WESTERN SPRUCE BUDWORM EFFECTS ON THROUGHFALL C, N, AND P FLUXES IN A CENTRAL WASHINGTON FOREST

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Western spruce budworm (Choristoneura occidentalis) outbreaks periodically disturb Western US conifer forests by defoliating canopies, which could alter the quantity and chemistry of throughfall delivered to the forest floor. Our objectives were to: i) quantify throughfall water, carbon (C), nitrogen (N), and phosphorus (P) fluxes under budworm-impacted canopies, and ii) examine the influence of herbivore intensity on flux magnitudes. In June 2015, we installed throughfall collectors in two watersheds experiencing high and background levels of herbivory. In each watershed, four plots, each with three throughfall collectors, were established (n=24) collectors), and two bulk rainfall collectors were installed in areas without canopy cover. Throughfall and rainfall were collected from late June to early November 2015. Samples were analyzed for dissolved organic carbon (DOC), ammonium (NH4-N), and soluble reactive phosphorus (SRP). Over the sampling period, throughfall fluxes ranged 8.57 to 47.59 kg/ha for DOC, 0.004-0.011 kg/ha for NH4-N, and 007 - 0.29 kg/ha for SRP. Percent throughfall was slightly, but not significantly, higher in the high (48%) compared to the background watershed (42%). There were no differences in solute concentrations among the watersheds. Net throughfall fluxes, the sum of canopy uptake and leaching and dry/fog deposition, differed significantly for NH4-N by herbivory level and through time for NH4-N and DOC but not SRP. Over time, net NH4-N throughfall fluxes showed a clear transition from net uptake of NH4-N to net leaching of NH4-N in the high herbivory watershed. There was also a clearn NH4-N pulse in the high herbivory watershed after the first, but not subsequent, rainfall events. In this N-limited forest, altered throughfall N may affect soil nutrient cycling and downstream water quality.

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CHAPTER 1

INTRODUCTION

Western spruce budworm (*Choristoneura occidentalis)* outbreaks periodically disturb coniferous forests in western US and southwest Canada (Nealis, 2009; Candau, 1998; Meigs, 2015). The spruce budworm is an endemic canopy herbivore that feeds preferentially on Douglas fir (*Pseudostega menzeisii*) and grand fir (*Abies grandis*), but it also feeds on a variety of other conifer species (Brookes et al., 1987). Although this phytophagous insect persists in forests at background levels during non-outbreak years, *C. occidentalis* has the ability to partially defoliate tree crowns within a season and to completely strip trees of their needles during a multi-season outbreak (Brookes et al., 1987; Candau, 1998). Previous research has shown that leaf-feeding canopy herbivores can influence various ecosystem processes by affecting the quantity and chemical composition of water delivered to the forest floor in throughfall (Lovett, 1995; Rinker, 2001; Fonte et al., 2005; Stadler and Michalzik, 2001; LeMellec, 2009; Pitman, 2010). Yet, despite their persistent role as a disturbance agent, little research has been conducted on spruce budworms in the seasonally dry coniferous forests of western North America.

Throughfall, precipitation that drips through plant canopies and carries materials from atmospheric deposition (wet, fog, and dry) and canopy processing to the forest floor (Ponette-González et al., 2016), is the most important pathway for chemical inputs to forest soils (Parker, 1983). Canopy herbivores have profound effects on these throughfall chemical fluxes (Stadler, 2000; Hunter, 2001; Kindlmann, 2004; Michalzik and Stadler, 2005; leMellec, 2009) through a variety of mechanisms. First, by eating leaves and needles, herbivores reduce leaf area index (LAI: the proportion of leaf area to ground area). Lower LAI results in reduced rainfall interception, increasing the amount of water delivered to the soil. Second, while some materials in the canopy originate from atmospheric deposition, canopy herbivores can alter dissolved and particulate matter inputs by transforming eaten foliage into frass, dropping of needle or leaf fragments, and egestion (Rinker, 2001). These processes have been found to alter concentrations of carbon (C) as well as the major plant-limiting nutrients nitrogen (N) and phosphorus (P) in throughfall (McDowell, 1998) in various ways.

1.1 Background Literature – Canopy Herbivores Affect Throughfall

1.1.1 Carbon

During feeding, herbivores damage needles, create webbing and egest frass (insect excreta). In the canopy, damaged needles and decomposing material are important sources of dissolved organic carbon (DOC) in throughfall (Seastedt et al., 1983; Schowalter, 1991; Stadler, 1998; Schwendemann et al., 2005). However, the contribution of canopy frass and webbing to throughfall flux and concentration of DOC is not clear.

Several studies show the influence of canopy insects on DOC in throughfall. For example, winter moth (*Operophtera brumata*) and mottled umber moth (*Eranis defoliaria*) infestation increased DOC fluxes (LeMellec, 2011). Concentrations of throughfall DOC measured beneath Norway spruce trees infested with aphids of the genus *Cinara* were high in midsummer when aphid populations were high (Kindlmann, 2004). Similarly, elevated throughfall DOC concentrations were found beneath lepidopteran folivore-infested deciduous trees during peak feeding activity (Stadler et al., 2001).

Because C is easily dissolved and transported in water, herbivore activity coupled with precipitation pulses tends to result in large DOC fluxes to the forest floor. For example, throughfall fluxes beneath hardwoods infested by lepidopteran larvae were 70% higher than in control (un-infested) trees (Michalzik and Stadler, 2005), and fluxes of DOC throughfall beneath Scots pine increased with population density of the pine lappet (*Dendrolimus pini)* (LeMellec et al., 2009).

The magnitude of C transported from the canopy via throughfall to the forest floor can have important effects on soil microbial processes, including the mineralization or immobilization of soil N (Chapin et al., 2002; Jones, 2005). In one study, variation in throughfall DOC fluxes explained 46% and 65% of the variation in DOC and DON fluxes to streams respectively (Michalzik, 2001). Quantifying the influence of herbivores on throughfall DOC is thus important for understanding ecosystem C and nutrient fluxes.

1.1.2 Nitrogen

Canopy herbivores can increase or? decrease throughfall nitrogen concentrations and fluxes (Hunter, 2003). Several studies have shown decreases in dissolved inorganic nitrogen (DIN, ammonium (NH_4^+-N) + nitrate (NO_3^--N)) concentrations (Stadler, 2001; Stadler and Michalzik, 2007) and fluxes (LeMellec, 2009) as a result of canopy herbivory. For example, throughfall NH_4^+-N and NO_3^--N concentrations collected in a Bavarian deciduous forest were 25-45% lower beneath folivore-damaged sessile oak (*Quercus petraea)* and European beech (*Fagus sylvatica)* trees than beneath undamaged trees in a control plot, suggesting that DIN had been immobilized by microbial processes in the canopy (Stadler, 2001). Another study, also in Bavaria, found decreased NH_4^+-N and NO_3^--N concentrations under European beech and sessile oak trees infested with larvae of the winter moth (*Operophthera brumata* L.) and the mottled umber moth (*Erannis defoliaria* Cl.) during peak feeding activity (Stadler, 2000). In a study measuring throughfall beneath hemlock and birch trees in the northeastern US, litter below birch trees had higher NH_4^+-N leachate concentrations than litter below hemlock trees exposed to hemlock woolly adelgid (*Adelges tsugae*) (Stadler, 2006). Similarly, NO_3^--N fluxes diminished during peak feeding activity in a study on a stocked Scots pine (*Pinus sylvestris)* forest in northern Germany (LeMellec, 2009). Some studies have found an increase in dissolved organic nitrogen (DON) concentrations with herbivory (Michalzik and Stadler, 2005; Stadler, et al., 2005; LeMellec, et al. 2011). Only some of these studies measured these changes against total N (LeMellec, et al. 2011, 2009; Stadler et al., 2006), thus it is not clear how herbivory alters the ratio of DIN to DON in throughfall.

1.1.3 Phosphorus

The primary source of P in rainfall and throughfall is generally thought to be dust originating from the weathering of rocks (Newman et al., 1995), although a recent review highlights the importance of primary biological aerosol particles (e.g., leaf fragments, excretions) as a source of P in rainfall (Tipping et al., 2014). To date, very few studies have examined the influence of canopy herbivory on P in throughfall, and none have been conducted in seasonally dry conifer forests. One study demonstrated that even moderate outbreak densities could increase P fluxes to the soil by >30% by augmenting greenfall (fragmented foliage), frass fall and throughfall to experimental plots in a tropical rainforest. Plots with throughfall carrying experimentally enhanced NO3, NH4 and PO4 reduced decay rate in litter. (Schowalter et al., 2011). Large inputs of phosphate (PO_4^{3-}) were found in throughfall during a defoliator outbreak, but these data and the details of the study remain unpublished (cited in Rinker, 2001). In Southern Appalachia, no significant differences in total P concentrations in throughfall were found between trees treated with insecticide to diminish canopy herbivory and untreated trees

(Seastedt et al., 1983). As evidenced here, much conflicting data exists in the literature and this study seeks to contribute data towards a better understanding of how herbivores alter P concentrations and flux in throughfall.

1.2 Significance and Research Questions

Many ecological studies focus on invasive species whose range or negative ecological effects may increase due to climate change climate (e.g., Stadler, 2006; Barron, 2008). However, the changing impacts of native species may be just as detrimental. The western spruce budworm is native to North America and outbreaks are evident in the dendrochronological record for hundreds of years (Ryerson, 2003; Campbell, 2006; Robert, 2012). These records suggest that budworm eruptions can still be major a force of disturbance in forests of the Pacific Northwest, similar to the whitemarked tussock moth (*Orygia leucostigma*) and the pine lappet (*Dendrolimus pini*) in their native ranges (Meehan, 2009; Bouchard, 2004; LeMellec, 2009; Lowman et al., 2012).

The Environmental Protection Agency projects a 20% to 30% decrease in precipitation in the Pacific Northwest under a business-as-usual model of carbon dioxide emissions over the coming decades (U.S. National Climate Assessment 2014). Spruce budworm outbreaks have been shown to be triggered by drought (Ryerson, 2003; Flower, 2013), and thus may become more frequent and widespread if summer precipitation continues to decrease in the Pacific Northwest as it has for the past forty years (Mote and Salathe, 2010; Abatzaglou et al., 2014). Budworm outbreaks of increasing magnitude could affect not only forest canopy structure and nutrient cycling between trees and soils, but also such changes may translate to changes in ecosystem processes such as nutrient export to streams (Frost and Hunter, 2001; Flower, 2010).

Given the role of throughfall in forest nutrient cycling, documented responses of throughfall chemistry to canopy herbivory, and projected changes in spruce budworm disturbance regimes, this research will: 1) quantify throughfall carbon (C), nitrogen (N), and phosphorus (P) concentrations and fluxes under spruce budworm-impacted canopies and 2) examine the influence of herbivore intensity on the magnitude of these fluxes.

CHAPTER 2

MATERIALS AND METHODS

2.1 Study Area

Data were collected in the Wenatchee National Forest and in the Teanaway Community Forest, which is cooperatively managed by the Department of Natural Resources, the Washington Department of Fish and Wildlife, and a community-based advisory committee. These areas are part of the Eastern Cascades Slopes and Foothills ecoregion of Central Washington State, which is home to 268 federally listed taxa, ten of which are listed as endangered.

The study area is characterized by a continental climate with dry summers (May-September) and wet winters (October-April). Between 2009 and 2014, mean annual precipitation was 881 mm with ~66% (range 47% - 80%) falling between October and April, mostly as snow, with peak rainfall between October and December. However, total precipitation in this mountainous region can vary considerably by aspect and elevation (range 762-1981 m). Average monthly temperature is 0.5 °C during the wetter season and 13 °C during the dry summer months (U.S. Department of Agriculture).

Before European settlement, mature fire-resistant stands of Douglas fir were open and park like due to frequent low-intensity ground fires known as underburns (Freund, 2015). The continental climate in the study region supports open conifer forests, but there are many transitional ecotones throughout the ecoregion where wetter western Cascade forest types overlap with drier eastern Cascade types based on aspect and elevation. West-facing slopes intercept more precipitation than east-facing slopes, so slightly different associations of plants grow on either side. Low-elevation sites are dominated by Douglas fir (*Pseudostega menzeiseii),* grand fir (*Abies grandis*), and Ponderosa pine (*Pinus ponderosa*) whereas high elevation sites have higher tree species diversity. Along with Douglas fir and grand fir, species include western larch (*Larix occidentalis*), lodgepole pine (*Pinus contorta*), and western hemlock (*Tsuga heterophylla*).

2.1.1 Western Spruce Budworms in the Wenatchee Mountains

The western spruce budworm (*C. occidentalis)* is a member of a native, eruptive lepidopteran whose larvae feed upon the needles of conifer trees throughout montane forests of western North America (Brookes et al., 1987; Alfaro, 2014). In central Washington, the western spruce budworm's preferential hosts are Douglas fir and grand fir, though it may feed less preferentially on a variety of other evergreen species. Though they exist at background levels in non-outbreak years, these canopy herbivores have the ability to defoliate the crown of their hosts within a season and to completely strip trees of their needles during a multi-season outbreak (Zhao, 2014).

Spruce budworm feeding begins in spring when warm temperatures cue the overwintering larvae to abandon their hibernacula (silken retreat from the elements) and tunnel into the foliage. The larvae molt into their third instar (life stage) after seven to 14 days and begin to feed on cone buds and new needle growth, preferentially at the crown of the tree where most of the new needle growth is. When new growth is exhausted, larvae begin to feed on old needles (Nealis, 2012). Needles begin to discolor as the larvae feed and grow. After 30-40 days of continuous feeding, the larvae construct a simple pupal case from which adults emerge after a few weeks in late summer (late July, August). Adults disperse to mate and lay eggs, and they die shortly thereafter. Though budworm host tree species have a variety of defense mechanisms

(Chen, 2001), drought-affected Douglas fir trees seem to be particularly vulnerable to budworm defoliation. Intensity of western spruce budworm has been steadily increasing since the beginning of the 20th century, possibly related to increased fire suppression which has increased stocks of Douglas fir and grand fir, climate change which has increased drought stress through hotter summers and drier winters, or some combination of the two (Lynch, 2008; Flower, 2014).

2.2 Throughfall Sampling

In June 2015, total of eight throughfall sampling plots in two different watersheds were established, one each experiencing high (Swauk drainage) and background (North Fork Teanaway drainage) levels of canopy budworm herbivory. Herbivory intensity was determined in the field qualitatively by visually assessing defoliated tree crowns, the prevalence of discolored dead needles in each area, and manually surveying buds for presence of actively feeding larvae. Four high herbivory plots were situated in the Wenatchee National Forest (average elevation 892 m) and four background herbivory plots were situated in the Teanaway Community Forest (average elevation 1004 m).

In each of the eight plots, a total of three throughfall collectors were installed (n=24 throughfall collectors). Collectors were established below individual trees of the species *P. menzeisii* or *A. grandis*. Throughfall collectors were constructed after Simkin et al. (2006) and Ponette-González et al. (2010). One throughfall collector was placed beneath each tree, half way between the trunk and the dripline. Each throughfall collector consisted of a heavy-duty polyethylene funnel securely attached atop a 1-m length cellular core pipe hammered into the ground. Inside the pipe, a length of tygon tubing was connected to the bottom of the funnel by a plastic connector. A small hole was drilled in the side of the pipe so that the tubing could connect to a heavy-duty polyethylene 4L container secured to the side of the pipe with wire. The tubing was positioned inside the lip of the container with a wrap of parafilm so that throughfall collected in the funnel could drip securely into the container below. In addition, two bulk rainfall collectors (collectors that remain open between sampling) were established in each watershed in a grassy area with no canopy cover (n=4 bulk collectors).

Through a collaboration with researchers at Central Washington University, throughfall and rainfall sampling began on June 25th, 2015. Throughfall and rainfall samples were collected on an event basis from the first rain on September 11, 2015, to first snowfall on November 8, 2015. Following collection, samples were returned to the lab and refrigerated at 4°C until filtering (Pall AE GFF, 1-um pore size) within 24 h of collection. Samples were frozen until analysis.

2.3 Chemical Analysis

In December 2015, ammonium (NH_4^+-N) and soluble reactive phosphate (SRP) were analyzed at Central Washington University. Throughfall was analyzed for DOC in Spring 2016. Dissolved organic carbon was measured after the sample was acidified with HCl (pH <2.0) to purge inorganic carbon from the sample as carbon dioxide (CO_2) . After acidification, DOC was measured via high temperature combustion followed by analysis of CO_2 using a nondispersive infrared sensor (NDIR).

Ammonium-N is a form of N that is readily available to plants and microbes for uptake and it can be leached from canopies and soils in solution. Ammonium was determined using the phenate method (Solorzano, 1969) wherein ammonia and NH_4^+-N react with hypochlorite in a slightly alkaline solution to form monochloramine. This reacts in the presence of phenol, nitroprusside and excess hypochlorite to form indophenol blue which was analyzed colorimetrically at 630 nm on a Beckman DU © 520 General Purpose UV/Vis spectrophotometer.

Soluble reactive phosphorus (SRP) is the form of P that can be taken up directly by plants or microbes, and it was measured using the ascorbic acid method (Wetzel and Likens 1991). Ammonium molybdate and potassium antimonyl tartrate react with orthophosphate in an acidic medium to form phosphomolybdic acid. Reduction by ascorbic acid follows and the resulting blue solution was colorimetrically analyzed at 885 nm on a Beckman DU © 520 General Purpose UV/Vis Spectrophotometer.

2.4 Flux Calculations and Statistical Analysis

For each sample event and plot, volume-weighted mean (VWM) bulk rainfall and throughfall concentrations were computed using the following equation:

$$VWM = \Sigma(conc_i * precip_i)/\Sigma precip_i \qquad (1)$$

where *i* is the sample event, conc is the solute concentration (mg/L) and precip is the rainfall or throughfall amount (mm). Plot-scale throughfall flux was calculated by multiplying VWM concentration by water volume. Net throughfall flux (NTF) is the sum of fog and dry deposition and canopy processing (leaching and uptake) was calculated as:

$$NTF=TF-BD$$

where TF = throughfall, BD = bulk rainfall deposition. Where NTF < 0, canopy? uptake is greater than dry deposition and leaching, and where NTF > 0, dry deposition and leaching are greater than canopy? uptake. Although NTF cannot separate the contribution of canopy herbivory from that of fog and dry deposition to chemical fluxes, the similar rainfall, elevation, and vegetation characteristics of the watersheds are likely to reduce differences in rates of dry deposition.

Wilcoxon tests were performed in JMP (student edition) to determine if there were significant differences in water volumes, solute concentrations, and solute fluxes between the high and background watersheds, among sites, and by sampling date at each site and watershed. This method was chosen due to the small size of the dataset. Because large volumes of water can cause high fluxes of DOC and N from the canopy (Wilcke, 2001), a Spearman's correlations were performed among the solute concentrations and between water volumes and net throughfall fluxes. No correlation between water volumes and solute fluxes could indicate interference by canopy processes.

CHAPTER 3

RESULTS

3.1 Water inputs

3.1.1 Bulk Precipitation

The Teanaway and Swauk watersheds are separated by a drainage divide, but the bulk rainfall data indicate that the high- and background-herbivory plots experienced similar rainfall over the study period. From June 25th to November 8th, 2015, total rainfall was 156.4 mm at the high herbivory watershed and 136.6 mm at the background herbivory watershed. The Natural Resources Conservation Service site at Blewett Pass (47.35 N, Longitude: 120.683056 W), the nearest meteorological station to the study sites (3-15 km distant), received 167.64 mm of water over the same time period. Median rainfall at Blewett Pass in the same period for the previous six years was 200.56 mm, with a mean rainfall of 187.92 mm.

No rainfall was measured at our sites from June 25th until September 10th, and precipitation at Blewett Pass between the same dates was 0.32 mm. Samples of roughly similar volume were collected from all sites after rainfall on September 11th, October 11th, and October 29th. These events were followed by a larger rainfall pulse on November 8th. Samples were only collected when there was enough water in the collector to provide a sufficient volume for chemical analysis. Thus, small amounts of rainfall in the collector likely evaporated before analysis was possible.

3.1.2 Throughfall

Over the study period, 86.2 ± 20.6 mm (SD) of throughfall was collected at the high herbivory and 63.5 ± 8.9 (SD) mm at the background herbivory sites (Table 1). There was more variability among the high herbivory than among the background herbivory sites and this was true across all sample dates. Throughfall volumes differed significantly by date (Wilcoxon, p=0.0001; Table 2) but not by herbivory level or site.

Table 1 *Total rainfall, throughfall, net throughfall (throughfall – rainfall) and percent throughfall water fluxes measured between June 25th and November 8th, 2015, in four high and four background herbivory sites in Wenatchee National Forest, central Washington, USA.*

Site	Herbivory Level	Rainfall (mm)	Throughfall (mm)	Net throughfall	Throughfall (%)
Billy Goat	High	156.4	56.2	-100.2	35.9
Blue	High	156.4	89.5	-67.0	57.2
Hovey	High	156.4	102.1	-54.4	65.2
Hurley	High	156.4	97.0	-59.5	62.0
Jack	Backgrnd	136.6	59.1	-77.5	43.3
Jungle	Backgrnd	136.6	65.4	-71.2	47.9
Moonbm	Backgrnd	136.6	75.2	-61.4	55.0
Standup	Backgrnd	136.6	54.6	-82.0	40.0

3.1.3 Net Throughfall Water Flux

One of the high herbivory sites, Billy Goat, had the lowest net throughfall (i.e., greatest interception), which was unexpected. At the high herbivory sites, canopy throughfall during the autumn rainy season ranged from 35.9% to 65.2% of incident rainfall. Percent throughfall at background herbivory sites was slightly lower, ranging from 39.9% to 55%, but this difference was not significant (Wilcoxon, p = 0.5; Table 2). There were significant differences in net throughfall by date (Wilcoxon p=0.005) attributed to more intense rainfall in November.

Table 2 *Results of Wilcoxon rank sum tests comparing rainfall, throughfall, and net throughfall by site, herbivory level, and date.*

	p value		
	Site	Herbivory Level	Date
Rainfall	0.4	0.7	0.1
Throughfall	0.8	0.6	0.0001*
Net TF	0.9	0.5	0.005*

3.2 Concentrations in Bulk Precipitation and Throughfall

3.2.1 Bulk Precipitation

Soluble reactive phosphorus concentration in rainfall was higher at the high (0.139 mg/L ± 0.16 SD) than at background sites (0.021 mg/L ± 0.03 SD) (Table 3), but the difference was not significant (Wilcoxon p=0.7). There was a general decrease in bulk SRP concentrations in both the high and background herbivory watersheds over time. Bulk rainfall concentrations of SRP (0.08 mg/L ± 0.13 SD) were always less than throughfall concentrations (2.39 mg/L ± 2.79 SD).

Table 3 *Volume-weighted means of soluble reactive phosphorus concentrations (mg/L) for four high and four low herbivory sites in Wenatchee National Forest, central Washington, USA measured between September 11th and November 8th, 2015.*

Site	Herbivory	September 11		October 11		October 29		November 8	
		RF	TF	RF	TF	RF	TF	RF	TF
Billy Goat	High		14.02		3.10		1.8		0.58
Blue	High		9.89		0.63		2.67		0.64
Hovey	High	0.375	2.52	0.019	1.60	0.119	1.07	0.043	0.41
Hurley	High		3.11		1.47		2.38		0.66
Jack	Background		3.13		2.61		1.00		0.59
Jungle	Background		3.92		3.04		2.38		0.96
Moonbeam	Background		2.72		4.13		1.03		0.44
Standup	Background	0.066	2.10	0.004	1.21	0.014	0.66	0.001	0.12

Mean ammonium concentration in bulk rainfall was 0.02147 mg/L (±0.03 SD) at the background water shed and 0.1389 mg/L (± 0.163) at the high watershed. Both watersheds saw decreases after September 11th in NH_4^+-N to near 0.005 mg/L for the remaining sample events (Table 4). With the exception of the first event, concentrations of ammonium tended to be lower in bulk precipitation than in throughfall.

Table 4 *Volume-weighted means of ammonium (NH_4^+N) concentrations (mg/L) at four low and four high herbivory sites between September 11th and November 8th, 2015 in the Wenatchee National Forest, central Washington, USA.*

Site	Herbivory	September 11		October 11		October 29		November 8	
		RF	TF	RF	TF	RF	TF	RF	TF
Billy Goat	High		0.48		0.22		0.04		0.02
Blue	High		0.48		0.08		0.05		0.03
Hovey	High	0.51	0.05	0.005	0.03	0.005	0.04	0.005	0.02
Hurley	High		0.35		0.03		0.03		0.02
Jack	Background		0.05		0.05		0.04		0.02
Jungle	Background		0.08		0.05		0.05		0.03
Moonbeam	Background		0.05		0.02		0.03		0.02
Standup	Background	0.022	0.06	0.005	0.03	0.006	0.04	0.005	0.02

Bulk DOC concentrations at the high and background watersheds decreased over time. The high herbivory watershed decreased more sharply and showed values less than the background watershed for the last three sample events. Because of the higher pulse on the first sample date the mean concentration at the high herbivory watershed was higher (15.88 mg/L ± 25.3 SD) than the background watershed (11.16 mg/L ± 9.97 SD) (Table 5).

Table 5 *Volume weighted means of dissolved organic carbon (mg/L) at four low and four high herbivory sites between September 11th and November 8th, 2015 in the Wenatchee National Forest, central Washington, USA.*

Site	Herbivory	September 11		October 11		October 29		November 8	
		RF	TF	RF	TF	RF	TF	RF	TF
Billy Goat	High		310.2		108.2		109.59		515.9
Blue	High		263.1		21.8		36.73		44.14
Hovey	High	53.7	113.6	4.91	96.26	4.71	104.14	0.2	30.09
Hurley	High		92.80		148.8		195.0		79.59
Jack	Background		148.4		308.5		220.7		36.46
Jungle	Background		229.8		291.2		274.7		30.98
Moonbeam	Background		121.7		97.57		104.1		28.40
Standup	Background	23.55	157.6	14.46	179.6	5.6	102.6	1.03	19.43

3.2.2 Throughfall Concentrations

Throughfall concentrations of all constituents varied more and were greater than in rainfall (Table 6). Throughfall concentrations of SRP, NH_4^+-N, and DOC were higher at Billy Goat and Blue compared to all other sites. Standup had the lowest volume weighted mean concentration of SRP and DOC and nearly the lowest concentration of NH_4^+-N in throughfall.

Table 6 *Volume-weighted means of SRP, NH_4^+-N and DOC concentrations in throughfall and rainfall (mg/L) at four low and four high herbivory sites in Wenatchee National Forest, central Washington, USA over the entire sample period (June 25th – November 8th, 2015).*

Site		SRP		NH_4^+-N		DOC	
	Herbivory	RF	TF	RF	TF	RF	TF
Billy Goat	High		1.838		0.061		374.7
Blue	High		1.304		0.053		50.15
Hovey	High	0.082	0.919	0.038	0.027	5.825	57.55
Hurley	High		0.917		0.027		94.62
Jack	Background		1.004		0.030		84.56
Jungle	Background		1.343		0.037		86.22
Moonbeam	Background		1.005		0.026		55.02
Standup	Background	0.012	0.414	0.007	0.028	6.735	53.62

Throughfall SRP concentrations were generally highest during the first sample period for both the high and background watersheds (Table 3). There was a general decrease for all sites over time. The volume weighted mean concentration of SRP at Billy Goat on September 11th was as much as 7-fold higher compared to the other sites, and this site displayed the most drastic decrease in concentrations, with SRP declining to 0.58 mg/L by the end of the sampling period. No significant differences were detected between high and background herbivory levels for SRP concentrations (Wilcoxon $p=0.7455$). There were significant differences among sites (Wilcoxon $p=0.0413$), most likely due to major pulses for Billy Goat and Blue on the first sample date.

There was a pulse of NH_4^+-N in throughfall at the high herbivory but not the background watershed on the first sampling date (Table 4). Throughfall concentrations generally declined over time, with the high and background herbivory watersheds receiving similar concentrations in throughfall by the end of the sampling period. Ammonium concentrations did not differ by site (Wilcoxon p=0.1370) or by herbivory level (Wilcoxon p=0.4930), but a significant difference over time was found (Wilcoxon p=0.0006).

Table 7 *Results of Wilcoxon rank sum test comparing concentrations among site, herbivory, date and type (rainfall or throughfall collector).*

	p value			
Chemical	Site	Herbivory Level	Date	Type
SRP	0.0413*	0.7455	0.0071*	0.0001*
NH_4^+-N	0.1370	0.4903	0.0006*	0.0019*
DOC	0.0371*	0.7972	0.0786	0.0001*

Throughfall volume weighted mean DOC concentrations ranged from 21.8 mg/L to 515.9 mg/L in the high watershed and from 19.43 mg/L to 308.5 mg/L in the background watershed. There was a general pattern of decreasing DOC concentrations over time except at Billy Goat where there was a pulse on November 8th (Table 5). While three out of four high herbivory sites decreased from September to October 11th, three out of four background herbivory sites increased over the same time period followed by decreases at all sites except Billy Goat.

3.3 Bulk Deposition and Solute Fluxes to the Soil

In the high herbivory watershed, total bulk deposition of SRP was 0.0156 kg/ha, 0.007 kg/ha for NH_4^+-N and 1.105 kg/ha for DOC. Total bulk deposition in the background herbivory watershed was 0.003 kg/ha for SRP, 0.002 kg/ha for NH_4^+-N, and 1.572 kg/ha for DOC. The highest average rainfall flux (Table 8) was seen in dissolved organic carbon at the background herbivory watershed (0.39 kg/ha) and the lowest average rainfall flux was for ammonium at the background herbivory watershed (0.0004 kg/ha). Bulk deposition of solutes falling in high and background watersheds were very similar to each other over time, with the exception of NH_4^+-N on September 11th which was 0.006 kg/ha in the high watershed and 0.0007 kg/ha in the background. This difference disappeared by October 11th, and values were similar to each other for the rest of the sampling period.

Table 8 Average fluxes (kg/ha) of SRP, NH_4^+-N and DOC in throughfall and rainfall at four low and four high herbivory sites in Wenatchee National Forest, central Washington, USA over the entire sample period (June 25th – November 8th, 2015).

Herbivory	**SRP**		**NH_4^+-N**		**DOC**	
	TF	**RF**	**TF**	**RF**	**TF**	**RF**
Background	0.183± 0.08 SD	0.0007± 0.001 SD	0.006± 0.001 SD	0.0004± 0.003 SD	13.2± 3.56 SD	0.393± 0.352 SD
High	0.268± 0.03 SD	0.0039± 0.002 SD	0.009± 0.002 SD	0.0018± 0.003 SD	25.9± 15.8 SD	0.276± 0.279 SD

3.3.1 SRP Throughfall Flux

Throughfall fluxes at the high herbivory watershed were greater than at the low herbivory watershed but the difference was not significant. Over time, SRP fluxes in throughfall were highly variable and there were no significant differences found over the entire sampling period by site (p=0.5645) or herbivory level (p=0.2948) and no significant differences by date found for high (0.2632) or background (p=0.6233) watersheds (Table 12).

Table 9 *Throughfall fluxes (kg/ha) of soluble reactive phosphorus at four low and four high herbivory sites in Wenatchee National Forest, central Washington, USA over the entire sample period (June 25th – November 8th, 2015).*

Site	Herbivory	Sept 11	Oct 11	Oct 29	Nov 8
Billy Goat	High	0.1115	0.0089	0.0673	0.0456
Blue	High	0.0913	0.0126	0.0824	0.1054
Hovey	High	0.1368	0.0255	0.0430	0.0808
Hurley	High	0.0160	0.0173	0.0721	0.1616
Jack	Background	0.0618	0.0200	0.0246	0.0774
Jungle	Background	0.0187	0.0154	0.0810	0.1417
Moonbeam	Background	0.0579	0.0629	0.0404	0.0656
Standup	Background	0.0187	0.0094	0.0254	0.0127

3.3.2 NH_4^+-N Throughfall Flux

Fluxes of NH_4^+-N in throughfall on September 11th were higher in the high herbivory watershed than in the background (Table 10), which showed similar values for background rainfall NH_4^+-N fluxes (Table 8). Both watersheds experienced a decrease at all sites on the second sample date and both watersheds increased at all sites on November 8th. Fluxes differed significantly by date for both the high ($p=0.0063$) and background watershed (0.0387). A significant difference between herbivory levels was also found ($p = 0.0458$) but not by site. (Table 12).

Table 10 *Throughfall fluxes (kg/ha) of ammonium at four low and four high herbivory sites in Wenatchee National Forest, central Washington, USA over the entire sample period (June 25th – November 8th, 2015).*

Site	Herbivory	Sept 11	Oct 11	Oct 29	Nov 8
Billy Goat	High	0.0038	0.0006	0.0014	0.0019
Blue	High	0.0044	0.0016	0.0016	0.0042
Hovey	High	0.0088	0.0005	0.0015	0.0039
Hurley	High	0.0018	0.0004	0.0010	0.0046
Jack	Background	0.0010	0.0004	0.0009	0.0032
Jungle	Background	0.0004	0.0002	0.0015	0.0049
Moonbeam	Background	0.0010	0.0004	0.0013	0.0031
Standup	Background	0.0004	0.0003	0.0015	0.0023

3.3.3 DOC Throughfall Flux

Of all the solute fluxes over the entire sample period, the largest difference between throughfall and rainfall in both watersheds was seen in DOC throughfall flux (Table 8). Even though throughfall concentrations of DOC were highest at Blue and Billy Goat on September 11th, DOC throughfall flux was highest for Hovey on that date as a result of higher throughfall (Table 11). High herbivory sites tended to decrease on October 11 and all increase through the last two sampling events. Background watershed sites had a less discernable pattern, all increasing on October 29th, but leveling out below DOC flux values on November 8th. The largest throughfall DOC flux of the four sampling dates is seen at Billy Goat on November 8th (40.732 kg/ha).

Table 11 *Throughfall fluxes (kg/ha) of dissolved organic carbon at four low and four high herbivory sites in Wenatchee National Forest, central Washington, USA over the entire sample period (June 25th – November 8th, 2015).*

Site	Herbivory	Sept 11	Oct 11	Oct 29	Nov 8
Billy Goat	High	2.4687	0.3099	4.0815	40.732
Blue	High	2.4290	0.4372	1.1342	7.2220
Hovey	High	6.6328	1.5320	4.1769	5.9486
Hurley	High	0.4756	1.7526	5.8976	19.4054
Jack	Background	2.9291	2.3571	5.4094	4.7812
Jungle	Background	1.0969	1.4739	9.3553	4.5656
Moonbeam	Background	2.5963	1.4847	4.0753	4.2573
Standup	Background	1.1953	1.3897	3.9528	2.0345

Table 12 *Results of Wilcoxon rank sum test comparing a) fluxes over the entire sample period and b) fluxes by date among site and herbivory level.*

	p value			
	a) Flux over entire period		b) Flux by date	
Chemical	Site	Herbivory Level	High	Background
SRP	0.5645	0.1748	0.2632	0.6233
NH_4^+-N	0.4067	0.0458*	0.0063*	0.0387*
DOC	0.4763	0.6785	0.1186	0.1323

3.4 Net Throughfall Fluxes

There were no significant differences between herbivory levels or sites for net throughfall fluxes of soluble reactive phosphorus, ammonium or dissolved organic carbon (Table 14), but net throughfall fluxes of dissolved organic carbon and ammonium changed significantly over time for both high and background watersheds. These findings indicate significant changes in canopy uptake of DOC and NH_4^+-N over time (Figure 1) but not between watersheds or among sites.

Table 13 *Net throughfall flux in kg/ha of SRP, NH_4^+-N and DOC at four low and four high herbivory sites in Wenatchee National Forest, central Washington, USA over the entire sample period (June 25th – November 8th, 2015).*

		Bulk Deposition			Throughfall Flux			Net Throughfall Flux		
Site	Herbivory	SRP	NH_4^+-N	DOC	SRP	NH_4^+-N	DOC	SRP	NH_4^+-N	DOC
B. Goat	High	0.0156	0.007	1.105	0.233	0.007	47.592	0.217	0.0006	46.487
Blue	High	0.0156	0.007	1.105	0.291	0.011	11.223	0.276	0.0046	10.117
Hovey	High	0.0156	0.007	1.105	0.281	0.008	17.623	0.265	0.0011	16.518
Hurley	High	0.0156	0.007	1.105	0.266	0.007	27.531	0.251	0.0006	26.425
Jack	Backgrnd	0.0028	0.001	1.572	0.183	0.005	15.476	0.180	0.0037	13.904
Jungle	Backgrnd	0.0028	0.001	1.572	0.256	0.007	16.491	0.253	0.0053	14.919
Moonm	Backgrnd	0.0028	0.001	1.572	0.226	0.005	12.413	0.223	0.0040	10.841
Standup	Backgrnd	0.0028	0.001	1.572	0.066	0.004	8.572	0.063	0.0027	6.9999

3.4.1 Net Throughfall Flux of SRP

Net throughfall fluxes of SRP were positive during all sampling dates, indicating net leaching of soluble reactive phosphorus from the canopy. After the first period, during which the high herbivory watershed experienced a pulse of SRP to the forest floor, both background and high herbivory watersheds experienced increasing net throughfall flux over the course of sampling period. Net Throughfall flux of soluble reactive phosphorus was the least variable over time at Standup and Moonbeam. The differences in net throughfall fluxes of SRP between background sites and high sites were less pronounced than for NH_4^+-N or DOC.

Table 14: Results of Wilcoxon rank sum test comparing a) net throughfall fluxes over the entire period and b) net throughfall fluxes by date among site and herbivory level.

	p value			
	a) net TF Flux over entire period		b) net TF Flux by date	
Chemical	Site	Herbivory Level	High	Background
SRP	0.5598	0.2744	0.0543	0.3373
NH_4^+-N	0.9988	0.9100	0.0045*	0.0053*
DOC	0.8558	0.7919	0.0149*	0.0132*

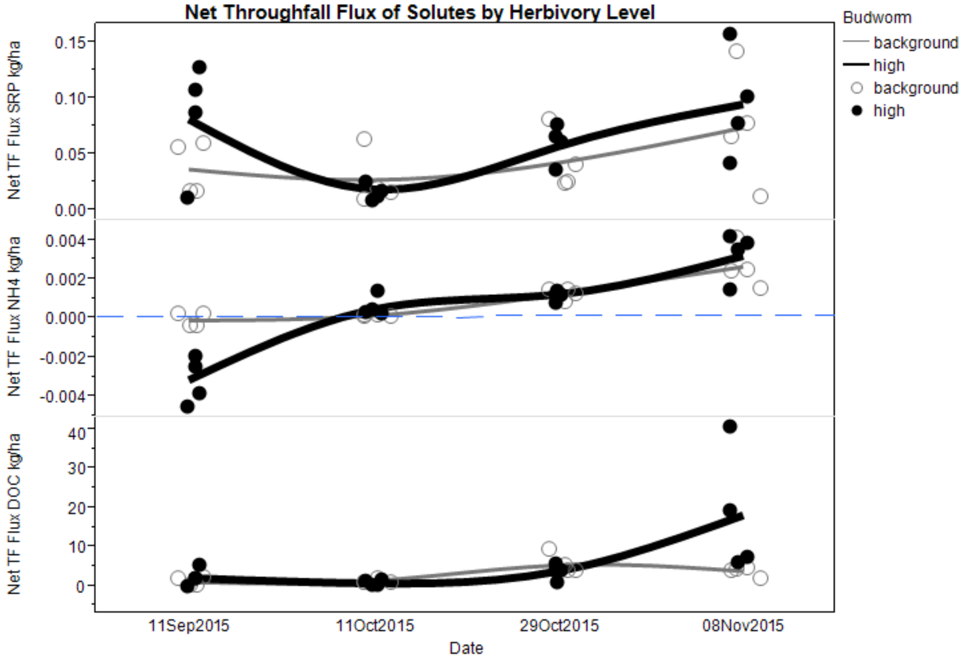

Figure 1 *Comparison of Net Throughfall fluxes of Soluble Reactive Phosphorus, Ammonium and Dissolved Organic Carbon in high herbivory and background herbivory watersheds over the four sampling dates*

3.4.2 Net Throughfall Flux of NH_4^+-N

Net throughfall of ammonium over time shows a distinct change towards positive values, indicating an increase in net leaching of ammonium (Figure 1) in both watersheds. However, this transition is more pronounced in the high herbivory watershed, as initial (September 11th) net throughfall rates were negative, indicating some mechanisms of NH_4^+-N uptake in the canopy. Background net throughfall flux of NH_4^+-N showed values similar to bulk deposition, hovering near zero. This indicates virtually no initial uptake of NH_4^+-N in the background watershed and an apparent though not significant difference in net throughfall flux of NH_4^+-N between watersheds. Over time, both watersheds transitioned to similar positive values, indicating similar rates of net leaching of NH_4^+-N.

3.4.3 Net Throughfall of DOC

Net throughfall of dissolved organic carbon was the least variable among sites (Table 13). Only Hurley had even slight uptake (-0.1911 kg/ha on September 11th). Hovey had the largest initial net flux of DOC (5.3 kg/ha) on the first sampling date. Net throughfall flux of DOC increased at all sites between October sampling dates, but by November 8th net throughfall fluxes of DOC increased substantially at the high herbivory sites, with huge values at Billy Goat (40.71 kg/ha) and Hurley (19.39 kg/ha). The background watershed had much lower net throughfall fluxes at this date with less variability.

3.4.5 Correlations

Positive correlations were found between throughfall water volume and net throughfall flux for all solutes at the background and high herbivory watershed (Table 15). This indicates the rinsing of water-soluble compounds from the canopy: as more water came into contact with leaf surfaces, more compounds came into solution and were transported via throughfall from the canopy. In the background watershed, net throughfall flux of NH_4^+-N was most strongly correlated with throughfall water volume, indicated by the Spearman's coefficient of 0.9559. In the high watershed, net throughfall flux of DOC was most strongly correlated with throughfall water volume, indicated by a Spearman's coefficient of 0.8353.

In the background herbivory watershed, all solutes in throughfall were significantly positively correlated to each other (Table 16). Ammonium and DOC were more strongly correlated to each other than with SRP, indicated by the Spearman's coefficient of 0.8206 for the two solutes. At the high herbivory sites, only NH_4^+-N and SRP concentrations were positively correlated, indicated by the Spearman's coefficient of 0.8176.

Table 15 *Results of Spearman correlation analysis of Net TF Flux of solutes and Throughfall water volume in mm.*

Background Herbivory		**Spearman's r**	**P value**
Net TF Flux SRP	TF Water vol mm	0.6029	0.0134*
Net TF Flux NH_4^+-N	TF Water vol mm	0.9559	<0.0001*
Net TF Flux DOC	TF water vol mm	0.7676	0.0005*
High Herbivory		**Spearman's r**	**P value**
Net TF Flux SRP	TF Water vol mm	0.5647	0.0227*
Net TF Flux NH_4^+-N	TF Water vol mm	0.7500	0.0008*
Net TF Flux DOC	TF water vol mm	0.8353	<0.0001*

Table 16 *Results of Spearman correlation analysis between concentrations of solutes in rainfall and in throughfall.*

Background Herbivory		**Spearman's r**	**p value**
NH_4^+-N mg/L	SRP mg/L	0.6882	0.0032*
DOC mg/L	SRP mg/L	0.6235	0.0099*
DOC mg/L	NH_4^+-N mg/L	0.8206	0.0001*
High Herbivory		**Spearman's r**	**p value**
NH_4^+-N mg/L	SRP mg/L	0.8176	0.0001*
DOC mg/L	SRP mg/L	0.4059	0.1188
DOC mg/L	NH_4^+-N mg/L	0.2206	0.4117

CHAPTER 4

DISCUSSION

Although in some cases, solute concentrations and fluxes varied by site and changed through time, only ammonium throughfall fluxes were found significantly different between herbivory levels. Additionally, net throughfall flux of ammonium was found to significantly differ over time, transitioning from net uptake to net leaching. Decoupling of dissolved organic carbon concentration correlations to soluble reactive phosphorus and ammonium in the high herbivory water shed but not background (Table 16) could indicate differences in canopy processes between the two watersheds.

4.1 Throughfall Water Volumes

It was expected that mean net throughfall would be lower (i.e., greater interception) at the background watershed due to fuller needle canopies in areas less impacted by budworms. However, there was only a slight, insignificant (8.5%) difference in water volumes between herbivory treatments. One tree at a site in the high herbivory watershed (Billy Goat) had the highest interception rate of all the sites. This site was host to large amounts of mistletoe, which can increase surface area and thus interception rates (Lowman et al., 2012). The only slight difference in throughfall volumes between high and background watersheds could indicate that the degree of patchiness (spatial variability) in stands hosting high populations of budworms is greater than in background watersheds resulting in no mean differences between treatments. Alternatively, the pattern may have resulted from a lack of difference in herbivory density.

4.2 Throughfall Concentrations

It was thought that differing distributions and feeding preferences (new needles) of larval caterpillars were likely to create a non-uniform leaf area index among trees in stands of high herbivory, and concentration data from outbreak stands would be expected to reflect this patchiness in concentration variation. A significant difference was detected in coefficient of variation (CV) among the sites (p=0.0377) and the watersheds (p= 0.0262) over time for concentrations of soluble reactive phosphorus, however the background watershed had slightly higher and less uniform CV than the high herbivory watershed.

Concentrations of dissolved organic carbon in throughfall were expected to be high when herbivore activity was high (LeMellec, 2011; Kindlmann, 2004; Stadler et al., 2001), which may be evident in the high values of DOC concentrations in the initial sample dates for both watersheds (although slightly higher for high herbivory). DOC concentrations saw decreasing values in both high and background herbivory, except for an apparent anomaly at Billy Goat, which is the most likely cause for the significant difference by site detected in DOC concentrations. There is a large amount of variability within the DOC throughfall concentration data by site, however all of the sites except for Billy Goat became lower and more similar over time.

Ammonium concentrations were expected to be low when feeding activity was high due to… (Stadler, 2001; Stadler and Michalzik, 2007). Though this was true for fluxes, ammonium concentrations were initially larger and decreased slightly over time (Table 4). Increasingly larger volumes of water resulted in higher fluxes as the rainy season progressed. This outcome reinforces how important analysis of flux (kilogram solute per unit area) is for interpreting throughfall data.

Due to conflicting evidence in the literature, there were no clear expectations for concentrations of soluble reactive phosphorus in throughfall. Results were variable, but a pattern of decreasing concentrations was detected for both watersheds, indicating impacts of seasonality and increasing precipitation on phosphorus concentrations. No significant differences were found due to herbivory level, and thus we cannot illustrate any direct impacts of canopy herbivore infestation on phosphorus cycling in the canopy. Our findings reflect the variability of phosphorus findings from the literature.

4.3 Solute Fluxes

4.3.1 Soluable Reactive Phosphorus

Though variability was high among sites, two pulses of SRP were evident during the sampling period. The first sample date could be attributed to herbivore excreta falling from the canopy, which has been connected to phosphorus inputs in previous studies (Lovett, 1995). Previous studies have shown that leaves tend to retain phosphorus (Runyan, 2013), and the SRP is not readily leached from the canopy in a predictable manner (Newman, 1995). Though our analysis demonstrated a positive correlation between SRP leaching and water volumes, the significance is far weaker than for the other solutes in both watersheds, and thus it can be deduced that water volume is not as strong a driver for SRP leaching as it is for the other two solutes. Fire is known to be a source of dry deposited phosphorus, contributing 1.3 Tg P/ha/yr (Wang, 2014). There were many intense fires within a hundred miles from our site the summer of sampling, however if the wildfires were the main source of dry deposition, elevated levels of SRP would be expected at all sites on the first sample date. However this was not the case so wildfires may not have been the primary cause of the elevated magnitudes of throughfall fluxes of SRP. If water and fire are not the main drivers, some other factor such as herbivory was likely responsible for change in net throughfall flux of SRP. Net throughfall SRP flux is about 40% greater at high sites than the low sites. Although this difference was not found to be significant, it does indicate that the source for phosphorus is associated with higher herbivory levels and could be coming from herbivore excreta. The second pulse of SRP was more gradual and there was little distinction between high and background sites. During the last sampling period, SRP concentrations were low and throughfall water fluxes were high, therefore this pulse was rain driven.

4.3.2 Ammonium

A significant difference in ammonium over time was found for both watersheds, apparent as a transition from net uptake to net leaching of ammonium at the high herbivory site, indicating a change in some process in the canopy. Additionally, mean rainfall flux of ammonium was higher (0.0063 kg/ha) than the mean throughfall flux (0.0031 ± 0.0012 kg/ha (SD) while background herbivory throughfall ammonium was more similar to rainfall. A significant difference in ammonium between the high and background herbivory watersheds was found ($p=0.0458$). This indicates that some process was causing increased ammonium uptake in high herbivory canopies and not in the background herbivory. Generally, nitrogen cycling in the canopy involves the microbial mineralization of organic nitrogen found in decomposing materials to ammonium followed by transformation into nitrate via nitrification by microorganisms (Chapin, et al., 2002). Specifically, potential fates of ammonium in the canopy include uptake by plant surfaces or epiphytic organisms as ammonium or nitrate (Clark et al 2005; Endres and Mercier, 2001) or assimilation by organisms. (Frost 2007). Cycling of nitrogen often depends on the presence of carbon compounds. For example, in a study on gypsy frass, labile C in the canopy was shown to contribute to microbial immobilization of N in herbivore excreta (Lovett, 1995). Canopy organic matter may retain a significant proportion of inputs from the recycling of plant litter (Matson, 2014). Nutrient cycling in canopy organic matter is likely more rapid than in soils due to exposure to evaporation and rinsing, with inorganic N potentially being taken up multiple times in a season (Fonte and Scholwalter, 2004), however the transformation of ammonium in organic matter in canopies may decrease at increasing elevations (Matson, 2014). Canopy processes may directly and indirectly affect processes in the soil. For example, uptake of N in the canopy may inhibit N uptake at the root (Rennenberg and Gessler, 1999). Additionally, direct input of excreta from canopy herbivores can cause ephemeral pulses of N to soil (Frost, 2007). Less understood is if decomposition of excreta stuck in the canopy can contribute to ammonium in throughfall.

Thus, high rates of microbial immobilization of NH_4^+-N were likely occurring in the high herbivory watershed due to large amounts of decaying material stuck in the canopy, leading to negative net throughfall of ammonium evident in the first sample period. In the background watershed, net throughfall of ammonium was very similar to rainfall. Though nutrient cycling and microbial activity were likely occurring here, background densities of herbivores did not cause enough of an increase in the rate of mineralization and immobilization of excess inputs to inhibit ammonium transport, resulting in significant differences in ammonium fluxes between watersheds. As time went on, precipitation volumes became large enough to slow decomposition rates and rinse NH_4^+-N out of the canopy before it could be taken up by microbes, leading to net leaching and increasingly positive net throughfall of ammonium. By the end of the sample period, net throughfall of ammonium values were similar in both high and low herbivory watersheds, suggesting that any differences in nutrient cycling occurring between them in the dry season could be washed away by large amounts of precipitation in the rainy season. Decreased losses of inorganic nitrogen via throughfall seen under trees affected by a lepidopteran folivore were likely due to the immobilization of ammonium by microbes (Stadler and Michalzik, 2006). Canopy soil respiration and nutrient cycling were likely increased by the presence and associated inputs of western spruce budworm during our sample period, similar to gypsy moths and hemlock wooly adelgids (Reynolds and Hunter 2001, Stadler, 2005). Without nitrate analysis, it is difficult to determine what cycling processes were dominant in the canopy at certain times, but general mineralization of organic nitrogen inputs followed by microbial mineralization could account for the evident net uptake of ammonium seen at the high herbivory watershed.

4.3.3 Dissolved Organic Carbon

In both high and background watersheds, higher throughfall was positively correlated with higher leaching of DOC, with the correlation slightly more significant in the high herbivory watershed. Even though there was only slightly more water, it drove an increase in the leaching rate. This explains why our net flux is greater in the high herbivory watershed even though differences in water volumes between herbivory levels were not significantly different. Slightly greater litter flux (1.021 $g/m^2/day$) was found at the background than at the high sites (0.626 $g/m^2/day$) sites even though there were fewer sample dates (n=5 versus n=8). Even though the difference was not significant (Wilcoxon p=0.1230), this could indicate a higher amount of uneaten needles falling from the canopy in the background watershed. More litter falling from the canopy could indicate more carbon leaching from the canopy, however, our data show higher dissolved organic carbon fluxes in the high herbivory watershed. Higher herbivore density implies larger amounts of herbivore excreta in the canopy which could lead to increased dissolved organic carbon transported in throughfall (Lovett, 1995; Stadler, 2001). Thus, water volume flux and excreta load could be the driving force in DOC flux at the high herbivory sites and not litter flux. Outbreak levels of western spruce budworm were not associated with NH_4^+-N and SRP flux correlations with DOC fluxes, though these correlations were present in the background herbivory watershed. Altered C:N ratios associated with herbivore damage have been shown to influence microbial action (Stadler & Michalzik, 2000; Michalzik, 2005; LeMellec, 2010), but more research is needed to determine if this association can hide correlations between DOC and NH_4^+-N concentrations.

4.4 Summarized Findings

Though there was only a slight (8.5%), difference in water volumes, significant differences in ammonium uptake between herbivory levels and over time indicated a transition from net uptake of NH_4^+-N (most likely by microbial immobilization) to net leaching of NH_4^+-N in the high herbivory sites as the rainy season progressed and water volumes increased. There were significantly higher rates of ammonium uptake in sites where feeding activity was high, suggesting increased microbial activity in stands affected by budworm outbreaks. SRP became more variable without distinction between watersheds, but showed a slight pulse in three out of four high sites on the first sample period, possibly driven by high quantities of herbivore excreta rinsed from the canopy. There were slight differences in DOC, driven mostly by water volume. Outbreak level western spruce budworm presence was associated with decoupling NH_4^+-N and SRP flux correlations with DOC fluxes, a significant difference in NH_4^+-N fluxes between watersheds and a pronounced transition from uptake to leaching of NH_4^+-N over time. Some degree of stand patchiness likely contributed to uncertainty in the data and thus further studies with higher number of replicates and multiple sample periods are crucial to understand the patterns of influence western spruce budworms have on C, N and P flux to the forest floor.

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