S., Bärlocher, Felix, Gessner, Mark O. 2005. Methods to Study Litter Decomposition: A Practical Guide.

Griffin, J. M. Turner, M. G. 2012. Changes to the N cycle following bark beetle outbreaks in two contrasting conifer forest types. Oecologia, 170, 551-565.

Goodale, C. L. Fredriksen, G. Weiss, M. S. McCalley, C. K. Sparks, J. P. Thomas, S. A. 2015. Soil Process drive seasonal variation in retention of 15N tracers in a deciduous forest catchment. Ecology, 96(10), 2653-2668.

Schlesinger, W. H. Dietze, M. C. Jackson, R. B. Phillips, R. P. Rhoades, C. C. Rustad, L. E. Vose, J. M. 2015. Forest Biogeochemistry in Response to Drought. DOI 10.1111/gcb.13105.

Chapman, S. K. Newman, G. S. Hart, S. C. Schweitzer, J. A. Koch, G. W. 2013. Leaf Litter Mixtures Alter Microbial Community Development: Mechanisms for Non-Additive Effects in Litter Decomposition. Plos, DOI 10.1371/journal.pone.0062671.

Schweitzer, J. A. Bailey, J. K. Hart, S. C. Whitham, T. G. 2005. Nonadditive Effects of Mixing Cottonwood Genotypes on Litter Decomposition and Nutrient Dynamics. Ecology, 86(10), 2834-2840.

Johnson, L. T. Tank, J. L. Dodds, W. K. 2009. The influence of land use on stream biofilm nutrient limitation across eight North American ecoregions. Can. J. Fish. Aquat. Sci., 66, 1081-1094.

Schweitzer, J. A. Bailey, J. K. Hart, S. C. Wimp, G. M. Chapman, S. K. Whitham, T. G. 2005. The interaction of plant genotype and herbivory decelerate leaf litter decomposition and alter nutrient dynamics. Oikos, 110(1), 133-145.

Kryskalla, J. R. Patton, C. J. 2003, Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory---Evaluation of Alkaline Persulfate Digestion as an Alternative to Kjeldahl Digestion for Determination of Total and Dissolved Nitrogen and Phosphorus in Water. USGS, 1-33.

Senf, C. Campbell, E. M. Pflugmacher, D. Wulder, M. A. Hostert, P. 2016.

A multi-scale analysis of western spruce budworm outbreak dynamics. Landscape Ecol, 32, 501-514.