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

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by

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ABSTRACT

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

by

Izak Roland Neziri

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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 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

9 Title of figure 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**

Forests make up approximately one third of the surface of the earth (Likens et al 1970). In these ecosystems, nutrients are generally retain a large portion of the nutrients within that system. The general flow of nutrients are precipitation to throughfall to soil, with those nutrients being uptaken by plants and converted into biomass. When these systems are subjected to disturbances, the cycling of forest nutrients is subject to changes and losses. Common disturbances include: fire, human activity such as pollution, clear cut logging, and human driven climate change, and insects.

Clear cutting has been a common logging practice for a long time, and in 1970, an experiment was done in the Hubbard Brook Experimental Forest to look at the effects of clear cutting on runoff and it was found that in the clear cut section of this forest, that annual runoff increased dramatically in that region (Likens et al, 1970). Excessive runoff can carry increased concentrations of nitrate to local watersheds, and in this case, nitrate concentrations increased drastically, as well as concentrations of other ions. In these clear cut watersheds, Likens and others also found that the disturbed watershed had higher stream temperatures, as there was less shade, and saw an increase in turbidity, which lead to an increase in dissolved oxygen concentrations. If less canopy cover due to clear cutting can lead to increased temperature, increased decomposition rates, and increased nutrients, then it is possible that defoliating insects can also cause similar changes to forest ecosystem watersheds by removal of canopy cover.

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 (Poirier 2017) (Murdock et al. 2012). 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, we are currently seeing changes in how these insects are affecting the ecosystem due to anthropogenic driven fire suppression and climate change (Abatzolou and Williams 2016). 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).

Naturally caused fires for centuries have shaped the structure of coniferous forests across the United States. Many types of coniferous forests including ones like Pondersoa Pine forests had spaced out trees with grasses and shrubs that grew underneath. These areas were subjected to frequent fires, but low intensity fires that helped shape these ecosystems (Klenner et al 2008). Since the 1930s, intense fire suppression throughout the West has led to thicker forests with increased canopy cover (Keane et al, 2002). These high frequency, low intensity wildfires that formerly maintained an open forest stand occur less often, leading to increased incidence of forest insect pests due to thickening tree stands. 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). Decreases in winter severity due to climate change has also led to increased pest outbreaks (Murdock et al. 2012). As climate change progresses, theses insect outbreaks are expected to intensify (Flower et al 2014), as outbreaks tend to occur more often during warmer, wet time periods.

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*) in this region, but also feed on spruce needles (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 (Flowers 2014). 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. If NO3- 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 NH4+ levels are decreasing (net nitrification) then it can be inferred that it is taking the form of nitrate (NO3-) that is then subjected to leaching (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 ecosystem dynamics, 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 us to better understand the consequences of outbreaks and how we can predict future outbreaks and changes that may occur. 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).

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.

To better understand how outbreak insects affect internal forest nutrient pathways, I studied how WSB herbivory 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 accelerate the movement of nutrients which 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 rain shadow 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 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*).

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; 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 (903 m a.s.l.) where sites were on a slope with light tree cover, Jungle Creek (824 m a.s.l.) where sites were often disturbed by free range cattle, Jack Creek (963 m a.s.l.) where sites were under moderately heavy tree cover, and Moonbeam Creek (973 m a.s.l. where sites were also under moderately heavy tree cover. The high budworm sites were located in the Swauk drainage in the Okanogan-Wenatchee National Forest in Washington state approximately 45 miles north of Central Washington University and east of the low budworm sites (Figure 1). These study sites were located near the following creeks: Cougar Creek (984 m a.s.l.) where sites were on a slope, Hurley Creek (978 m a.s.l.) where sites were located further away from the stream in comparison to other sites due to the stream being less accessible in a confined valley, Hovey Creek (1050 m a.s.l.) where sites were under moderately heavy tree cover, and Blue Creek (1055 m a.s.l.) where sites were also further away from the stream due to difficulty of access. 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.

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.

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). 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.

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

**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.

**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. Net increases in NH4+ indicate net mineralization, Net increases in NO3- indicate net nitrification, and net decreases in either ion indicate immobilization of that ion (Griffin and Turner, 2012).

A 2M KCl extraction method (Keeney and Nelson 1987) 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.

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 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.

Chemical Analyses for Throughfall and Soil

Samples were analyzed for NO3-+NO2- (hereafter referred to as NO3-) using the cadmium reduction method (U.S. Environmental Protection Agency (EPA) 1993) and NH4+ using the phenate method (Solórzano, 1969) on a Seal AQ1 Discrete Analyzer (Seal AQ1, Seal Analytical; Mequon, Wisconsin, USA). 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). DOC samples were measured using infrared methods (American Public Health Association (APHA) 1995) after acidifying the samples to a pH of less than 2 to remove inorganic carbon on a total organic carbon analyzer (TOC-L Total Organic Carbon Analyzer, Shimadzu, Kyoto, Japan) (Arango et al, 2019).

Statistical Analysis

All data was analyzed in R version 3.6.1 (R Core Team 2019). Throughfall composition was analyzed using to see how patterns varied by budworm herbivory level and time. Frass and litterfall was compared using a generalized least squares (GLS) model 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, and net nitrification/mineralization through time. LM models were also used to regress decomposition rates of both deciduous and conifers leaf litter verses total N and verses total water. 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 help reduce residual patterns. 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 on data to determine which sample events differed significantly. All statistical tests were evaluated against  = 0.05.

**III**

**RESULTS**

Throughfall Chemistry



**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 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 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 2B). 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 2).



**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) (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 the two rainfall events with the most water collected.

Frass and Litter Deposition

A picture containing map, boat, photo, sitting

Description automatically generated

**Figure 4:** The deposition of frass and litter in high impact and low impact sites.

In high impact sites, frass content was greater than in low impact sites during peak herbivory times (GLS p=0.04). Once the budworm feeding season ended, as indicated by the vertical dashed line, frass input for high impact and low impact sites are the virtually the same. Low budworm site collectors contained more leaf litter than high impact sights during the cooler months where the highest concentration of litter fell during the Oct sampling dates.

Decomposition Rates



**Figure 5:** Decomposition rates (-*k*) of deciduous and coniferous leaf litter in high and low budworm sites.

![A close up of a map

Description automatically generated]()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 DIN deposited by throughfall was positively associated with the deciduous decomposition rate (R2=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 6:** Regression analysis of throughfall DIN and deciduous decomposition rate.

Soil Chemistry



**Figure 7:** 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 (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 nitrification for that recorded NO3- spike, but it did not differ between high and low.



**Figure 8:** 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 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 9:** 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 the 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 increased N loss from the canopy, especially for NH4+, WSB did not affect throughfall SRP and DOC. Instead, higher concentrations of SRP and DOC in throughfall were seen in two heavy rainfall events 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 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. Soil phosphorus concentrations were clearly higher in high compared to low budworm sites. Unexpectedly, budworms did not influence net nitrification rate.

Throughfall Nitrogen

I hypothesized that budworms would increase the amount of DIN in throughfall, and throughout this study, there was an interaction between WSB level 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 increase during defoliation events, and then decrease in the fall in the absence of defoliation, supporting my findings for WSB (Grüning et al, 2017). On a fourth date, 11 Sept 2015, I also observed higher ammonium concentrations in high budworm stand, 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 in the first major rain event in months. This was observed in subtropical wet forests in Puerto Rico (Heartsill-Scalley et al. 2007). In this study, all throughfall fluxes where positively correlated with rainfall. As rainfall decreased, throughfall decrease and as rainfall increased throughfall increased. In contrast, 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. In a regenerating southern Appalachian forest, regression analysis of NH4+ and NO3- canopy change rates showed that these nutrients are positively correlated and instead claim that they are being absorbed from precipitation suggesting uptake of ammonium, and could explain what I am seeing in the high budworm canopy sites (Potter et al, 1991).

Similar to ammonium, budworms activity interacted with sample date to affect nitrate concentrations, so a generalized conclusion cannot be drawn. Interestingly, 13 Jul 16 and 21 Jul 16 have higher nitrate in high budworm stands that coincided with higher ammonium, which could suggest canopy nitrification, similar to findings in 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 and instead offer other possible sources of nitrate could be from leaf leaching of partially consumed or damaged leaves in the canopy from mature trees. Mature tree leaves become less hydrophobic as they age, allowing 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 affects of nitrogen fertilizer on a mature spruce-hemlock forest in Maine also suggests that canopy nitrification was not responsible for nitrate increases, and instead suggested that it was due to dry deposition of nitrate being washed off by rain events (Gaige, et al 2007). 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 budworm effect. Winter moths have been shown to increase canopy N during herbivory outbreaks in Oak forests in Germany and claimed 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 growing season could also have potential for plant uptake during that time. An experiment using Gala apples showed that photosynthesis rates do increase, leading to new growth after defoliating events, 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).

Throughfall SRP and DOC

The observed pattern of throughfall SRP data does not support my hypothesis of increased SRP in high WSB sites, but sample event was significant for all sampling events. During 8 Nov 15 and 21 July 16, there were two large rainfall events, and here I saw higher concentrations of SRP. I would have 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). Not all studies have shown that herbivory always 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 did, which is 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 for PO4-, but attributed imprecise method design for a potential reason for not being up to pick up small changes (Seastedt et al. 1983).

There were also significant differences at all sample events for DOC, but no differences for budworm impact. Again, I would have expected higher DOC concentrations in high budworm impact sites as studies have shown that insect herbivory leads to increased throughfall DOC and N (LeMellec et al, 2011). Aphids and seasonal interactions have also shown increases in DOC (Kindlmann and Stadler 2004), and while I did not see a budworm impact, I did see differences in DOC based on sample date which means that there is still a possibility that budworms play a role in DOC concentrations in the canopy. During four sample events tended to be higher in high impact sites. Lepidopterous larvae have also been shown to increase DOC leachate in oak, spruce, and beech tree canopies (Stadler and Michalzik, 2001).

Frassfall and Litterfall

Peak litter fall occurred for both high and low budworm areas during late fall, but litter fall being greater for low budworm sites during that time. Peak frass fall was during the summer, and was greater in high budworm sites. Native herbivores have shown peak frass input in the southern Appalachians between the months of June and August, and attribute the differences between those months to elevation, as the growing season differs at elevation (Hunter et al, 2003). This study looked at throughfall nitrogen inputs and found that peak throughfall ammonium occurred in May, and that peak nitrate occurred in August, both in low concentrations, but were positively correlated with frass deposition in May and June. While this study showed that special-temporal effects also played a large role, when they did see positive correlations between frass inputs and nutrient availability, they were relatively strong correlations. Frass is more susceptible to leaching, as there as less complex organic molecules that need broken down (Hunter et al, 2003) allowing for quick microbial immobilization of ammonium, and nitrate export to local watersheds.

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, as well as more DOC and nitrogen simulating bacterial growth, (Lovett et al., 1995). After collecting my last decomposition litter bag, results showed the opposite effect; litter in high budworm sites decomposed at a slower rate than litter in low budworm sites. Similar results were found in cottonwood leaf litter decomposition rates in response to galling aphids (*P. betae*). It was found that galled leaf litter decomposed 34-40% slower than non-galled leaf litter (Schweitzer et al 2005), leading to decreased leaf litter quality. Climate does not have much of an effect on late stage decomposition, so even with less foliage cover, greater amounts of light reaching the forest floor, and warm dry months, the rate of decay should not increase (Berg and Meentemeyer 2002). 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. My findings are consistent with literature in that decomposition rates are lower in low budworm impact areas compared to high due to high budworm sites having lower quality leaf litter, and having high concentrations of N overall, lessening fungal decomposition ability, and suggest that WSB could have the potential to alter ecosystem nutrient dynamics if their current outbreak cycles continue.

Soil Moisture and Organic Matter

Sample event influenced soil moisture, but I would have expected budworms to also affect soil moisture, which I did not see. I would have thought that as defoliation occurred, the canopy would have more openings for moisture to reach the forest floor during rain events and increasing soil moisture, and decreasing soil moisture during warmer dry periods by letting more sunlight in through those openings. Large rainfall events led to more soil moisture. On 11 Oct 15 and 4-Aug-16, there was more soil moisture in high impacted sites, and on 8-May-16 and 6-Nov-16 there was more soil moisture in low impacted sites. These differences could be attributed to microclimate differences between the two regions.

The mean for high budworm sites did not differ significantly for soil organic matter percentage. I hypothesized that more frass input would lead to higher soil organic matter content. Grasslands worldwide have shown that soil organic carbon can increase, decrease, or stay the same in the presence of 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).

Soil Nutrients

I hypothesized that soil N would increase in the presence of budworms, and this did not occur in ammonium. Sample event significantly influenced ammonium concentrations, but bud worms did not have an effect. Similar to throughfall, there was a higher concentration of ammonium on 8 May 2016 in the low budworm sites, which is in the early growing season. Herbivores have been show to limited nitrogen availability in grasses and woody shrubs in an oak savannah by decreasing the amount of nitrogen fixing legumes (Ritchie et al, 1998), which could also explain why concentrations were lower in the high budworm sites.

Soil nitrate had an interaction between sample event and budworm herbivory. There were two pulses of nitrate, one at the end of the growing season (August 2016) and one right before winter (November 2016). 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. There was a large rainfall event just prior to the August sampling and this has been shown in the North China Plain to leach nitrates from the soil (Wang et al, 2010), with potential for run off to streams (Wang, 2020). Similar to throughfall, soil nitrate was in general, in very low concentrations, indicating low inputs from the canopy, with the exception of 8 May 2016 and June 2016, where pulses of throughfall nitrate were not seen in the soil, indicating rapid microbial immobilization or plant uptake into biomass.

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 been seen in tropical forests experiencing herbivory (Metcalfe et al, 2013). Herbivores can increase phosphorous inputs by adding frass, molts, and partially consumed litter (Metcalfe et al, 2013).

Because increases in SRP samples from throughfall were not seen, it suggests that the WSB in highly impacted areas are adding more phosphorous, potential in the form of frass or damaged leaf litter than can be taken up by soil microbes. A study with potted Douglas fir seedlings should that soils containing high levels of Basalt, WSB increased soil P concentrations (Kolb et all, 1999), suggesting that budworms can increase soil P in systems that are not limited by P. The central cascade region is high in basalt, and would suggest this is not a P limited system.

Increases in SRP have the potential to be washed into the nearby streams during rain events and 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.

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 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**

American Public Health Association (APHA). 1995. Standard methods for the examination of water and wastewater. 19th edition. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, D.C.

Andresen, L.C., Michelsen, A. Off-season uptake of nitrogen in temperate heath vegetation. Oecologia 144, 585–597 (2005). https://doi.org/10.1007/s00442-005-0044-1

Arango, C., Ponette-González, A., Neziri, I., & Bailey, J. (2019). Western spruce budworm effects on throughfall N, P, and C fluxes and soil nutrient status in the Pacific Northwest. *Canadian journal of forest research, 49*, 1207-1218. doi: [10.1139/cjfr-2018-0523](http://dx.doi.org/10.1139/cjfr-2018-0523)

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

Bray, R.H., and Kurtz, L.T. 1945. Determination of total, organic, and available forms of phosphorus in soils. Soil Sci. 59(1): 39–46. doi:10.1097/00010694194501000-00006.

Bordeleau, L.M., Prévost, D. Nodulation and nitrogen fixation in extreme environments. Plant Soil 161, 115–125 (1994). https://doi.org/10.1007/BF02183092

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.

Chen, C. W., Hudson, R. J. M., Gherini, S. A., Dean, J. D. & Goldstein, R. A. (1983). Acid rain model:

canopy module. Journal of Environmental Engineering, 109, 585-603.

Flower, A., Gavin, D.G., Heyerdahl, E.K., Parsons, R.A., and Cohn, G.M. 2014. Drought-triggeredwesternsprucebudwormoutbreaksintheinteriorPaciﬁc Northwest: a multi-century dendrochronological record. For. Ecol. and Manage. 324: 16–27. doi:10.1016/j.foreco.2014.03.042.

Flower, A., Gavin, D.G., Heyerdahl, E.K., Parsons, R.A., and Cohn, G.M. 2014. Drought-triggered western spruce budworm outbreaks in the interior Paciﬁc Northwest: a multi-century dendrochronological record. For. Ecol. and Manage. 324: 16–27. doi:10.1016/j.foreco.2014.03.042.

Gaige, E., Dail, D.B., Hollinger, D.Y. et al. Changes in Canopy Processes Following Whole-Forest Canopy Nitrogen Fertilization of a Mature Spruce-Hemlock Forest. Ecosystems 10, 1133–1147 (2007). https://doi-org.ezp.lib.cwu.edu/10.1007/s10021-007-9081-4

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.

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

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.

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.

Harvey, J.E. Axelson, J.N. Smith, D.J. 2018. Disturbance-climate relationships between wildfire and western spruce budworm in interior British Columbia. Ecosphere 9(3): e02126. 10.1002/ecs2.2126.

Hunter M.D. 2001. Insect population dynamics meets ecosystem ecology: effects of herbivory on soil nutrient dynamics. Agriculture and Forest Entomology 3, 77-84.

Hunter, M.D. Linnen, C.R. Reynolds, B.C. Effects of endemic densities of canopy herbivores on nutrient dynamics along a gradient in elevation in the southern Appalachians. Pedo biologia 47, 231-244.

Keeney, D.R., and Nelson, D.W. 1987. Nitrogen — Inorganic forms, sec. 33-3, extraction of exchangeable ammonium, nitrate, and nitrite. In Methods of Soil Analysis: Part 2, Chemical and Microbiological Properties. Agronomy, A

Arango et al. 1217

Published by NRC Research Press

Can. J. For. Res. Downloaded from www.nrcresearchpress.com by Clay Arango on 09/05/19 For personal use only.

Series of Monographs, No. 9 pt.2. Edited by A.L. Page. Soil Science Society of America, Madison, Wisconsin, USA. pp. 648–649.

Kindlmann, P. & Stadler, B. (2004) Temporal fluctuations in throughfall carbon concentrations

in a spruce forest. Ecological Modelling 176, 381-388.

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.

leMellec, A., Gerold, G. & Michalzik, B. (2011) Insect herbivory, organic matter deposition and

effects on belowground organic matter fluxes in a central European oak forest. Plant &

Soil 342, 393-403

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.

Likens, G.E., Bormann, F.H., Johnson, N.M., Fisher, D.W. and Pierce, R.S. (1970), Effects of Forest Cutting and Herbicide Treatment on Nutrient Budgets in the Hubbard Brook Watershed‐Ecosystem. Ecological Monographs, 40: 23-47. doi:10.2307/1942440

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.

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.

Lovett, G.M. & Ruesink, A.E. (1995) Carbon and Nitrogen Mineralization from Decomposing

Gypsy Moth Frass. Oecologia 104, 133-138

Metcalfe D.B. Crutsinger, G.M. Kumordzi, B.B. Wardle, D.A. 2016. Nutirent fluxes from insect herbivory increase during ecosystem retrogression in boreal forest. Ecology, 97(1) 124-132.

Miller-Rushing, A. J. Primack, R. B. 2008. Global Warming and Flowering Times in Thoreau’s Concord: Community Perspective. Ecology, 89(2), 332-341.Nadelhoffer, K. J. Aber, J. D. Melillo, J. M. 1984. Seasonal patterns of ammonium and nitrate uptake in nine temperate forest ecosystems. Plant and Soil 80, 21–335. <https://DOI.org/10.1007/BF02140039>

Piñeiro, G. Paruelo, J.M. Oesterheld, M. Jobbágy, E.G. (2010).

Pathways of Grazing Effects on Soil Organic Carbon and Nitrogen, Rangeland Ecology & Management, 63 (1), 109-119, https://doi.org/10.2111/08-255.

Poirier, L.M. 2017. Production of epicormic buds by Douglas-fir in central British Columbia, Canada, following defoliation by western spruce budworm (Lepidoptera: Tortricidae). J.Entomol. Soc. Brit. Columbia 114. 73-76

Potter, C.S. Ragsdale, H.L. Swank, W.T. 1991. Atmospheric Deposition and Foliar Leaching in a Regenerating Southern Appalachian Forest Canopy. Journal of Ecology. 79, 97-115.

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reynolds, B. C. Hunter, M. D. Crossley, D. A. Jr. 2000. Effects of Canopy Herbivory on Nutrient Cycling in a Northern Hardwood Forest in Western North Carolina. Selbyana, 21(1,2): 74-78.Ritchie, Mark E. Tilman, David Knops, Johannes M. H. HERBIVORE EFFECTS ON PLANT AND NITROGEN DYNAMICS IN OAK SAVANNA Ecology 79

doi:10.1890/0012-9658(1998)079[0165:HEOPAN]2.0.CO;2 165 177 1998

Stadler, Bernhard & Solinger, Stephan & Michalzik, Beate. (2001). Insect herbivores and the nutrient flow from the canopy to the soil in coniferous and deciduous forests. Oecologia. 126. 104-113. 10.1007/s004420000514.

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.

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.

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.

Schowalter, T. (1999). Throughfall Volume and Chemistry as Affected by Precipitation Volume, Sapling Size, and Defoliation Intensity. The Great Basin Naturalist, 59(1), 79-84. Retrieved July 8, 2020, from www.jstor.org/stable/41713087

Seastedt T.R., Crossley, D.A., Hargrove, W.W. 1983. The Effects of Low-Level Consumption by Canopy Arthropods on the Growth and Nutrient Dynamics of Black Locust and Red Maple Tress in the Southern Appalachians. Ecology, 64(5), 1040-1048.

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.

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.

Stadler, B., Solinger, S. & Michalzik, B. (2001) Insect Herbivores and the Nutrient Flow from

the Canopy to the Soil in Coniferous and Deciduous Forests. Oecologia 126, 104-113.

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.

Tukey, H. (1966). Leaching of Metabolites from Above-Ground Plant Parts and Its Implications. Bulletin of the Torrey Botanical Club, 93(6), 385-401. doi:10.2307/2483411

U.S. Environmental Protection Agency (EPA). 1993. Determination of nitrate– nitrite nitrogen by automated colorimetery. Method 353.2, Revision 2.0. Environmental Monitoring Systems Laboratory, Ofﬁce of Research and Development, Cincinnati,Ohio.

Solórzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. 14: 799–801. doi:10.4319/lo.1969.14. 5.0799.

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.

Huanyuan Wang, Xiaotang Ju, Yongping Wei, Baoguo Li, Lulu Zhao, Kelin Hu, 2010.

Simulation of bromide and nitrate leaching under heavy rainfall and high-intensity irrigation rates in North China Plain, Agricultural Water Management,97(10),1646-1654,

Zhong-Jun Wang, Si-Liang Li, Fu-Jun Yue, Cai-Qing Qin, Sarah Buckerfield, Jie Zeng,

Rainfall driven nitrate transport in agricultural karst surface river system: Insight from high resolution hydrochemistry and nitrate isotopes,

Agriculture, Ecosystems & Environment,

Volume 291,

2020

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

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.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., and Smith, G.M. 2009. Mixed effects models and extensions in ecology with R. Springer, New York.