**Methods**

This project was part of an overarching research grant and is intended to help provide more data on WSB activity and their effect on PNW ecosystems. The main question of the grant being addressed was; are the WSB affecting aquatic food webs in local streams. To help answer that question, I looked at two smaller questions that led back to that main focus. The first question that was investigated was; are WSB changing that rate of decomposition of conifer litter on the forest floor in the grant’s study site. My project will be testing against the null hypothesis that there is no change to see whether WSB are affecting the rate of decomposition. A second question will also be looked at to support the data gathered on the rate of decomposition. The second question is; are the WSB changing net nitrification in the soils of the areas being investigated. This will also be tested against the null hypothesis of no change.

**Experimental Design**

A nested study design was used to look at both the rate of decomposition and net nitrification. Two areas were investigated; one with high WSB impact and one with low WSB impact. Within each area, I looked at 4 different streams, one in the high impact area and one in the low impact area for a total of 8. At each one of those sites I will have three replications ranging from upstream to downstream. Each replication will be approximately 30 meters apart. At each site there were twenty 20x20cm mesh bags (García-Palacios et al. 2016) for a total of 480 bags. Ten will contain sugar maple (*Acer saccharum*) leaves which are being used as a control (?) due to budworms only eating conifer needles. The other ten bags contained a mixed conifer needle sample of Douglas Fir, Grand Fir, and Ponderosa Pine; the most abundant species in the study area. The maple leaves will be sorted to be as whole as possible to best represent how they would fall to the forest floor.

**Throughfall**

Throughfall collectors were installed under the tree canopy close to each decomposition bag line. Two rainfall collectors were set up in an area with no canopy cover in the low budworm activity sites and two in the high impacted sites as controls. During rain events, water came through the canopy into a funnel ( \_ mm) with polywool filtering out any litter that happened to fall into the aparatus as well as filtering the water itself. The funnel was attached to a hose with nylon mesh ( \_ μm) between the two and was held in place with parafilm. Water traveled through the tubing into an acid washed 4 L jug. Water was then transferred to an acid washed nalgene bottle and was taken to the lab, filtered through a 0.5μm fiberglass filter using vacuum filtration. The volume of each collector was also measured in order to calculation mg of nutrient per L of water. Samples were frozen until analysis. All samples were run on the Seal AQ1 Discrete Analyzer using EPA equivalent methods.

**Decomposition**

I used ~3-5 grams per bag of air dried leaves (Benfield, 1996). In the conifer bags I used ~1.2 grams of Ponderosa Pine and then ~4.8 grams of mixed Douglas Fir and Grand Fir that have been air dried. The bottom side of the mesh bags were made of a smaller sieve size ( \_ mm) (Schweitzer et al, 2004) than the top ( \_ mm) to prevent the loss of its contents but still allows small herbivores to enter the bags as would happen in nature. These were assembled by stapling the two sieve sizes together and by reinforcing them with super gluing the corners. Once the leaves were placed into the bags, the bags were then placed into red peanut bags to help further protect them. The conifer bags were placed into Ziplocs before being filled to collect any lost material that can then be weighed and subtracted from the initial mass. The bags were then strung together in a line that was held in place by a 2ft piece of rebar on either side. This was to prevent the bags from being moved by the wind and by runoff. A coin flip determined which bags (conifers or deciduous maple) were placed upstream and downstream at each site. Handling loss was applied to the mass of the material by deploying twenty bags, ten deciduous and ten coniferous and extracting them immediately to see if there was any mass loss from deployment and extraction. After extracting bags from the field, they were air dried in paper bags (Genung et al. 2013) on a clothesline to evaporate out any moisture that may throw off the actual returning mass (Schweitzer, 2005). Once the bags were air dried, they were sorted morphologically and by color to remove any noticeable debris that may have ended up in the sample (Chapman et al. 2013). Due to pine needles falling into my bags it will be hard to determine what was originally in the bag and what has fallen into it so the maples was sorted and used as a correction factor for how much fir/pine entered the bags. One conifer bag and one maple bag will be cut and bagged in a Ziploc (to prevent mass loss) per site for a total of 48 bags per sampling time.

**Soil Analyses**

The measurements taken per soil sampling were A horizon soil temperature, and O Horizon soil temperature. A soil core was extracted from each replication each sampling time. Each core was then homogenized in a Ziploc bag. Soils were analyzed for moisture content, percent organic matter, ammonia, nitrate, organic N, inorganic P, and organic P. Testing for soil moisture will help give an understanding of how much water is being retained in the soil over the course of this study (Liu et al, 2015).

*Moisture Content and Percent Organic Matter:*

Sieved soil at 2 mm was placed into an ashed aluminum pan and weighed immediately. Pans were then placed in a drying oven until all moisture had evaporated out. Pans were cooled to room temperature and weighed again. The difference was then used to calculate percent moisture. The same pan samples were then placed in a muffle furnace to combust all carbon compounds. Samples were then again cooled to room temperature and rehydrated with Milli-Q water to rehydrate and colloids containing water molecules. Excess water was evaporated out by placing samples in the drying oven again. Pans were cooled to room temperature and weighed. The difference was then used to calculate percent organic matter.

*Phosphorous Analysis*

The Bray P1 method was used to extract phosphorus from each soil sample (Hamilton, 1997) (Patton and Kryskalla, 2003). To do this, one gram of air dried soil was added to 10 mls of the Bray P1 extractant solution (NH4F and HCl) and was shaken at 100 Rpm for 15 minutes. The sample was then filtered with a syringe through a 0.5 µm fiberglass filter and stored in the freezer until analysis. Samples were analyzed for inorganic and organic phosphorous on a Seal AQ1 Discrete Analyzer using EPA equivalent methods.

*Nitrogen Analyses*

A 2M KCl extraction method was used to extract nitrogen from each soil sample (?). 10 grams of air dried soil were added to 70 mls of 2M KCl and were shaken at 100 Rpm for 2 hours. The sample was then filtered with a syringe through a 0.5 µm fiberglass filter and stored in the freezer until analysis. Samples were analyzed for NO3- on a Seal AQ1 Discrete Analyzer using EPA equivalent methods.

Each site also contained a resin bag made of bleached nylons (to prevent color leaching that may affect results) filled with 30 grams of ion exchange beads to measure the activity in soils and was also extracted using 2M KCl and were analyzed for NO3- on a Seal AQ1 Discrete Analyzer using EPA equivalent methods.

**Frass and Litter Measurements**

To quantify what was considered a highly impacted site vs a lowly impacted site, frass was collected. Funnels with a 60 cm diameter made of tarp and garden hose connected to a one liter Nalgene bottle were placed on the ground under trees affected by budworms. The funnel collected all frass that fell, as well as all litter. The collection was dried, sorted, and then weighed.

**Location**

The low budworm impact sites for this study took place in the Teanaway Community Forest in Washington State approximately 40 miles from Central Washington University. The samples were placed around 3500ft and was done on public land. The creeks that were analyzed here were: Stand Up Creek, Jungle Creek, Jack Creek, and Moonbeam Creek. The high budworm impact sites took place near the Swauk drainage in the Wenatchee National Forest in Washington State approximately 45 miles from Central Washington University. The samples were placed around 3200ft on public land as well. The creeks that were looked at here were: Cougar Creek, Hurley Creek, Hovey Creek, and Blue Creek.

**Statistical Analysis**

I used a two-sample t-test to compare the two treatments; coniferous litter vs deciduous. This was done for both the rate of decomposition and for net nitrification. I used generalized least squares models and linear mixed effects models (Senf et al. 2016) to compare the variances within each of the 8 sites and each of the 3 replications within to compare percent soil moisture, percent organic matter, NO3-, NH4+, SRP, N:P ratio, total inorganic N, and net nitrification/mineralization. Data was analyzed using R.