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Monitoring the influx of marine derived Nitrogen and characterizing soil food webs of riparian zones of the Elwha River Watershed, WA, USA.

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Monitoring the Influx of Marine Derived Nitrogen and Characterizing Soil Food Webs of Riparian Zones of the Elwha River Watershed, WA, USA.

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Abstract

Monitoring the Influx of Marine Derived Nitrogen and Characterizing Soil Food Webs of Riparian Zones of the Elwha River Watershed, WA, USA.

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Nitrogen is often the most limiting nutrient to productivity in terrestrial ecosystems, and can have large effects on ecosystem processes. Two sources of nitrogen to Pacific Northwest riparian areas are marine derived nitrogen (MDN) via anadromous pacific salmon and symbiotic nitrogen fixation via *Alnus rubra*. The recent removal of two large dams on the Elwha River, WA, opened up ~60 km of previously inaccessible river habitat for pacific salmon. I used naturally abundant stable nitrogen isotopes (denoted as ‰ $\delta^{15}N$) to establish baseline data to monitor the influx of MDN to riparian zones of Elwha River tributaries, post dam removal. I sampled riparian soil and vegetation along three tributaries, representing either the lower (undammed reference), middle (accessible since 2012), or upper Elwha (no anadromous salmon control). I was not able to detect MDN in soil or vegetation at any of the tributaries, including the reference tributary. However, the understory vegetation at the middle tributary had a higher $\delta^{15}N$ than the other tributaries (1 ‰, $p < 0.05$), which may be due to MDN inputs, or upstream anthropogenic nitrogen sources. Periodical monitoring of these sites, and establishing sites further upstream on the main stem of the Elwha River will allow us to trace the return of MDN to the watershed.

I also compared soil food webs of *A. rubra* and a non-nitrogen fixing riparian tree species, *Acer macrophyllum*, by using nematodes as a focal organism. *Alnus rubra* soil food webs had more predaceous nematodes than *A. macrophyllum* stands, but this difference decreased with increased sand in the soil ($p = 0.034$). This could be due to resource quality, as the C:N ratio of *A. rubra* leaf litter was lower than that of *A. macrophyllum* ($p < 0.001$). I then compared riparian soil food webs to those of adjacent upland sites. Total nematode and bacterivorous nematode abundance increased with soil moisture, but only in upland soils ($p = 0.004$, $p = 0.001$, respectively). This varied response could be due to riparian and upland soils hosting different taxonomic groups not seen by classifications used here.

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Introduction

Nitrogen is often the most limiting nutrient in many different ecosystems and regions (Vitousek and Howarth 1991, Elser 2007), and nitrogen addition can have large effects on ecosystems processes. One significant form of nitrogen addition to many aquatic and riparian ecosystems of the northern Pacific Ocean is marine derived nitrogen (MDN) via anadromous pacific salmon.

Anadromous fish are born in freshwater, but spend majority of their life in the ocean, where they gain over 90% of their biomass (Hunt 1999, Kline et al. 1990, Kline et al. 1993). At maturity, they migrate into freshwater to spawn, where semelparous species die. Anadromous fish spawning migrations can deposit nitrogen into freshwater ecosystems and adjacent riparian zones. The stable isotope ratio (^{15}N:^{14}N) of MDN supplied by anadromous fish is detectably different than that of freshwater, terrestrial nitrogen, and other nitrogen sources (Owens 1998, Kline et al 1990, Kline et al 1993, Galloway et al 2004).

Marine environments are naturally more enriched in ^{15}N than freshwater and terrestrial ecosystems (Owens 1998, Galloway et al 2004). This is in part due to fractionation of nitrogen during evaporation at the ocean surface, where the lighter isotope, ^{14}N, vaporizes preferentially (Owens 1998). In addition, ^{15}N bioaccumulates predictably as it travels up the food web, resulting in salmon that are enriched in ^{15}N

relative to their spawning habitats (Owens 1998). This unique nitrogen isotope signature allows MDN to be traced from anadromous fish through ecosystems and food webs.

Post spawning migration, MDN is transferred to riparian systems via flooding, predation, and hyporheic exchange. Anadromous fish carcasses are deposited on land by flooding and predators, where nitrogen is released into the surrounding environment. The importance of predation in the transfer of fish carcasses, hence MDN, to riparian and terrestrial zones is influenced by predator density (Hilderbrand et al 1999, Quinn et al 2008). In a single spawning event, bears removed more than 50% of pacific salmon from an Alaskan stream (Gende et al 2004).

Deposited carcasses are fed upon by vertebrates, macroinvertebrates and bacteria. (Cederholm et al 1989, Meehan et al 2005). Organisms that feed on salmon carcasses will release MDN from salmon tissue, and incorporate it into the soil, where it can be assimilated by plants (Cederholm et al 1989, Meehan et al 2005). Nutrient additions that simulated salmon carcass content showed that *Thuja plicata*, western red cedar, was able to assimilate a late season pulse of nitrogen (Drake et al 2006)

Hyporheic exchange, the exchange of water and its solutes between surface water and groundwater, is also an important mechanism for the transfer of MDN to terrestrial systems. During a spawning migration in an Alaskan stream, the surface water ammonium concentration immediately increased from 2 mg N/L, ultimately peaking at 147 mg N/L (O'Keefe and Edwards 2003). An increase in the ammonium concentration

of hyporheic zones corresponds with the increase in surface waters during salmon migrations (O'Keefe and Edwards 2003). Construction of spawning nests, redds, by anadromous salmon facilitates and increases the dispersal of MDN into the hyporheic zone (Buxton et al 2015). Hyporheic zones are not scoured by floods, and contain heterotrophic communities, which allows for the seasonal persistence of MDN (O'Keefe and Edwards 2003).

Plants living in the riparian and terrestrial zones adjacent to streams with anadromous fish assimilate MDN (Helfield and Naiman 2001, Helfield and Naiman 2002, Reimchen et al 2003, Bartz and Naiman 2005). Helfield and Naiman (2001) showed that the *Picea glauca* adjacent to salmon bearing streams in Alaska had a larger trunk diameter than those growing upstream of waterfalls, which prevent salmon passage. *Picea glauca*, *Salix alaxensis*, and *Arctagrostis latifolia* had higher levels of MDN in their foliage downstream of the same waterfalls (Helfield and Naiman 2002).

However, the effect of MDN on riparian ecosystems may not be equal across landscapes. Evidence from *Alnus sp.* suggests plants that form a symbiotic relationship with a nitrogen fixing bacteria assimilate minimal amounts of MDN (Helfield and Naiman 2002). Isotope ratios of nitrogen fixers closely resemble that of atmospheric nitrogen, which is depleted in ^{15}N when compared to soils and MDN (Helfield and Naiman 2002). Therefore, terrestrial and riparian zones with a high density of plant-nitrogen fixers may incorporate proportionately less MDN, and be less impacted by the presence of anadromous fish.

Soil processes, such as denitrification, can also affect the spatial distribution of MDN by altering the soil ^{15}N:^{14}N ratio. Denitrification discriminates against ^{15}N, and leaves the soil more heavily enriched in the heavier isotope (Nadelhoffer and Fry 1994). This can result in ^{15}N values that mimic an MDN signature, and can confound results of MDN studies (Pinay et al 2003). Few MDN studies address this issue (but see Vizza et al 2017), and clarifying how denitrification alters the ^{15}N:^{14}N ratio in the study system is imperative to effectively measure MDN (Pinay et al 2003). Directly measuring denitrification in the field is a difficult process, and often beyond the scope of MDN studies, but comparing soil characteristics that are known to influence denitrification rates can help make qualitative comparisons.

Anadromous fish populations have been decreasing at alarming rates across the world (Brown et al 1994, Yoshiyama et al 1998, Grech et al 2000, Limburg and Waldman 2009, Pess et al 2014). There are many factors contributing to this decline, but one major factor is the construction of dams along fish spawning streams and rivers (Pess et al 2014). The decrease of accessible spawning habitats caused by dams is directly related to population decline (Han et al 2008, Pess et al 2014). Dams inhibit the transfer of MDN to streams, which can limit primary and secondary productivity.

In the Pacific Northwest, USA, anadromous fish migrations are below 10% of historic levels, causing a nutrient decline in many areas (Gresh et al 2000). Similar studies have found decreased anadromous fish populations in California (Brown et al

1994, Yoshiyama et al 1998), and the northeastern and northwestern Atlantic ocean as well (Limburg and Waldman 2009). In response to major declines of anadromous fish populations, other ecological impacts, and the aging dam infrastructure, many dams have been removed, and more are pending removal (Poff and Hart 2002).

Recently, the Elwha and Glines Canyon Dams were removed from the Elwha River, WA, USA, and are the largest dam removals to date (Pess et al 2008). Built in 1913 and 1927, these dams prevented anadromous fish passage for about 100 years. Post dam removal, anadromous fish have increased access to freshwater spawning habitats, and are known to rapidly colonize newly accessible areas upstream of dams (Pess et al 2014, Tonra et al 2015, Izzo et al 2016). Despite their rapid colonization, the impacts of increased MDN on freshwater and terrestrial ecosystems may be slower to manifest (Drake et al 2006).

Dam removals provide a rare opportunity to assess how the reintroduction of anadromous fish affects the local ecosystem. Anadromous fish are an important nutrient source to riparian and terrestrial ecosystems, but most of the studies of anadromous fish and MDN are restricted to a few watersheds with very large spawning migrations. More studies are needed to evaluate how variable and widespread this process is. Monitoring the return of MDN to the Elwha River watershed will provide insight into the timeframe of MDN recovery.

The goal of this research was to establish baseline isotope data for riparian

vegetation and soils of tributaries on the Elwha River, and a nearby reference tributary, Salt Creek. Salt Creek feeds directly into the Strait of Juan de Fuca, and hosted salmon spawning migrations during the period that the Elwha River was dammed (McHenry and McCoy 2004). I tested the hypothesis that Salt Creek samples would be more enriched in ^{15}N than samples from tributaries of the Elwha River, indicative of MDN presence.

Methods

Study Site

This study was conducted on the Olympic Peninsula, WA, within the Elwha River and Salt Creek Watersheds (Figure 1.1). Both watersheds feed into the Strait of Juan de Fuca. Salt Creek is about 9 miles east of the Elwha River, hosted anadromous spawning populations while the Elwha River was dammed (McHenry and McCoy 2004), and serves as an undammed reference in this study. The Elwha River is approximately 70 kilometers long, with 160 kilometers of tributaries, and has a drainage basin of 831 square kilometers. Salt Creek includes 37.5 kilometers of streams that are accessible to anadromous fish, and has a drainage basin of 49 square kilometers (McHenry and McCoy 2004).

Study design

A large amount of sediment was released by dam removal, which buried downstream riparian areas along the Elwha River (Warrick et al 2015). Therefore, I collected samples on tributaries of the Elwha River, including Indian Creek, Hurricane Creek, and Wolf Creek. Indian creek is 9 kilometers long with a drainage basin of 129

square kilometers. Its confluence with the Elwha River is just upstream of the former Elwha Dam, and represents the “middle Elwha”. Anadromous salmon have been spawning in Indian Creek since the Elwha Dam was removed in 2012. Hurricane creek is upstream of the former Glines Canyon Dam, and is inaccessible to anadromous salmon. It represents the “upper Elwha” in this study. These areas all receive different amounts of annual precipitation (Figure 1.2, Duda et al 2008), but the area of nearby Port Angeles did not have any rainfall for over 30 days prior to soil collection (Wunderground, 2018).

Within each of the three sites, I identified five *A. rubra* stands, interspersed with five non-nitrogen fixing *Acer macrophyllum* stands, which were within 5 m of the stream edge (“riparian”). I also located five *A. macrophyllum* stands that were greater than 25 m away from the stream (“upland”). All stand canopies were at least 5 m apart. I could not locate a sufficient number of suitable stands at Hurricane Creek, and some were placed at the nearby Wolf Creek (Figure 1.1).

Field Methods

Field sampling was conducted in July 2017. Within each stand, I collected the following sub-samples: five soil cores (2.5 cm X 10 cm), four canopy tree leaves (*A. rubra* or *A. macrophyllum*), four leaf litter, and four canopy tree roots. Leaf litter was sampled in two ways: 1) indiscriminate litter sample, 2) litter specific to the canopy tree. To have a vegetation standard between the three different stand types, I also collected a frond tip from four *Polystichum munitum*, western sword fern, individuals at each stand.

Lab methods

All soil was placed in an 8 °C cooler upon collection, and then into an 8 °C cold storage facility upon return from field sampling. I dried all vegetation samples in a 50 °C drying oven shortly after collection. I also dried a portion of each soil sample to determine soil moisture by weight, where I recorded any mass loss during drying as soil moisture.

To determine the nitrogen isotopic signature and percent nitrogen of each sample, dry vegetative samples were ground to a powder with a Wig L Bug, and dry soil was ground with a mortar and pestle. To limit the number of samples submitted for SIA, the sub-samples within each separate tree stand were equally mixed together. For any one group of samples (i.e. soil), 225 different sub-samples were obtained, but 45 separate samples were submitted. I shipped dry samples of soil, tree leaves, tree litter, fern fronds, and plant roots to the University of New Mexico Center for Stable Isotopes. Soil and plant samples were analyzed with a Thermo Scientific Delta V coupled to a Costech 4010 elemental analyzer.

This value is often reported as $\delta^{15}N$, which is calculated from the following equation. Here, R_{std} is the ^{15}N:^{14}N ratio of atmospheric nitrogen, and R_{sample} is the ^{15}N:^{14}N of the sample in question.

$$\delta^{15}N‰ = \left(\frac{R_{sample} - R_{std}}{R_{std}} \right) (1000\,\delta‰)$$

Soil texture analysis was modified from the micro-pipette method (Miller and Miller 1987). Each soil sample was dried at 50 °C, passed through a 2 mm sieve, ground to a powder, and treated with 10 mL of 10% H_2O_2 (Aqua Solution, Inc.) to digest organic

matter. I then added 35 mL of 5% sodium hexametaphosphate (Gilson Company, Inc.) to each sample, and placed the samples on a rocker table overnight to disperse soil particles. After shaking, each sample was allowed to rest for one minute to let sand particles settle. Then a 5 mL pipette sample was taken from a depth of 2.5 cm to represent the clay+silt fraction of the soil. After 2 hours, another sample was taken in the same manner, and represents the clay fraction.

After sampling for silts and clays, I passed the remaining sample solution through a 50 μm sieve to collect the sand fraction. Because the organic matter pre-treatment with H_2O_2 was not 100% effective, remaining organic matter is caught in the sieve with the sand. After drying, all sand collected was placed in a muffle furnace at 450 °C to burn off remaining organic matter. The proportion of each sample that is sand, silt, and clay was then determined using the following equations:

% Organic matter= (weight loss from H_2O_2 treatment + weight loss from burning) / total soil X 100%

% sand= (sand (g)/ total soil (g)) X 100%

% clay= (clay / (clay+silt)) X (total soil (g) - sand (g)) X 100%

% silt= 100% - %clay + %sand

Statistical Analyses

All statistics were done with R Programming (R Core Team, 2016). I determined if differences in soil texture, moisture, and organic matter were significant between tributary and location (riparian and upland) with linear mixed models via the R package "lme4" (Bates et al 2015). Stand was a random effect for soil models. I used type III ANOVA to test if isotopic ratios and percent nitrogen differed between each tributary,

between stands of *A. rubra* and *A. macrophyllum*, and between riparian and upland plots. Tukey's honestly significant differences test was used post hoc for pairwise comparisons. I used a linear model to test if soil moisture had an effect on soil $\delta^{15}N$, and displayed the result via the R package "effects" (Fox and Hong 2009).

Results

There was no difference between soil moisture of riparian areas and upland areas, though Indian Creek riparian soils had a greater moisture content than the other two tributaries (Table 1.1, $p=0.006$). Salt Creek had significantly less organic matter than the other two tributaries ($p < 0.001$). Riparian and upland soils did not differ in their proportion of soil organic matter. (Table 1.1). Proportion of nitrogen did not differ between upland and riparian soils, but Indian Creek had more nitrogen than the other tributaries ($p < 0.001$).

Riparian soils had more sand than upland areas ($p < 0.001$), except for Indian Creek where riparian and upland soil had equals amounts of sand (Table 1.1). Upland soils had a greater proportion of silt than riparian soils ($p = 0.002$), except for Indian Creek where riparian and upland soils did not differ in their silt content (Table 1.1). Upland soils also had a greater clay content ($p < 0.001$), but similar to silt and sand, there was no difference between upland and riparian soils at Indian Creek (Table 1.1).

I did not detect any difference in the $\delta^{15}N$ of any tree foliage between sites (Figure 1.3). Regardless of its location within a site, stream side or upland, *Acer macrophyllum*

foliage had a significantly lower $\delta^{15}N$ than *A. rubra* foliage (Figure 1.3, $p = 0.006$). There were no differences in total foliar percent nitrogen between sites, locations within a site (upland vs stream), or vegetation types (*A. rubra* vs *A. macrophyllum*). There were no differences between $\delta^{15}N$ or total nitrogen of any tree roots between sites, vegetation type, or location.

$\delta^{15}N$ of *Polystichum munitum* foliage marginally differed between site (Figure 1.4, $p = 0.085$), but not between stands of *A. macrophyllum* or *A. rubra* nor between riparian plots and upland plots (Figure 1.4). However, if the upland sites are removed from the model, riparian *P. munitum* at the middle tributary had a higher $\delta^{15}N$ than the other two tributaries ($p = 0.032$). Soil $\delta^{15}N$ and *P. munitum* foliage $\delta^{15}N$ were weakly correlated ($p = 0.059$, $r^2 = 0.061$).

Soil $\delta^{15}N$ did not differ between the three tributaries. Also, soil $\delta^{15}N$ did not differ between stands of *A. rubra* and *A. macrophyllum*, nor between stream edge and upland plots (Figure 1.5). Soils with a higher moisture content had a lower soil $\delta^{15}N$ (Figure 1.6, $p = 0.043$), though the effect was small ($r^2 = 0.07$). Soil percent sand did not correlate with soil $\delta^{15}N$. Soils at the middle tributary had a higher proportion of nitrogen compared to the other two tributaries (Figure 1.7, $p < 0.001$), but did not vary between stand types. Total amount of soil nitrogen did not differ between *A. rubra* stands or *A. macrophyllum* stands (Figure 1.7). Soil organic carbon followed the same trends as soil nitrogen, where the middle tributary had a greater amount of soil organic carbon than the other two

tributaries (Figure 1.8, $p < 0.001$). Further, proportion of soil nitrogen and soil organic carbon were strongly correlated ($p < 0.001$, $r^2 = 0.881$).

Indiscriminately collected *A. macrophyllum* litter in the upland had a lower $\delta^{15}N$ than riparian *A. macrophyllum* and *A. rubra* litter (Figure 1.9, $p = 0.030$). Litter samples from *A. macrophyllum* stands had significantly lower nitrogen than samples collected from *A. rubra* stands (Figure 1.10, $p < 0.001$). $\delta^{15}N$ did not vary between tributaries, and percent nitrogen was marginally significant ($p = 0.063$). There was no difference between the general litter nitrogen content of streamside plots and upland plots (Figure 1.10).

Similarly, litter that was specific to upland *A. macrophyllum* had a lower $\delta^{15}N$ than riparian *A. macrophyllum* and *A. rubra* litter (Figure 1.11, $p < 0.001$). *A. rubra* specific litter had significantly more nitrogen than that of *A. macrophyllum* (Figure 1.12, $p < 0.001$). There was no difference between nitrogen content of streamside or upland litter specific to *A. macrophyllum* (Figure 1.12).

Discussion

I did not detect MDN in any samples from any of the Elwha River tributaries that I sampled at, or at the undammed reference tributary, Salt Creek. However, foliage of riparian *P. munitum* had a higher $\delta^{15}N$ at Indian Creek than the other tributaries and my reference tributary. This difference was not observed for upland *P. munitum*, suggesting that differences between the riparian zones are in part due to being adjacent to the stream.

This could be a result of MDN inputs since dam removal in 2012 or any upstream anthropogenic inputs which could also raise the $\delta^{15}N$.

Fertilizer $\delta^{15}N$ ranges from -5 to +2 ‰ (Choi et al 2003), and Elwha River anadromous fish $\delta^{15}N$ ranges from 11.7 - 15.9 ‰ (Tonra et al 2015). *Polystichum munitum* foliage $\delta^{15}N$ ranged from -0.5 to -2.5 ‰ in this study, and therefore could be increased as a result MDN or fertilizer inputs. However, whether or not this is due to MDN or fertilizers cannot be analyzed with the data collected for this study. Denitrification can also elevate the soil $\delta^{15}N$ (Pinay et al 2003), which could also elevate the $\delta^{15}N$ of *P. munitum* foliage.

Denitrification is most prevalent when the silt and clay content of soil is greater than 65% (Pinay et al 2000), and can result in soil $\delta^{15}N$ values that are similar to a MDN signal (Pinay et al 2003). Indian Creek had significantly greater soil moisture, a finer soil texture, and a higher soil organic matter and nitrogen content than the other tributaries, which could explain the greater $\delta^{15}N$ of *P. munitum* foliage. However, few soil samples at Indian Creek were above the 65% threshold, and those that were tended to be upland soils. I did not detect any differences in $\delta^{15}N$ between riparian and upland soils or between any tributaries in this study, so it does not appear that denitrification is making an appreciable impact on the $\delta^{15}N$ of soil. Though, this data should be kept in mind for future monitoring of MDN in the Elwha River watershed.

I did detect isotopic differences between *A. rubra* and *A. macrophyllum* foliage, though there was no difference between their litter. Currently, I do not know how the $\delta^{15}N$ of non-nitrogen fixing vegetation at salmon bearing streams changes from leaf drop

through decomposition. Here, the $\delta^{15}N$ of *A. macrophyllum* litter changed relative to live foliage. Therefore, it may be difficult to detect MDN in the soil of salmon spawning streams if the $\delta^{15}N$ of litter of nitrogen fixing vegetation and non-nitrogen fixing vegetation is too similar.

Because large dam removal is a relatively new phenomenon, the time frame of anadromous fish populations' response to newly accessible habitat is not well known. However, the years since dam removal on the Elwha River and evidence from other dam removals suggest that anadromous fish rapidly colonize upstream habitats (Pess et al 2014, Tonra et al 2015, Izzo et al 2016).

Post dam removal on the Elwha River, Tonra et al (2015) detected MDN in the American Dipper, a bird that commonly feeds on fish and fish eggs, upstream of the former Elwha Dam. This suggests that MDN is present upstream of the former dam site, though this birds diet includes salmon eggs, which are enriched in MDN. The birds other main food source, macroinvertebrates, were not enriched in MDN. The incorporation of MDN into non-anadromous residents of the aquatic ecosystems that don't feed directly on salmon may be slower (Tonra et al 2015). Further, the influx of MDN into the riparian areas may be even slower to manifest (Drake et al 2006), as it relies on several different transfers of nutrients.

This influx relies on three major processes: flooding, predation, and hyporheic exchange. The relative importance of these in the transfer of MDN to riparian systems is

not known, and is likely to be highly variable. Floods deposit salmon carcasses into the floodplains, where they breakdown and leave MDN is incorporated into the system. Dams often limit the magnitude of downstream flooding (Poff and Hart 2002), which could be why Perry et al (2017) were unable to detect MDN in riparian soils of the undammed lower Elwha River. The tributaries I utilized in this study may not have the large flooding regime that other salmon bearing rivers have.

Predation also deposits salmon carcasses into floodplains. However, large predators that are known to hunt anadromous salmon do not have large populations in the vicinity of the Elwha River and Salt Creek. Potential salmon predator populations are being monitored, as they are expected to respond to potential increases in salmon spawning migrations (Sager-Fradkin et al 2006). Despite the probability that salmon carcasses are not directly being deposited in the floodplains, other processes may also be important for the influx of MDN into riparian zones

Hyporheic exchange is a known mechanism for the transfer of MDN into riparian areas (O'Keefe and Edwards 2003, Buxton et al 2015). During spawning migrations, surface waters increase in nitrate concentration, which is mirrored by adjacent hyporheic zones (O'Keefe and Edwards 2003). The hyporheic zone has a slower flow rate than the adjacent water system, so MDN can persist in this area much longer than in surface waters (O'Keefe and Edwards 2003). This provides more time for plants and microbes to assimilate MDN, where it will then be cycled in the system. However, the number of

studies of hyporheic exchange of MDN is limited, and may require much larger populations than the tributaries I utilized in this study currently support.

In addition to collecting nitrogen isotope data on tributaries of the Elwha River, I also generated data for the Salt Creek watershed. My initial aim for utilizing Salt Creek was as an undammed reference site. However, I did not detect any evidence of the presence of MDN in any of my Salt Creek samples. While it has never been dammed, Salt Creek has its own issues that may interfere with MDN influx to riparian zones. Historically, Salt Creek has served as an anadromous salmon spawning watershed, but in past decades the number of spawning salmon has been decreasing (McHenry and McCoy 2004). Salt Creek anadromous salmon populations are not as heavily monitored as Elwha River populations, with the most recent available data being from 2003 (McHenry and McCoy 2004). From 1995 until 2004, the number of winter steelhead redds ranged from 120 to 384 redds over an 8 Km reach (McHenry and McCoy 2004).

The Salt Creek basin has been subject to logging, culverts, grazing, and many other human impact, and is currently undergoing its own restoration (McHenry and McCoy 2004). Logging removed a large amount of woody vegetation from riparian areas on Salt Creek, resulting in decreased amounts of large woody debris in the creek. Large woody debris provides spawning habitat for salmon. In addition, several culverts have been established in the Salt Creek watershed (McHenry and McCoy 2004). While culverts are not impassable, they may inhibit spawning salmon.

I intentionally placed sampling plots on Salt Creek downstream of any culverts. The downstream influence of culverts is not well known, but they may limit flooding, which can be important in the transfer of MDN from freshwater to riparian vegetation and soils. While the land adjacent to Salt Creek has vegetation that is indicative of riparian floodplains, including *Rubus sp.*, *Oemleria cerasiformis*, and *Oplopanax horridus*, some areas of the tributary have incised banks. Bank incision is a common symptom of tributary and riparian degradation, and Salt Creek floodplains may be disjointed from their adjacent tributary (McHenry and McCoy 2004).

Many studies investigating MDN in riparian areas are conducted in watersheds that host spawning migrations that are much larger than the Elwha River and Salt Creek watersheds currently support. For instance, Lynx Creek, AK, is the focus of many MDN studies and has a mean spawning run size of 3,000 fish (Rogers and Rogers 1998), and a length of about 2 km. Monitoring the return of anadromous fish, and the influx of MDN to riparian areas, will allow us to determine how important the magnitude of salmon populations are for detecting MDN. Also, it will allow us to determine the time needed for MDN to move from marine systems to freshwater and riparian systems in an amount that can be detected via isotope methodologies.

Despite the large body of evidence that anadromous salmon provide a large portion of nitrogen to riparian areas, their effect on riparian ecosystem processes, such as nutrient cycling and decomposition, are not well known. I established baseline nitrogen isotope data that will be useful in monitoring the return of MDN to the riparian areas of

the Elwha River watershed. Continued monitoring via periodical sampling is necessary to assess how important anadromous fish migrations are for these processes.

Monitoring the areas I have addressed in this study, more tributaries within these areas, and areas further upstream on the Elwha River, will help us to further clarify the role anadromous fish play in riparian ecosystems. In addition, establishing a reference site that hosts anadromous salmon, and where MDN can be detected, is pivotal in monitoring the return of MDN to the Elwha River. Future studies should also investigate the presence of MDN in the freshwater ecosystems of the Elwha River by utilizing stable isotope methodologies, but also by examining surface water and hyporheic zone nitrogen concentrations before, during, and after spawning migrations.

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Chapter 2: Influence of Soil Abiotic Characteristics and Nitrogen Fixing Vegetation on Riparian Soil Food Webs of the Olympic Peninsula, WA, USA.

Introduction

Riparian zones, the interface between surface waters and terrestrial ecosystems, are important for a several reasons, including that they filter pollutants and fertilizer runoff (Gumiero et al 2011, Hoffman et al 2012), are biodiversity hotspots, and provide shade and carbon inputs for aquatic systems (Naiman and Decamps 1997). Despite the importance of riparian zones, riparian soil food webs are understudied compared to soil food webs of agricultural, grassland, and forested ecosystems.

The soil food web is an essential component of ecosystem function, as it plays a pivotal role in decomposition and nutrient cycling (Wagg et al 2014). In addition, most riparian soil community studies occur in riparian buffers to agriculture, and are influenced by anthropogenic disturbances (Sanchez-Moreno et al 2011, Briar et al 2012, Raich and Schultz 2015). Studying relatively undisturbed riparian soil food webs may lead to a better understanding of soil food webs as a whole because riparian systems differ from other terrestrial systems in their biotic and abiotic characteristics, and disturbance regime (Hodson et al 2014).

These differences in disturbance regime lead to notable differences in soil abiotic characteristics (Naiman and Decamps 1997, Bechtold and Naiman 2006). Commonly, riparian areas have different soil texture than adjacent upland sites, due to deposition during flooding, channel migration, and erosion (Naiman and Decamps 1997, Bechtold

and Naiman 2006). Also, sediment particles experience sorting during flooding, so riparian soil texture is often more heterogeneous than adjacent upland soils (Bechtold and Naiman 2006).

Soil texture influences soil moisture retention, where soils with a smaller average particle size tend to retain more water (Rawls et al 2003, Saxton and Rawls 2006). Soils with higher organic matter also have increased moisture retention (Rawls et al 2003, Saxton and Rawls 2006). The groundwater table also tends to be closer (more elevated) to the soil surface in riparian zones than adjacent upland sites (Naiman and Decamps 1997). This often results in soils with a higher moisture content, which has varied effects on the soil food web (Ferris et al 2001, Renco et al 2015).

The differences in riparian disturbance regime and soil characteristics lead to plant and animal communities that differ from adjacent terrestrial systems (Naiman and Decamps 1997, Bechtold and Naiman 2006). How riparian soil food webs differ from adjacent upland ecosystems is relatively unstudied when compared to vegetation and other animal communities. It is also unclear how they are influenced by nitrogen.

Because nitrogen is a limiting nutrient in many areas (Vitousek and Howarth 1991, Elser et al 2007), nitrogen addition can have large effects on ecosystems. The effect of nitrogen addition on soil food webs is well studied, but the response of the soil community to nitrogen addition is varied (Sjursen et al 2005, Wei et al 2012, Zhao et al 2014, Chen et al 2015). Investigations of the effect of nitrogen on riparian soil food webs

are even fewer (Ettema 1999). In addition, many studies utilize nitrogen fertilizers or organic amendments (Ettema 1999, Wei et al 2012, Zhao et al 2014, Chen et al 2015). While this is important to study how anthropogenic inputs influence soil communities, they may not accurately represent natural nitrogen addition processes, such as symbiotic nitrogen fixation.

Symbiotic nitrogen fixation, where a nitrogen fixing bacteria living within plant tissues fixes atmospheric nitrogen, is an important mechanism for the input of nitrogen to an ecosystem (Vitousek et al 2013). The spatial distribution of symbiotic nitrogen fixing plants will therefore influence the spatial distribution of nitrogen, which may affect the soil community. Nitrogen from these plants typically enters the soil food web via leaf litter decomposition, but also from root decomposition and root exudates (Vitousek et al 2013).

Soil texture and water content are known to influence denitrification rates. Soil texture directly influences denitrification, where soils with fine textures and high moisture have increased denitrification rates (Pinay et al 2003). Denitrification can lead to large amounts of nitrogen loss from soil (Nadelhoffer and Fry 1994, Pinay et al 2003). Texture also influences mineralization and retention of nitrogen in riparian ecosystems (Bechtold and Naiman 2006). The combined influence of soil abiotic characteristics and nitrogen fixing vegetation on riparian soil food webs can be determined by using nematodes as focal organisms.

Nematodes are often used as a surrogate for the soil food web as they occupy many trophic levels. Nematodes mineralize nitrogen, where they excrete excess nitrogen into the soil and make it more accessible to plants (Chen and Ferris 1998, Ferris et al 1999, Carrillo et al 2016, Gebremikael et al 2016). Based upon their mouthpart morphology, nematodes are often grouped into the following functional feeding groups: bacterivore, fungivore, plant parasite, omnivore, and predator (Bongers 1990, Yeates 1999).

For this study, I had two main objectives: 1) Characterize the riparian soil food webs of tributaries on the Olympic Peninsula, WA, USA, and compare them to adjacent upland soil food webs, and 2) Compare the soil food webs of *Alnus rubra*, a nitrogen fixing tree, to the soil food web of a non-nitrogen fixing tree, *Acer macrophyllum*. I hypothesized that soil texture would differ between riparian and upland soils due to differences in disturbance regime, resulting in different nematode communities. In the riparian zones, I expect that the abundances of nematode functional feeding groups would differ between stands of *A. rubra* and *A. macrophyllum*, as a result of increased nitrogen inputs in alder stands.

Methods

Study Site

This study was conducted in the Elwha River wand Salt Creek watersheds on the Olympic Peninsula, WA, USA. Both feed into the Strait of Juan de Fuca (Figure 1.1). The Salt Creek drainage basin is 49 square kilometers (McHenry and McCoy 2004).

Within the Elwha River watershed, I sampled at Indian Creek and Hurricane Creek. Indian creek is approximately 9 kilometers long with a drainage basin of 129 square kilometers. Some samples from Hurricane Creek were collected at the adjacent Wolf Creek. Both collectively form a drainage basin of about 14.2 square kilometers. These locations were chosen as part of a larger project concerning the return of marine derived nitrogen to the Elwha River. Precipitation varies between my three study tributaries (Figure 1.2, Duda et al 2008), but it did not rain for over 30 days prior to soil collection (Wunderground, 2018).

Study Design

To assess how the soil food web differs between riparian areas and adjacent upland sites, I sampled soil from ten *A. macrophyllum* tree stands at each tributary. Five stands were less than 5 m from the stream edge ("riparian"), and five stands were in the upland, greater than 25 m from the stream edge ("upland"). I also sampled soil from five riparian *A. rubra* stands to assess how the presence of a nitrogen fixing tree would influence the soil food web. Each stand was greater than 5 m away from other stands, and each *A. macrophyllum* stand was greater than 5 m away from any individuals of *A. rubra*. The edge of a stand was considered to be the edge of its canopy.

Field Methods

I collected all samples for this study in July 2017. At each stand, I collected five soil samples to a depth of 10 cm. Additionally, I collected four leaves from the canopy (*A. rubra* or *A. macrophyllum*), four leaf litter samples, and four canopy tree roots. I

further split leaf litter into indiscriminate leaf litter, and leaf litter specific to the canopy tree.

Lab methods

All soil was placed in an 8 °C cooler upon collection, and a portion was used to determine soil moisture by comparing weight before and after drying at 50 °C. Leaves, roots, and leaf litter were dried in at 50 °C shortly after collection. I extracted nematodes from soil with Baermann funnels (Baermann 1917, Barker 1985), and placed samples in 8 C cold storage until processing. I counted total nematode abundance, and identified a subset to their functional feeding group (Yeates 1993). Tylenchidae were placed in their own functional group of 'tylenchus', as their feeding habits are variable (Yeates et al 1993).

To determine the percent nitrogen and percent carbon of each sample, I submitted dry samples of soil, tree leaves, tree litter, and plant roots to the University of New Mexico Center for Stable Isotopes. All vegetation samples were ground with a Wig L Bug, while soil was passed through a 2 mm sieve and ground with a mortar and pestle. The sub-samples within each separate tree stand were equally mixed together into one composite sample. For any one group of samples (i.e. soil), 225 different sub-samples were obtained, but 45 separate samples were submitted. These samples were analyzed with a Thermo Scientific Delta V coupled to a Costech 4010 elemental analyzer.

I followed the micro-pipette method, with slight modifications, for soil texture analysis (Miller and Miller 1987). After drying, each ground soil sample was treated with 10 mL of 10% H_2O_2 (Aqua Solution, Inc.) to digest organic matter. Some samples with very high organic matter need more than 10 mL. After 48 hrs, I dried the samples and added 35 mL of 5% sodium hexametaphosphate (Gilson Company, Inc.) to each one. After rocking overnight, each sample was allowed to rest for 1 minute to let sand particles settle. Then a 5 mL pipette sample was taken at a depth of 2.5 cm, and again at 120 minutes to represent the clay + silt fraction, and the clay fraction of the soil, respectively. I dried these samples at 50 °C. Then I passed the remaining sample solution through a 50 μm sieve to collect the sand fraction. The H_2O_2 organic matter pre-treatment is not 100% effective, so sieved samples were dried at 50 °C and placed in a muffle furnace at 450 °C to burn off remaining organic matter. The proportion of each sample that is organic matter, sand, silt, and clay was then determined using the following equations:

% Organic matter= (weight loss from H_2O_2 treatment + weight loss from burning) / total soil X 100%

% sand= (sand / total soil) X 100%

% clay= (clay / (clay+silt)) X (total soil - sand) X 100%

% silt= 100% - %clay + %sand

Statistical Analyses

I conducted all statistical analysis in R programming (R Core Team 2016). Soil characteristics were compared between tributary and location (riparian or upland) with mixed linear models that used individual stand as a random effect. Because soil nitrogen

was determined from composite samples, it was compared between site and location with ANOVA, and did not have a random effect.

I also used mixed linear models to test if nematode communities differed between each site, between each stand type, and as a result of varied soil characteristics. I modelled the log transformation of total nematode, bacterivore, tylenchus, and omnivore abundance with mixed linear models. For predators, fungivores, and plant parasites, I used a hurdle model approach. Presence/absence was modelled with a generalized mixed linear effect model which used a binary distribution. Then, I modelled non-zero abundance with a generalized mixed linear model with a gamma distribution.

All mixed linear models and generalized mixed linear models were made in the R Programming package "lme4" (Bates et al 2015) and used individual stand as the random effect. I used the Akaike information criterion (AIC) to choose the best model for all models. To test for significance of predictor variables for each model, I used type III ANOVA. R^2 values for abundance models with a gamma distribution (predator, fungivores, and plant parasites) are not available in any R programming packages that I am aware of, and were determined via the methods described in Nakagawa et al 2017.

Results

Soil Characteristics

The moisture content of soil did not differ between riparian areas and upland areas, though Indian Creek riparian soils had a greater moisture content than the other

two tributaries (Table 1.1, $p = 0.006$). The proportion of soil organic matter in soils did not differ between riparian and upland areas, but Salt Creek had less organic matter than the other two tributaries in both the riparian and upland areas (Table 1.1, $p < 0.001$). Indian creek riparian soils had more nitrogen than other riparian soils ($p < 0.001$), but there was no difference between riparian and upland soils.

Riparian soils had more sand than upland areas ($p < 0.001$), except for Indian Creek where riparian and upland soil had equals amounts of sand (Table 1.1). Upland soils had a greater proportion of silt than riparian soils ($p = 0.002$), except for Indian Creek where riparian and upland soils did not differ in their silt content (Table 1.1). Upland soils also had a greater clay content ($p < 0.001$), but similar to silt and sand, there was no difference between upland and riparian soils at Indian Creek (Table 1.1). Soil organic matter was negatively correlated with the proportion of sand in the soil ($p < 0.001$, $R^2 = 0.70$).

Leaf litter of riparian *A. rubra* had a significantly lower carbon to nitrogen ratio than leaf litter of both riparian and upland *A. macrophyllum* (Figure 2.1, $p< 0.001$). This relationship remained the same for indiscriminate litter samples from each stand type ($p = 0.006$), though the comparison between riparian *A. rubra* and riparian *A. macrophyllum* was only marginally significant ($p = 0.066$).

Nematode Community

Total nematode abundance did not differ between the three tributaries. I modeled total nematode abundance as a function of the interaction of soil moisture and location ($R^2 = 0.30$). Nematodes were more abundant in the uplands than in riparian soils ($p = 0.001$). Total nematode abundance increased with soil moisture ($p = 0.071$), but this effect was strongest in the uplands (Figure 2.2, $p = 0.004$).

Bacterivore abundance did not differ between the three sites or between *A. rubra* and *A. macrophyllum* stands. I modelled bacterivore abundance as a function of the interaction of location and soil moisture (Figure 2.3, $R^2 = 0.17$). Bacterivores were more abundant in upland sites (Figure 2.3, $p = 0.017$). Bacterivore abundance increased with soil moisture ($p = 0.015$), but more so in the uplands (Figure 2.3, $p = 0.001$).

Tylenchus abundance did not differ between the three tributaries, nor between *A. rubra* and *A. macrophyllum* stands. Tylenchus abundance was modelled as a function of the interaction between soil moisture and location (Figure 2.4, $R^2 = 0.40$). Soil moisture had a significant effect on tylenchus abundance ($p < 0.001$), however, there was no difference between riparian and upland soils. Upland tylenchus were more affected by soil moisture than riparian tylenchus (Figure 2.4, $p= 0.011$).

Omnivore abundance did not differ between *A. rubra* and *A. macrophyllum* stands, nor between tributaries. Omnivore abundance was significantly negatively

affected by the proportion of soil that is sand (Figure 2.5, $p = 0.018$, $R^2 = 0.29$). Despite the effect of sand, omnivore abundance did not differ between riparian and upland zones.

I analyzed predator, plant parasite, and fungivore data with hurdle models because of the large number of zeros present in the data for those functional groups. I modelled plant parasite presence as a function of location and soil organic matter (Figure 2.6, $R^2 = 0.40$). Parasite presence was greater in the upland than at the stream edge (Figure 2.6, $p= 0.009$). Presence also decreased with increases in soil organic matter (Figure 2.6, $p = 0.008$), though the interaction with location was not significant ($p = 0.14$). When present, plant parasite abundance was best modelled by the interaction of soil moisture and vegetation type (Figure 2.7, $p < 0.001$, $R^2 = 0.36$). On their own, soil moisture and vegetation type did not influence overall parasite abundance.

Fungivore presence was significantly affected by soil organic matter (Figure 2.8, $p < 0.001$), where presence decreased with increases in organic matter ($R^2 = 0.19$). Fungivore abundance was best modelled by soil organic matter, though it was not significant.

Predator presence was significantly correlated with the proportion of sand in the soil (Figure 2.9, $p = 0.028$), and soil organic matter (Figure 2.10, $p = 0.002$), though the interaction was not significant ($R^2 = 0.26$). Predator abundance was most explained by the interaction of vegetation type and proportion of sand in the soil ($R^2 = 0.41$). Predator abundance was greater in *A. rubra* stands than in *A. macrophyllum* stands (Figure 2.11, p

= 0.009). Predator abundance decreased with proportion of sand in the soil ($p < 0.001$). This effect was more pronounced in *A. rubra* stands than in *A macrophyllum* stands (Figure 2.12, $p = 0.034$).

Discussion

This study represents the first investigation into the influence that nitrogen fixing vegetation has on riparian soil food webs. It also adds to the limited body of literature concerning riparian soil food webs, and is one of few studies that focuses on relatively undisturbed riparian zones. There were clear differences between the riparian and upland soil food webs, and closer taxonomic evaluation of nematodes may make this relationship more clear. Obtaining a broad understanding of soil food webs in riparian zones that are not subject to anthropogenic disturbances will provide context for the large body of research concerning human impacts on riparian soil food webs.

The difference in soil texture between riparian and upland soils is likely due to the unique disturbance regime that riparian zones experience (Naiman and Decamps 1997). Interestingly, soil moisture did not differ between riparian and upland soils. The three tributaries utilized in this study receive different amounts of annual precipitation (Figure 1.2, Duda et al 2008), but soil moisture did not follow this trend. This study simply shows a snapshot of what is happening in these systems, and it is possible that I sampled at a time, July, where soil moisture does not normally differ between these two areas. Also, the area of the nearby town of Port Angeles had not received rain for over 30 days prior to my soil sampling (Wunderground.com, 2018). Riparian soils had a higher proportion

of sand than upland soils, suggesting they are less able to retain water (Rawls et al 2003, Saxton and Rawls 2006). This may also explain why I did not observe any differences in soil moisture between riparian and upland soils.

Along with influencing soil moisture, soil texture also influences the soil food web. It is generally hypothesized that coarse soil textures promote high abundance of predaceous nematodes. However, soil texture preference differed between life history strategies of predators in California riparian woodlands (Hodson et al 2014). In this study, I found that predator presence tended to be higher in areas with a greater proportion of sand. In contrast, I found that predator and omnivore abundance was negatively correlated with increased sand. This may be due to sandier soils not being able to retain as much organic matter as finer soils (Rawls et al 2003, Saxton and Rawls 2006).

Organic matter leaches out of sandy soils, and in this study proportion of sand was negatively correlated with soil organic matter. Higher amounts of organic matter in the soil are thought to increase the abundance of higher trophic levels such as predators and omnivores (Neher 2010). Therefore, there could be some confounding factors between these two hypotheses. The relationship between soil texture, soil organic matter, and soil moisture is varied (Saxton and Rawls 2006), and may depend on other factors I did not address in this study.

In this study, total nematode abundance was most affected by soil moisture, but this relationship differed between riparian and upland sites. The response to soil moisture seems to be driven by the bacterivores, and to a lesser extent, Tylenchus. Bacterivores accounted for more than 40% of the nematodes in my soil samples, and Tylenchus accounted for nearly 20%. Nematode community response to changes in soil moisture is varied. For instance, soil moisture manipulations alone could not explain any changes in a grassland nematode community (Papatheodorou et al 2004), but total nematode abundance can vary with seasonal differences in precipitation (Alon and Steinburger 1999). This variation could also be due to soil temperature (Alon and Steinburger 1999, Papatheodorou et al 2004).

I found that soils of *A. rubra* supported a greater abundance of predaceous nematodes. This effect was not found for any other trophic level. Other manipulative studies have showed varied responses of the soil food web to nitrogen amendments, (Alon and Steinburger 1999, Chen et al 2015), and the exact relationship between nitrogen and soil communities is still unclear. It may be that the lower trophic levels of the soil food web may not be nitrogen limited, but the higher levels are. The data presented here seems to fall in line with this hypothesis.

While I did not detect differences in the soil nitrogen content under *A. rubra* and *A. macrophyllum, A. rubra* litter had a lower C:N ratio, indicating better litter quality. Litter of the genus *Alnus* is known to decompose faster than many other tree species (Fyles and Fyles 1993, Horodecki and Jagodziński 2017), and increases decomposition

rate of other litter types (Fyles and Fyles 1993). Therefore, the influence of *A. rubra* on soil food webs may not be a direct result of potential nitrogen increases, but could also be due to increased litter decomposition rates.

My study did not examine the concentration of different types of nitrogen in the soil, but rather I analyzed total nitrogen. Most of this nitrogen is bound in the organic matter of the soil, so I are not able to tease apart the effect of soil organic matter and soil nitrogen. However, inorganic nitrogen tends to leach out of the soil column quite rapidly (Rothwell et al 2008), so the organically bound nitrogen may be a driving force in increased predator abundance.

Future investigations of riparian soil food webs should sample soils on a continuous basis, allowing for the incorporation of natural seasonal fluctuations of the soil community into statistical models. Also, increasing the number of soil abiotic characteristics, vegetation types, and sampling locations will better My understanding of riparian soil food webs. Utilizing closer taxonomic groups such as family will allow for the use of metabolic footprints (Hodson et al 2014). More research into soil food webs is needed so generalizations can be made about their structure, and how they are influenced by the unique soil properties and vegetation communities of riparian zones.

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Table 1: Percent moisture, organic matter, total nitrogen, sand, silt, and clay for riparian and upland soils at each tributary. Parentheses show 95% confidence intervals.

Site	Location	% Moisture	% Organic Matter	% Total Nitrogen	% Sand	% Silt	% Clay
Hurricane Creek	Riparian	15.4 (± 3.7)	5.2 (± 4.8)	0.25 (± 0.24)	54.2 (± 7.7)	12.9 (± 3.0)	28.3 (± 4.9)
	Upland	19.7 (± 4.7)	12.7 (± 6.2)	0.52 (± 0.34)	21.8 (± 9.9)	21.1 (± 3.9)	44.4 (± 6.3)
Indian Creek	Riparian	25.8 (± 3.9)	17.5 (± 5.1)	0.98 (± 0.24)	40.0 (± 8.1)	10.4 (± 3.3)	37.5 (± 5.2)
	Upland	22.5 (± 5.2)	14.0 (± 6.8)	0.60 (± 0.34)	46.5 (± 10.9)	9.7 (± 4.3)	29.8 (± 6.9)
Salt Creek	Riparian	15.3 (± 3.7)	2.7 (± 4.8)	0.23 (± 0.24)	50.6 (± 7.7)	12.1 (± 3.0)	34.8 (± 4.9)
	Upland	20.3 (± 5.2)	3.4 (± 6.8)	0.32 (± 0.34)	27.1 (±10.9)	23.2 (± 4.3)	46.2 (± 6.9)

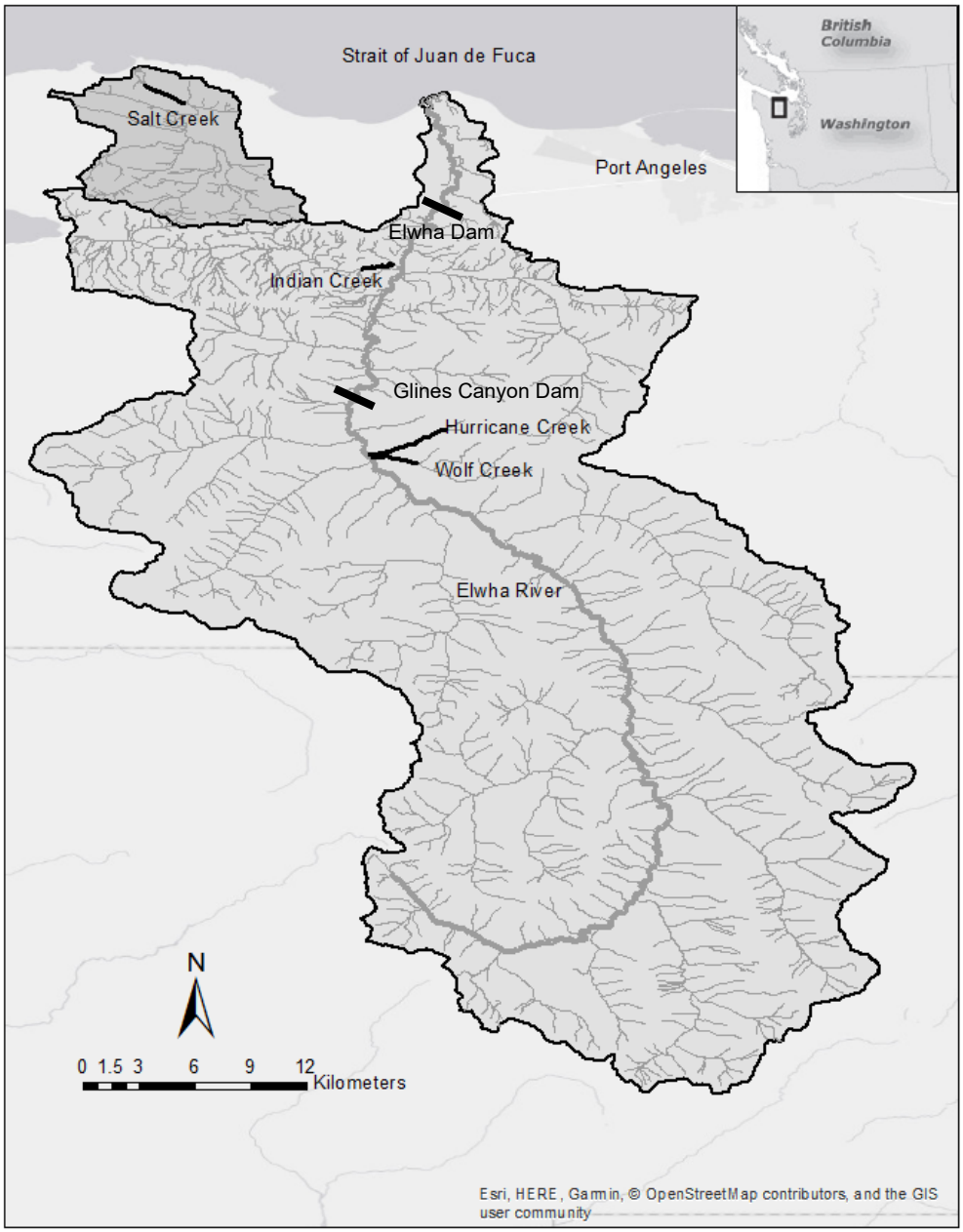

Figure 1.1: Map of the Elwha River watershed (light grey) and Salt Creek watershed (dark grey), Olympic Peninsula, WA. Main stem of the Elwha River is dark grey. Sampling location along each of the study tributaries is highlighted in black. Black bars denote approximate locations of former Elwha and Glines Canyon Dams. Inset of Washington State retrieved from Pess et al 2008.

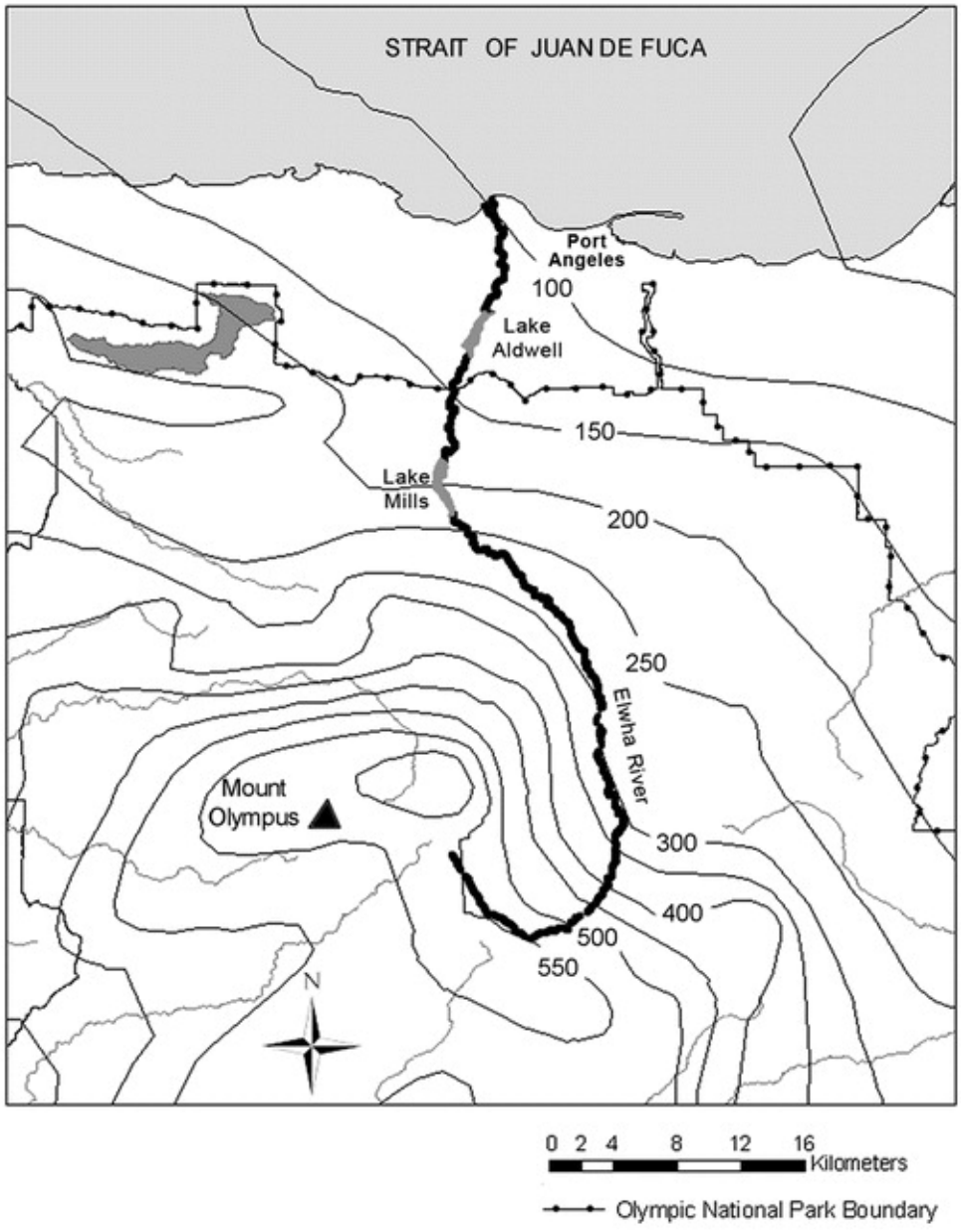

Figure 1.2: Isolines of annual precipitation (cm) estimates of the Elwha River basin. Retrieved from Duda et al 2008.

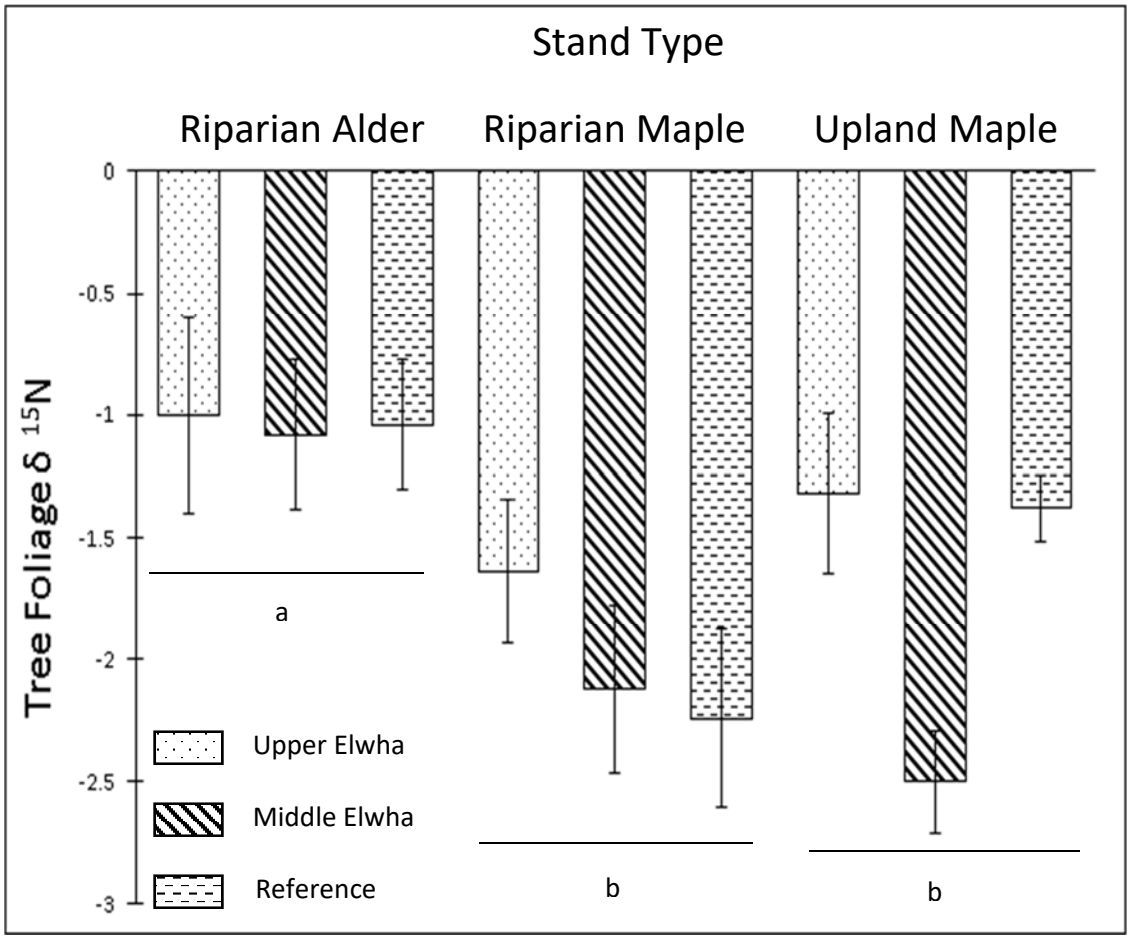

Figure 1.3: Comparison of average tree foliage δ15N between stand type and site. Legend refers to the tributary from which samples were collected. For each bar, n=5. Error bars represent ± 1 standard error. Different letters denote significance between stand types at $p < 0.05$.

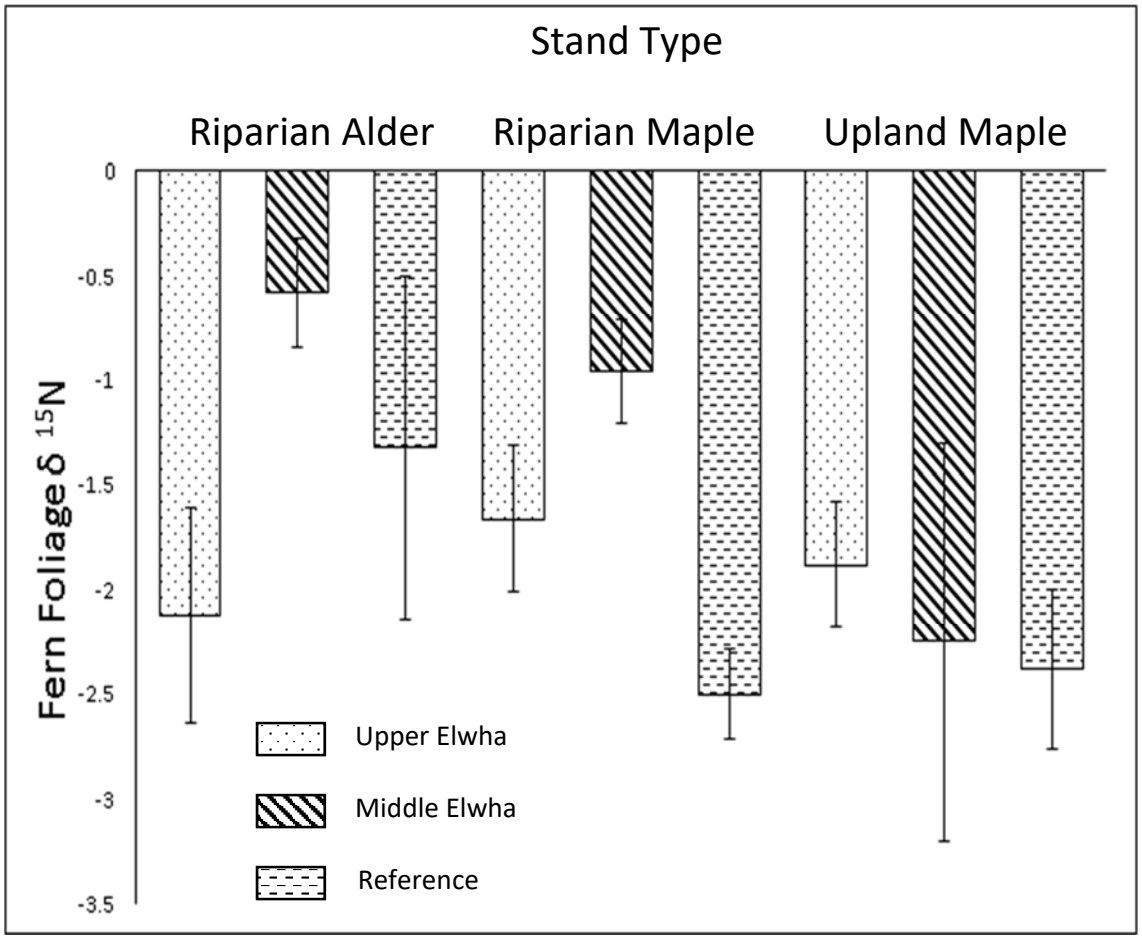

Figure 1.4: Comparison of average P. munitum foliage δ15N between stand type and site. Legend refers to the tributary from which samples were collected. For each bar, n=5. Error bars represent ± 1 standard error.

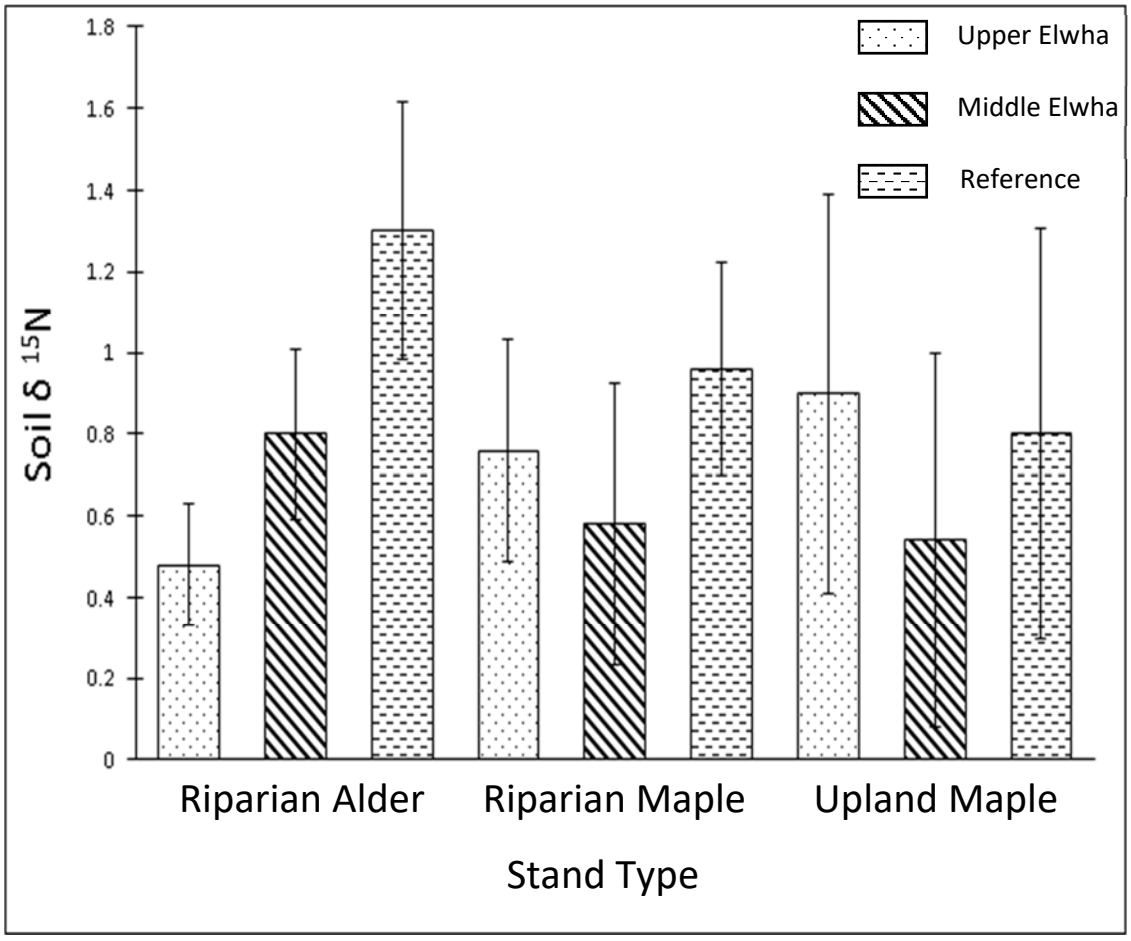

Figure 1.5: Comparison of total soil δ15N between site and stand type. Legend refers to the tributary from which samples were collected. For each bar, n=5. Error bars represent ± 1 standard error.

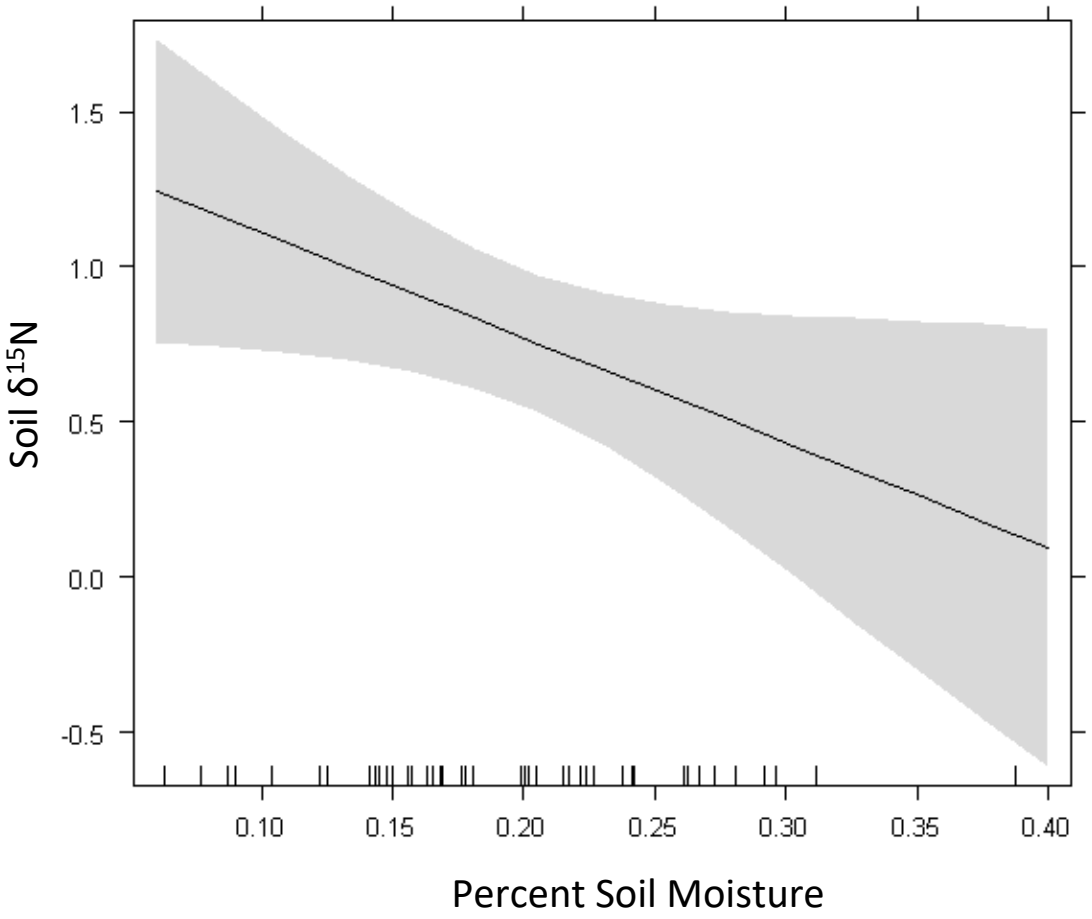

Figure 1.6: Soil δ15N as a function of percent soil moisture. Shaded area represents 95% confidence interval.

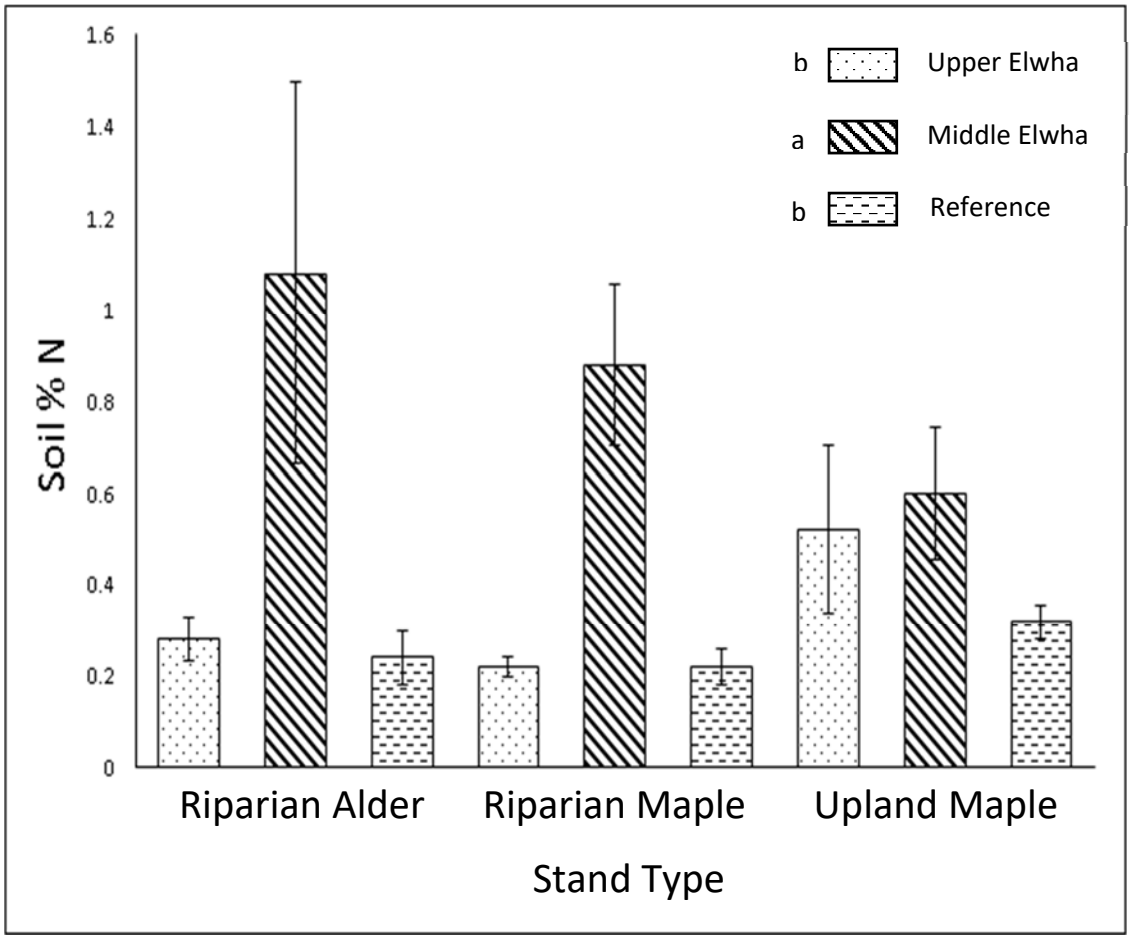

Figure 1.7: Comparison of total soil percent nitrogen between site and stand type. Legend refers to the tributary from which samples were collected. For each bar, n=5. Error bars represent ± 1 standard error. Letters denote significant differences at $p < 0.05$. Letters next to legend denote significance between tributary at $p < 0.05$.

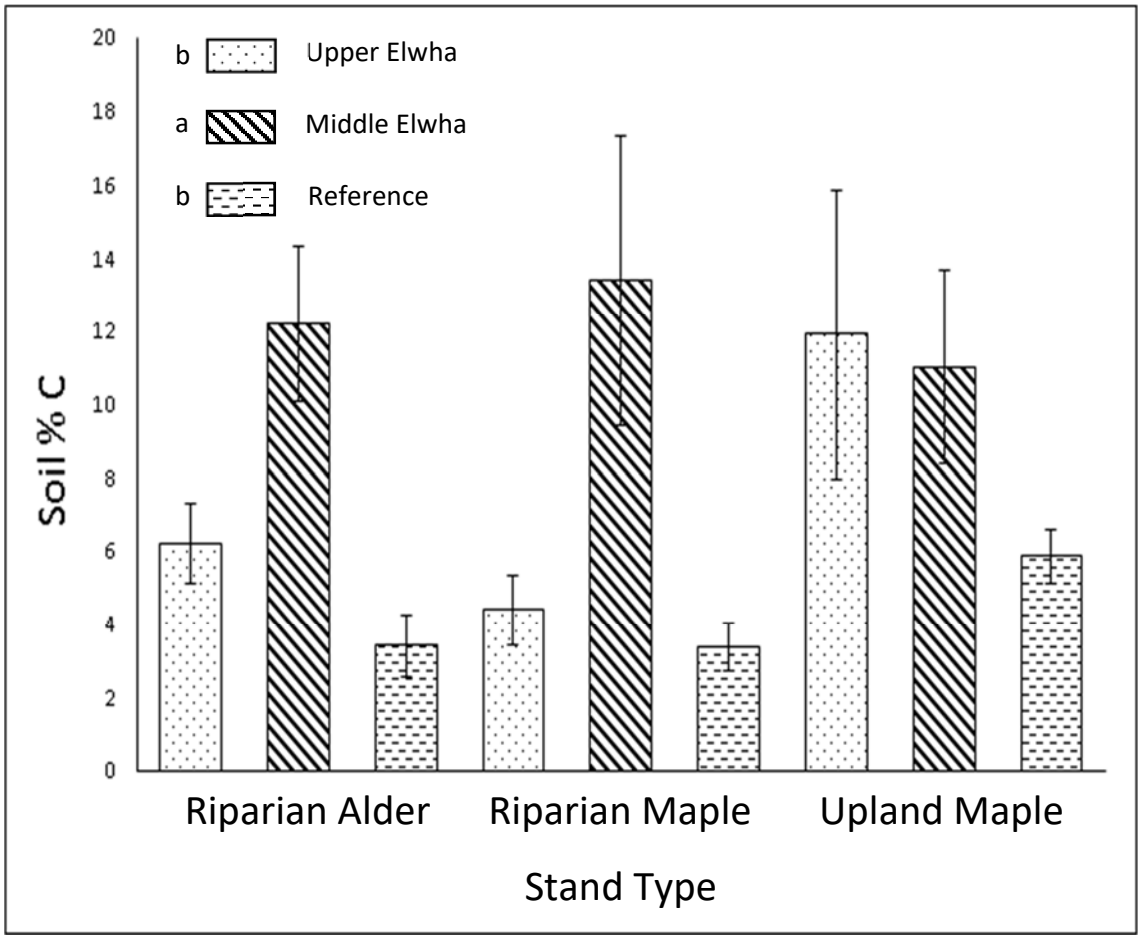

Figure 1.8: Comparison of total soil organic carbon between site and stand type. Legend refers to the tributary from which samples were collected. For each bar, n=5. Pairwise differences not significant. Letters next to legend denote significance between tributary at $p < 0.05$.

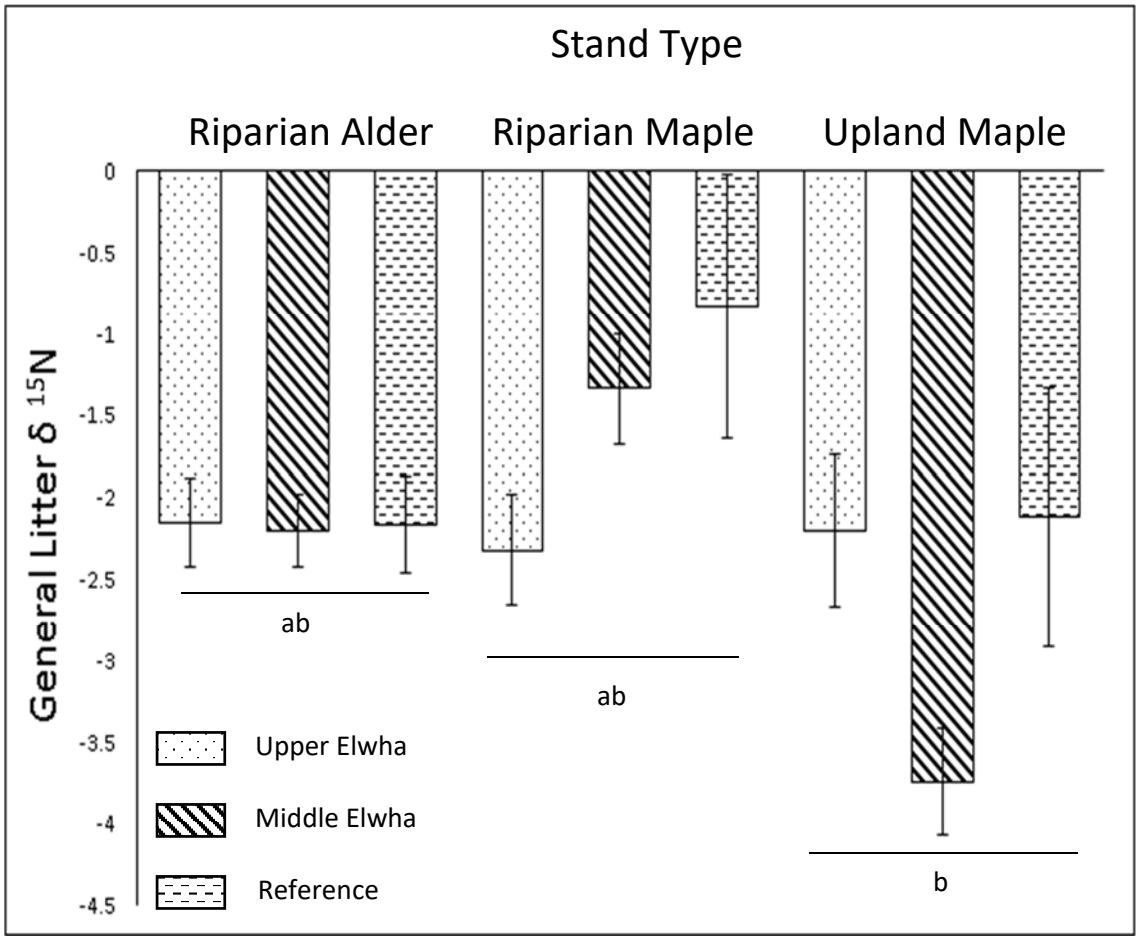

Figure 1.9: Comparison of the $\delta^{15}N$ of indiscriminately collected litter samples between site and stand type. Legend refers to the tributary from which samples were collected. For each bar, n=5. Error bars represent ± 1 standard error.

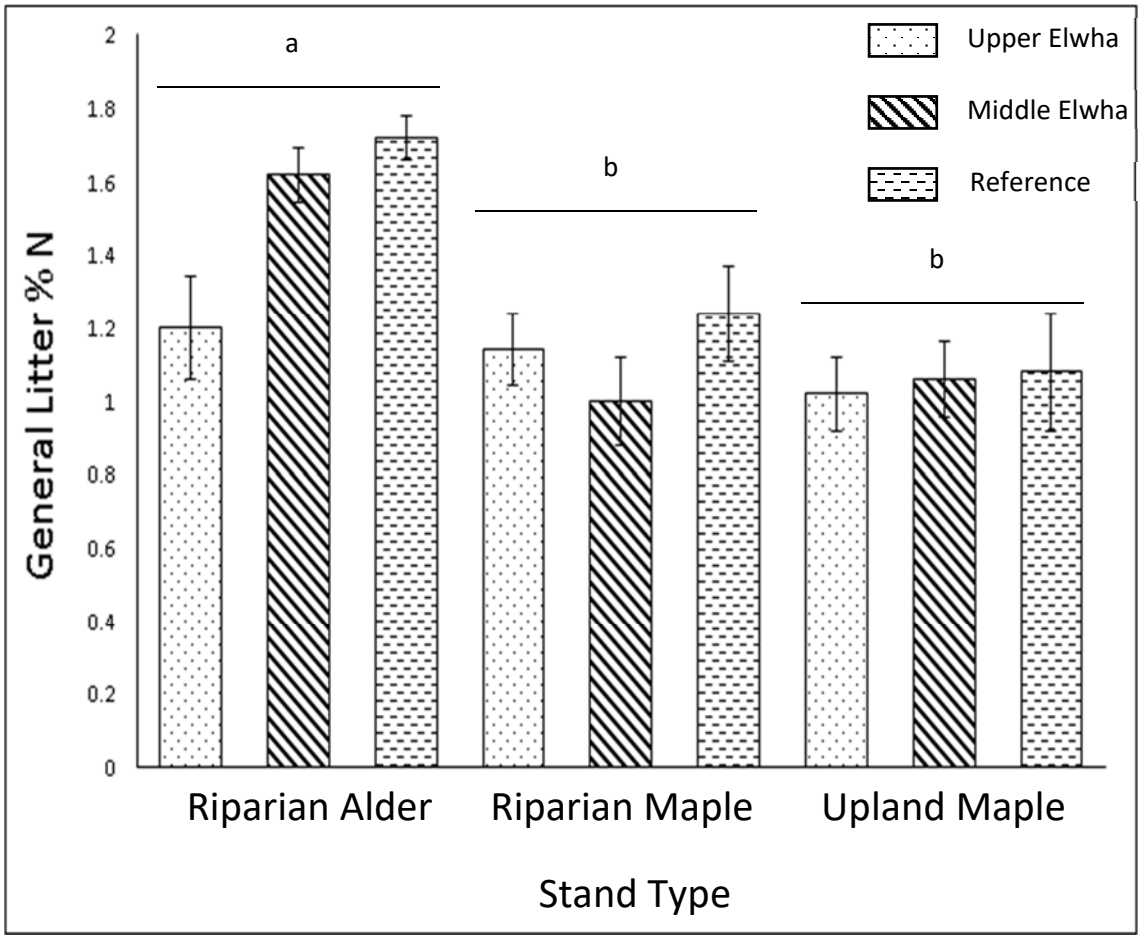

Figure 1.10: Comparison of the nitrogen content of indiscriminately collected litter samples between site and stand type. Legend refers to the tributary from which samples were collected. For each bar, n=5. Error bars represent ± 1 standard error.

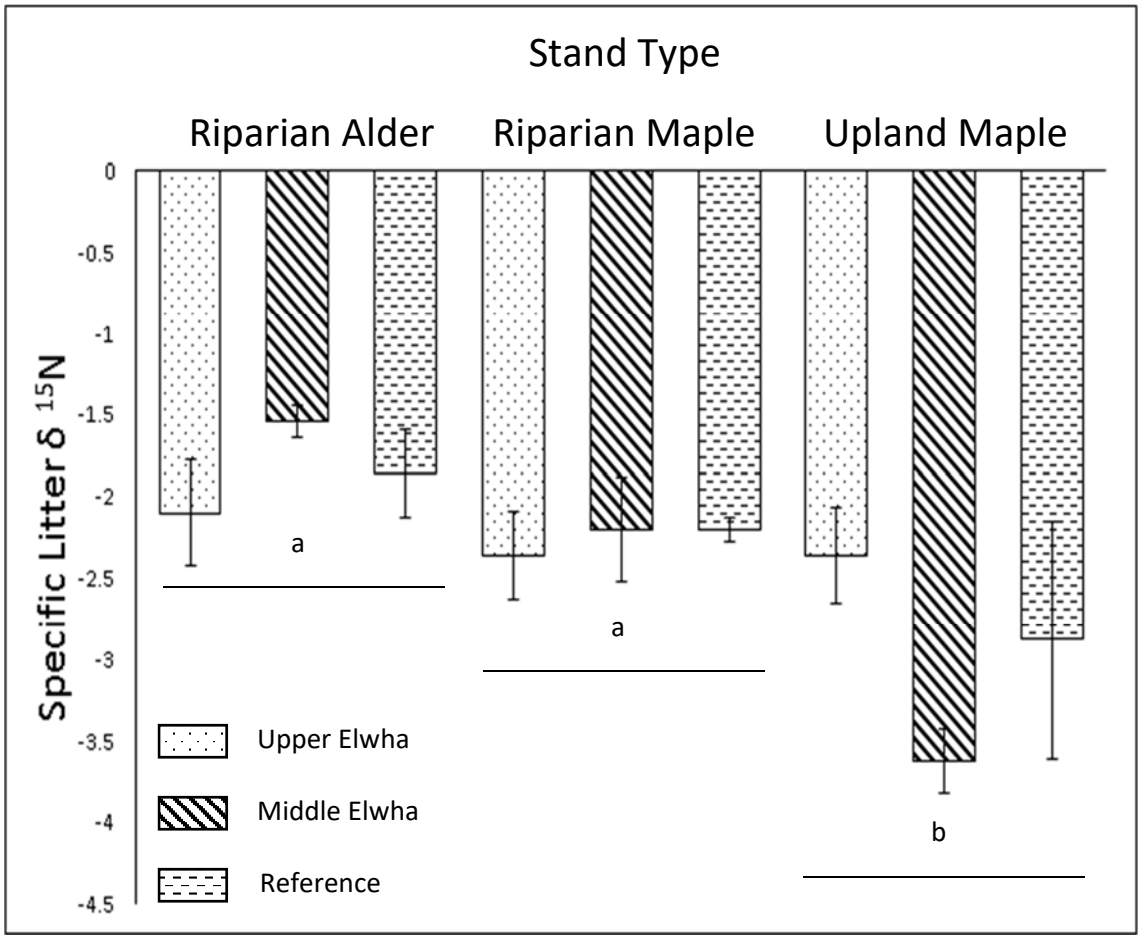

Figure 1.11: Comparison of the $\delta^{15}N$ of indiscriminately collected litter samples between site and stand type. Legend refers to the tributary from which samples were collected. For each bar, n=5. Error bars represent ± 1 standard error. Letters denote significant differences between stand types at $p < 0.05$.

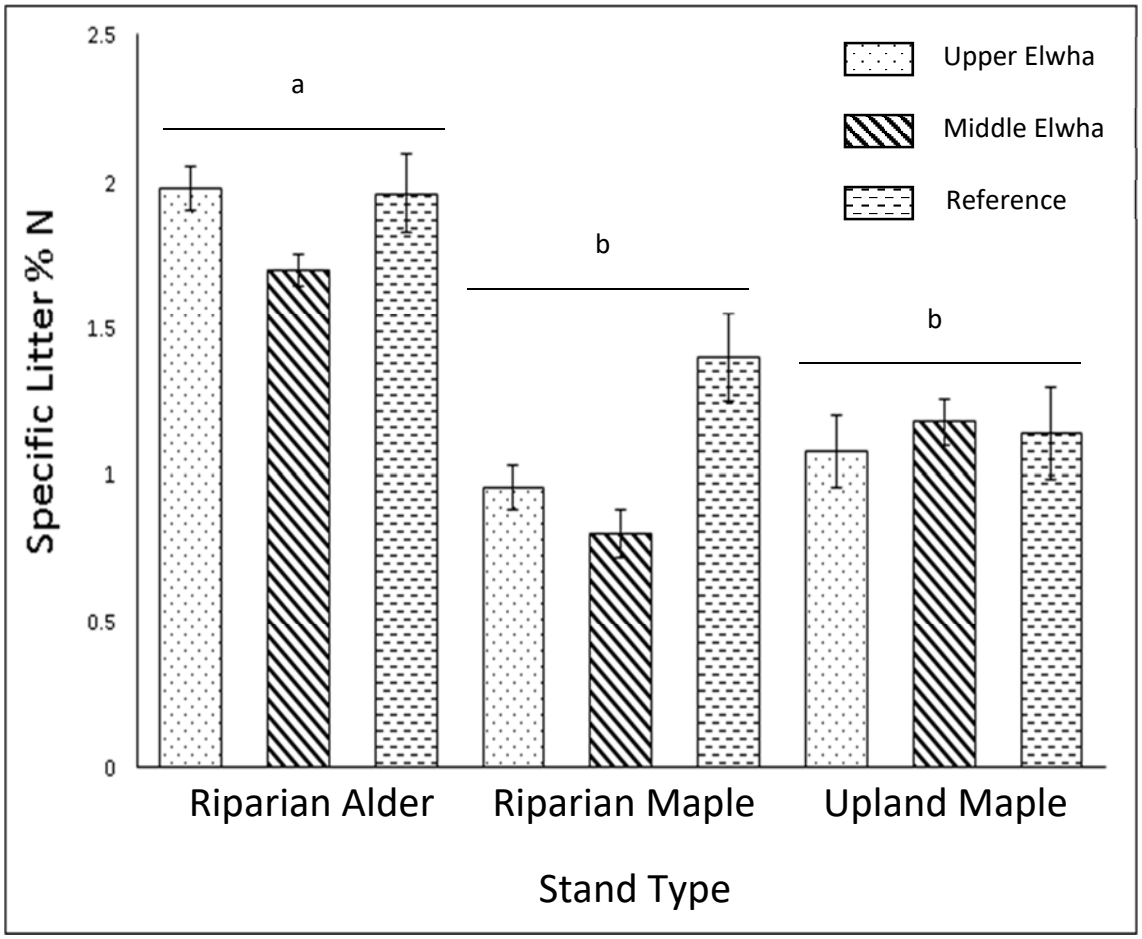

Figure 1.12: Comparison of the nitrogen content of litter samples, specific to the canopy tree, between site and stand type. Legend refers to the tributary from which samples were collected. For each bar, n=5. Error bars represent ± 1 standard error. Letters denote significant differences between stand types at $p < 0.05$.

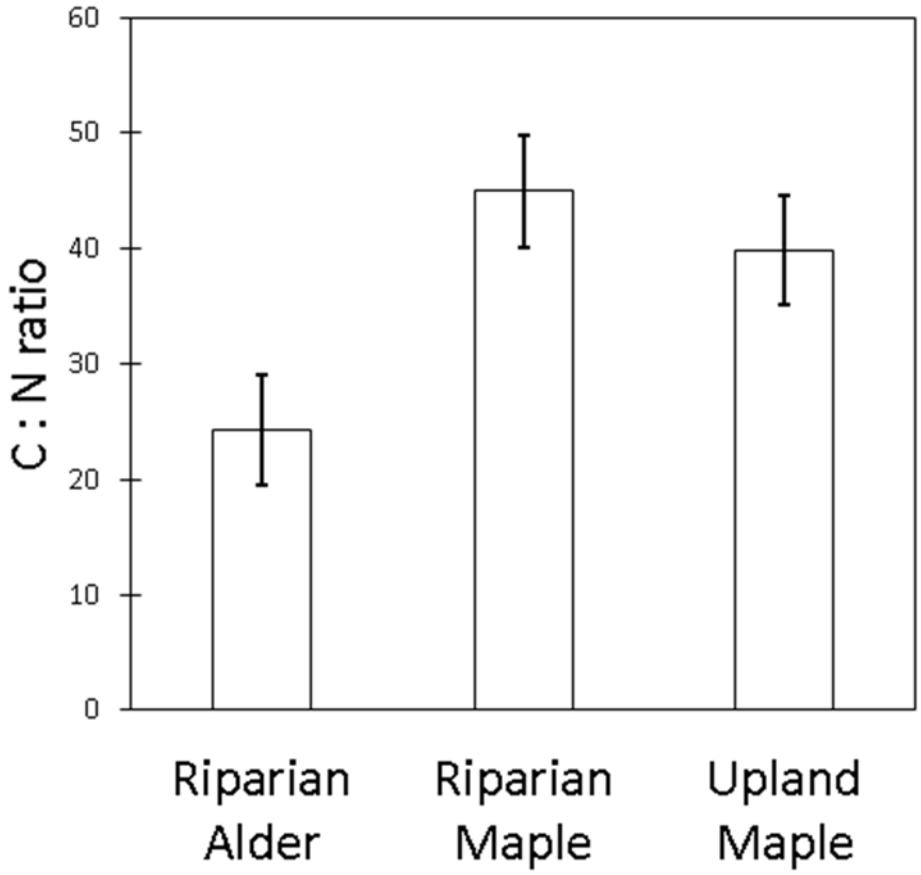

Figure 2.1: Comparison of specific leaf litter between the three stand types. Bars represent 95% confidence intervals. Different letters denote significance at $p < 0.05$.

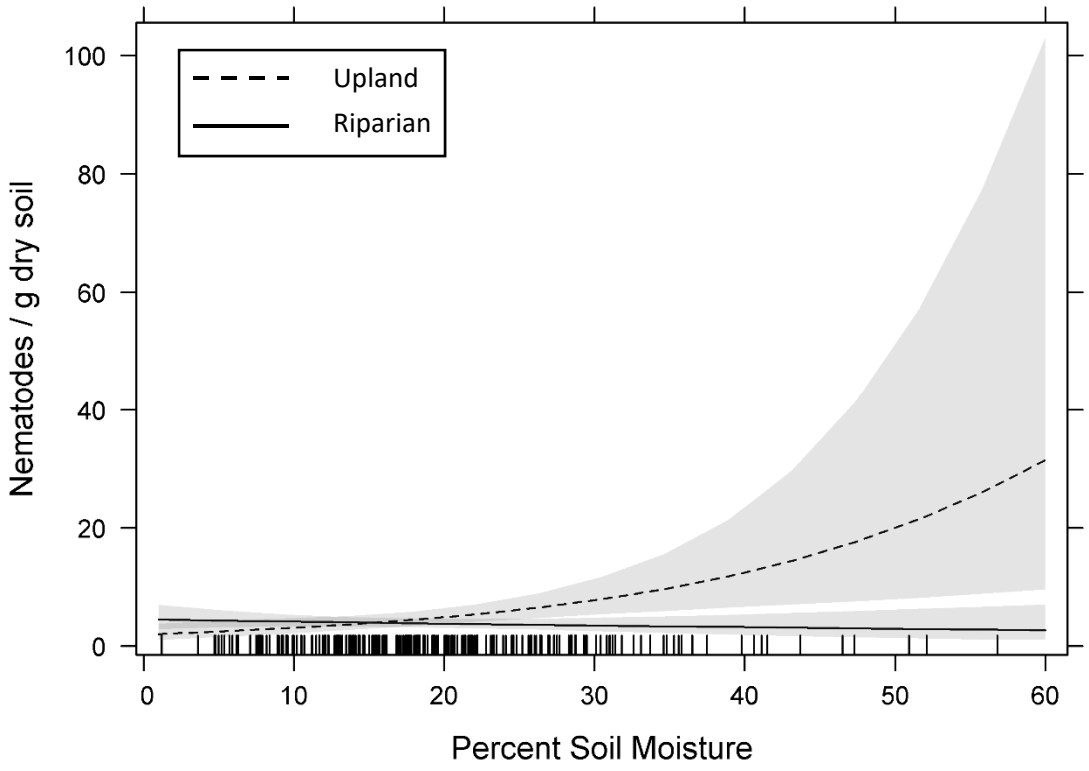

Figure 2.2: Total nematode abundance as a function of the interactive effect of soil moisture and stand type, riparian or upland. Shaded areas represent 95% confidence intervals. Ticks above x-axis show percent soil moisture data points.

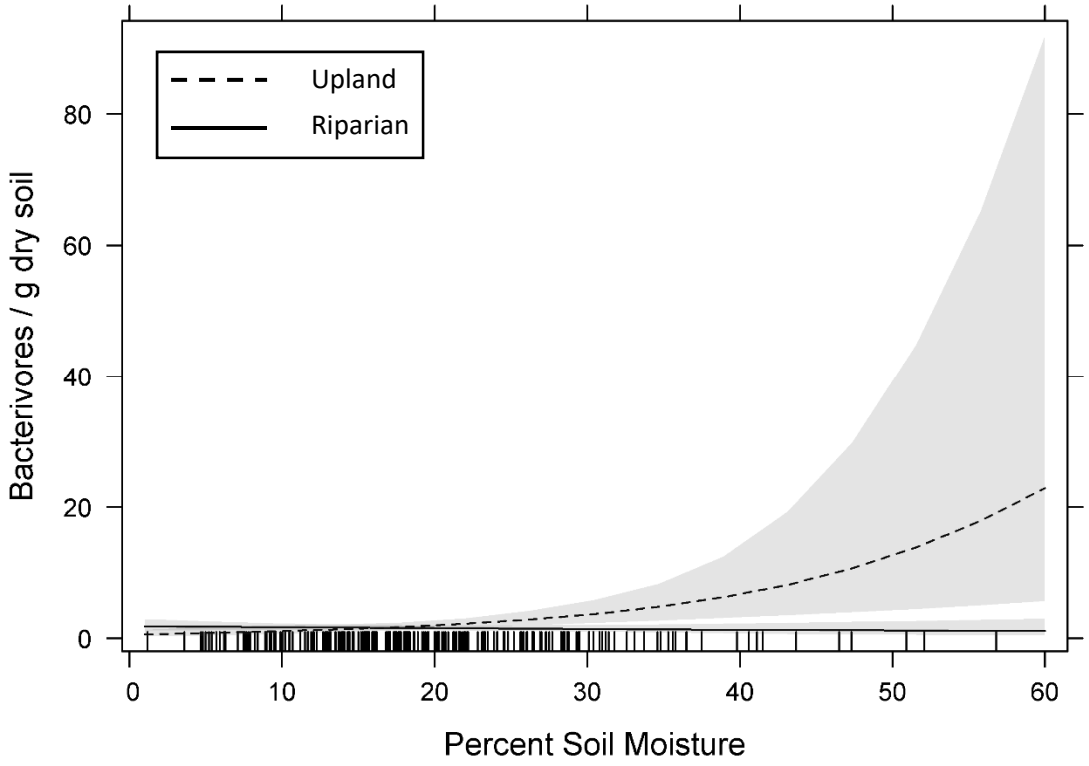

Figure 2.3: Bacterivore abundance as a function of the interactive effect of soil moisture and stand location, riparian or upland. Shaded areas represent 95% confidence intervals. Ticks above x-axis show percent soil moisture data points.

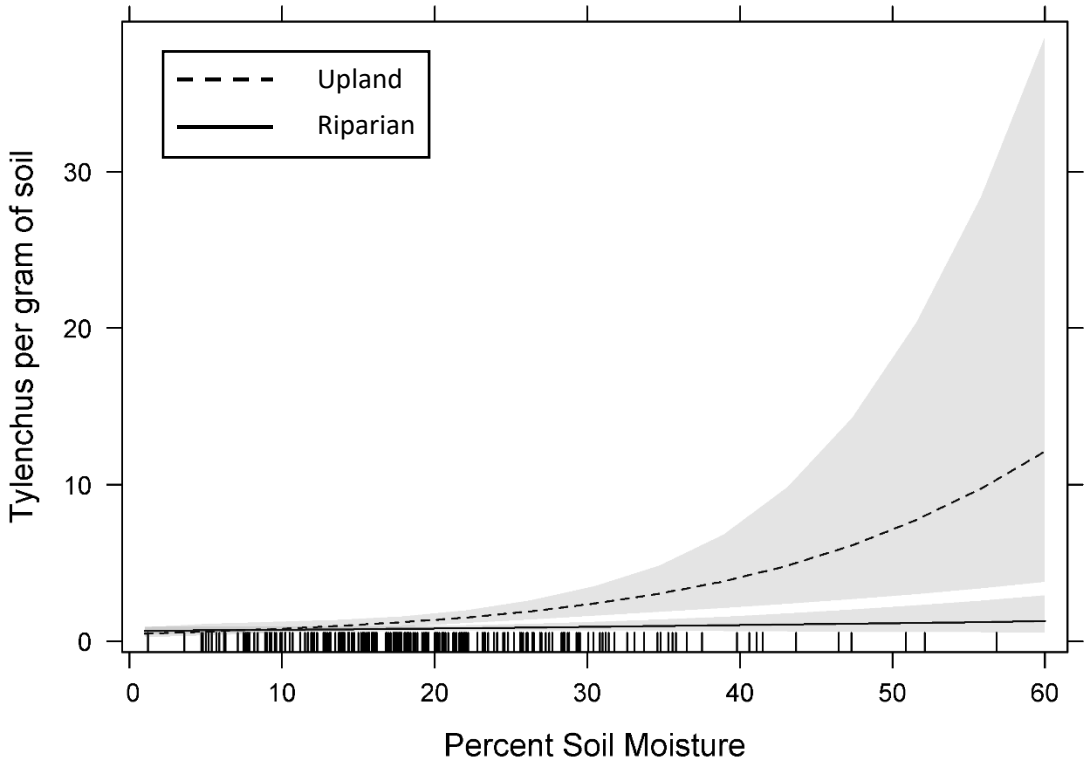

Figure 2.4: Tylenchus abundance as a function of the interactive effect of soil moisture and stand location, riparian or upland. Shaded areas represent 95% confidence intervals. Ticks above x-axis show percent soil moisture data points.

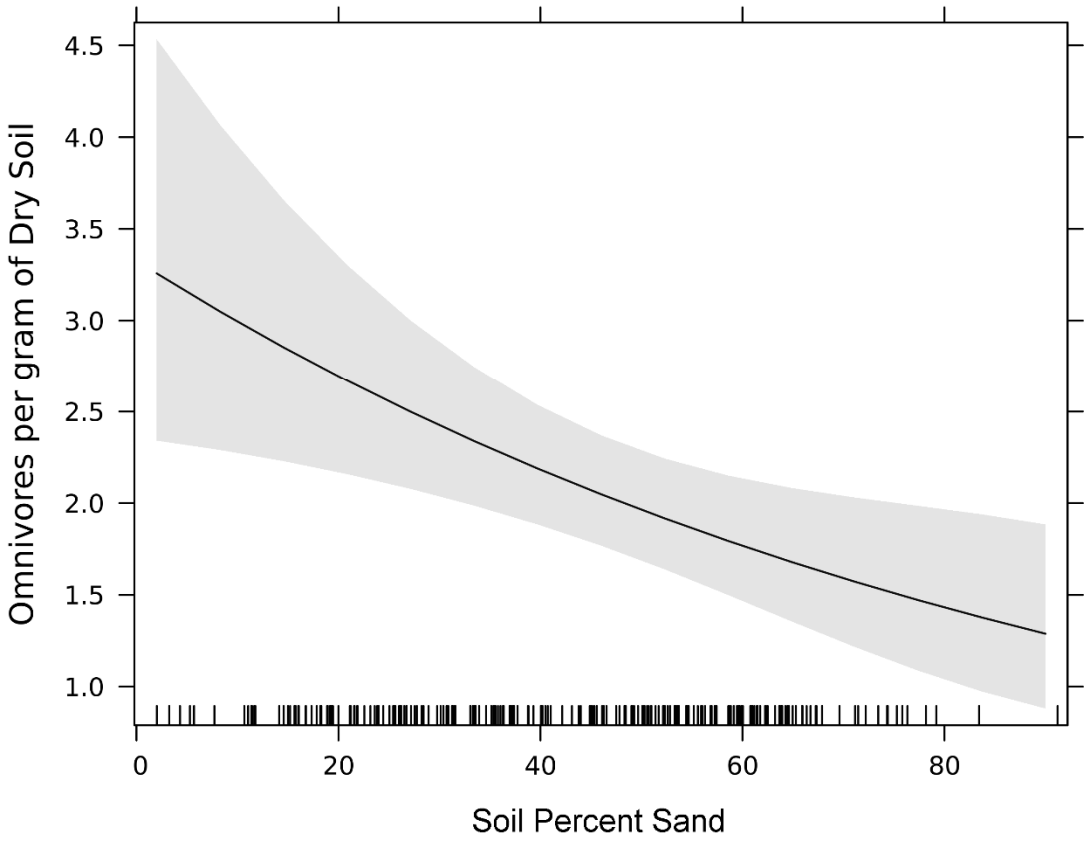

Figure 2.5: Omnivore abundance as a function of the proportion of sand in the soil. Shaded area represents 95% confidence intervals. Ticks above x-axis show soil percent sand data points.

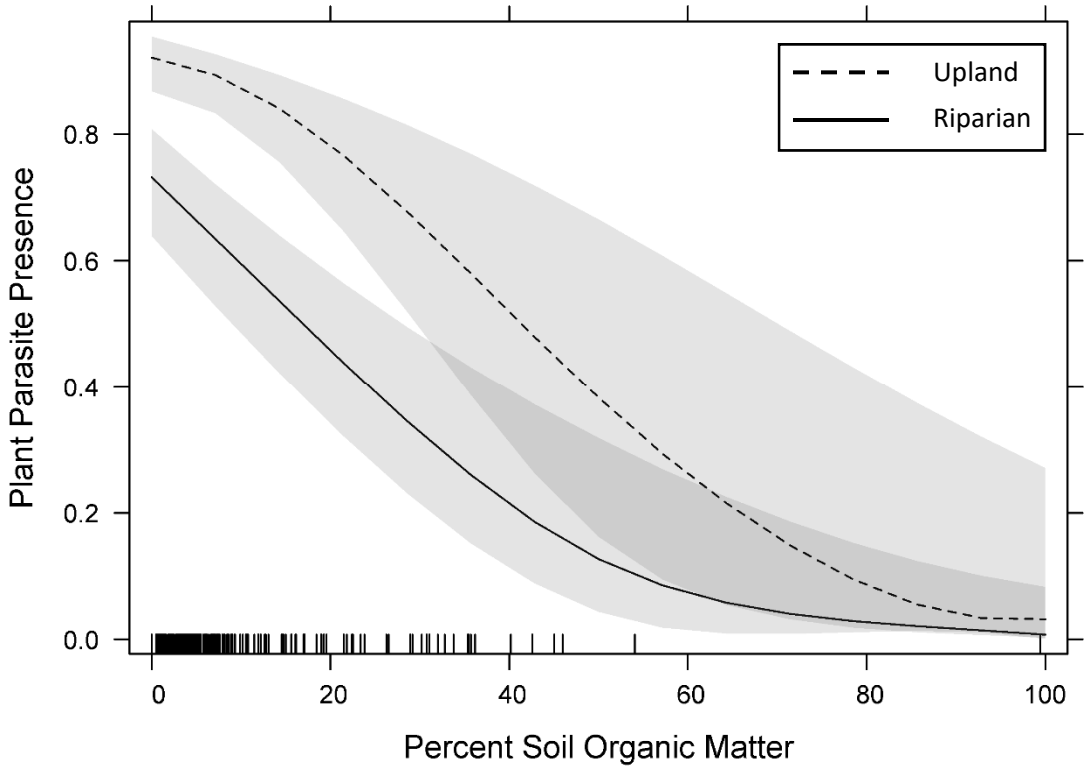

Figure 2.6: Probability of the presence of plant parasite as a function of location and soil organic matter. Shaded areas represent 95% confidence intervals. Ticks above x-axis show percent soil organic matter data points.

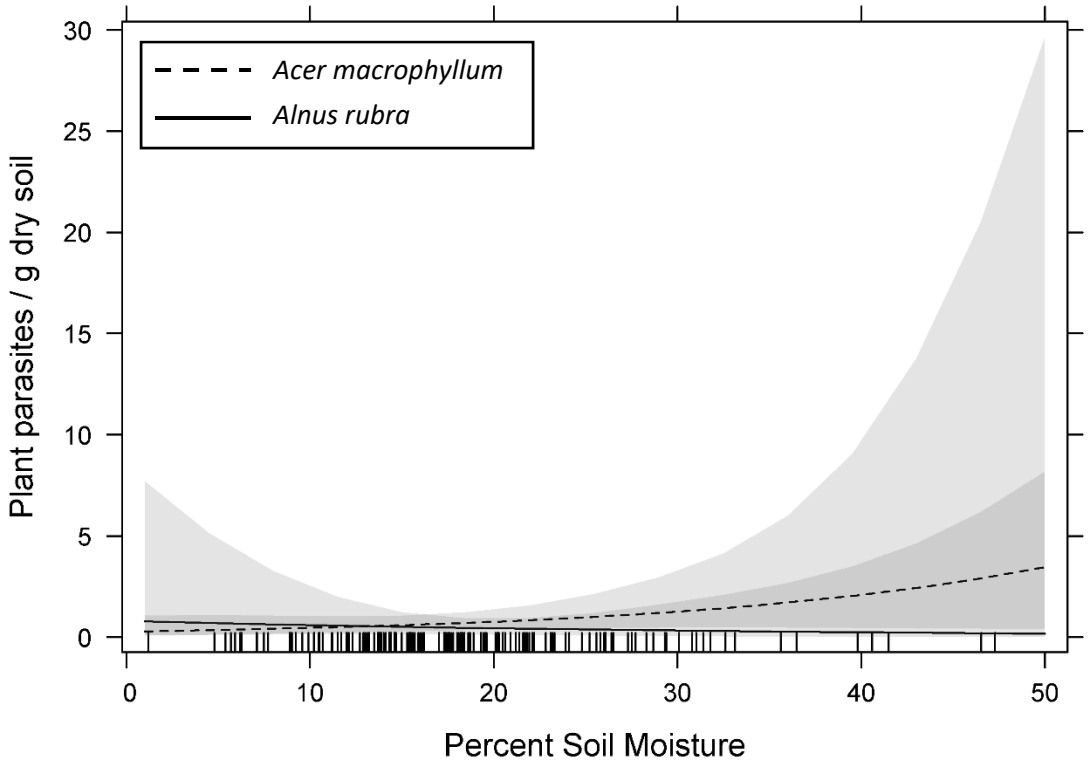

Figure 2.7: Abundance of plant parasites as a function of the interaction of vegetation type and soil moisture. Shaded areas represent 95% confidence intervals. Ticks above x-axis show percent soil moisture data points.

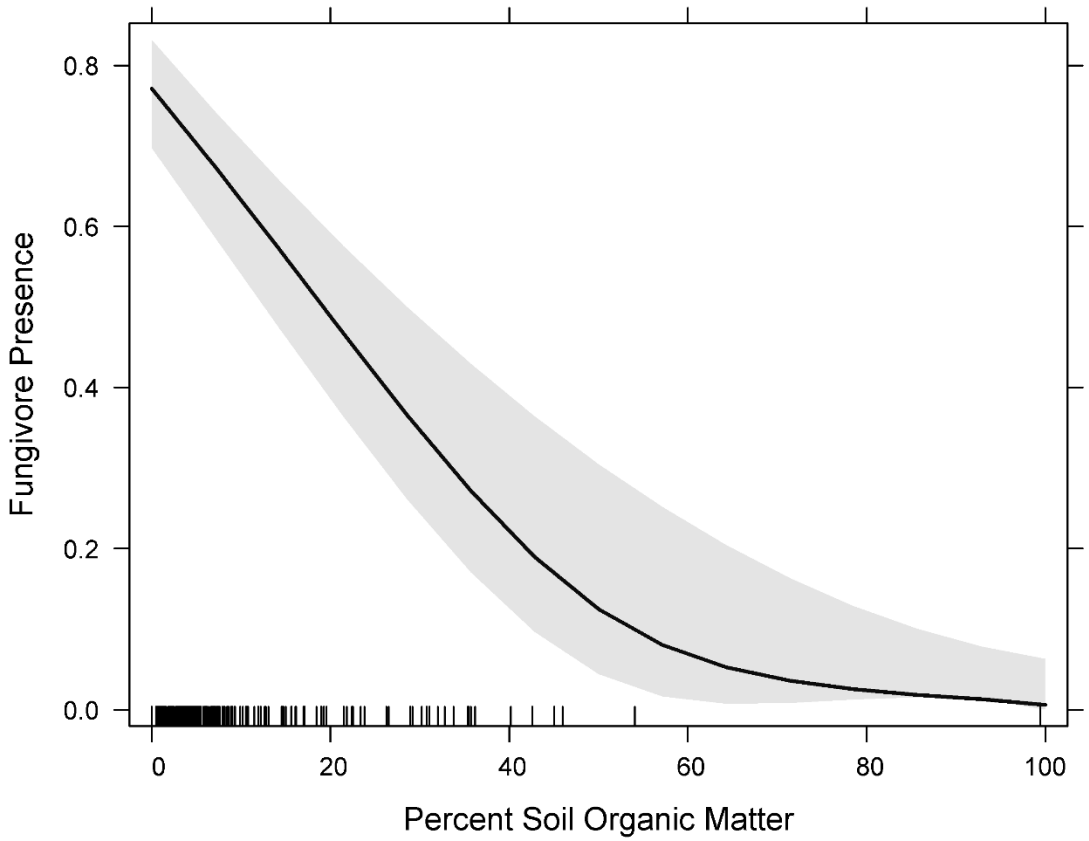

Figure 2.8: The probability of fungivore presence as a function of soil organic matter. Shaded area represents 95% confidence intervals. Ticks above x-axis show percent soil organic matter data points.

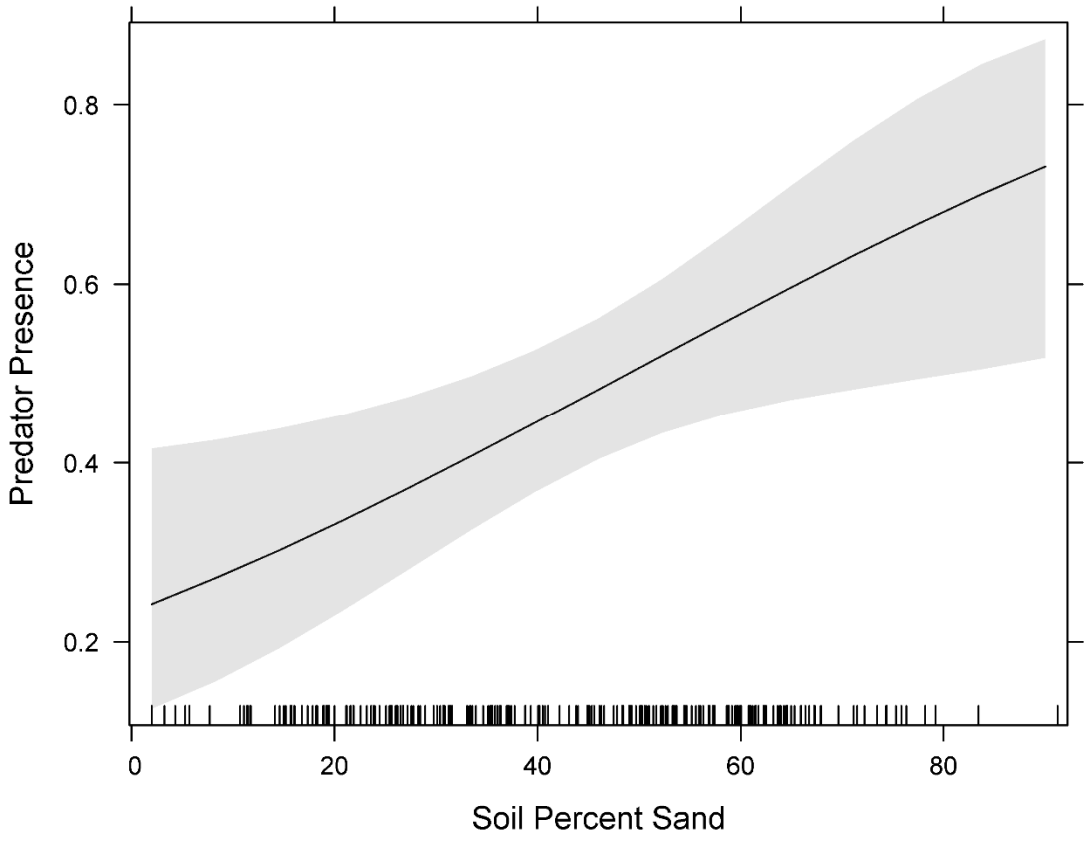

Figure 2.9: The probability of predator presence as a function of proportion of sand in the soil. Shaded area represents 95% confidence intervals. Ticks above x-axis show soil percent sand data points.

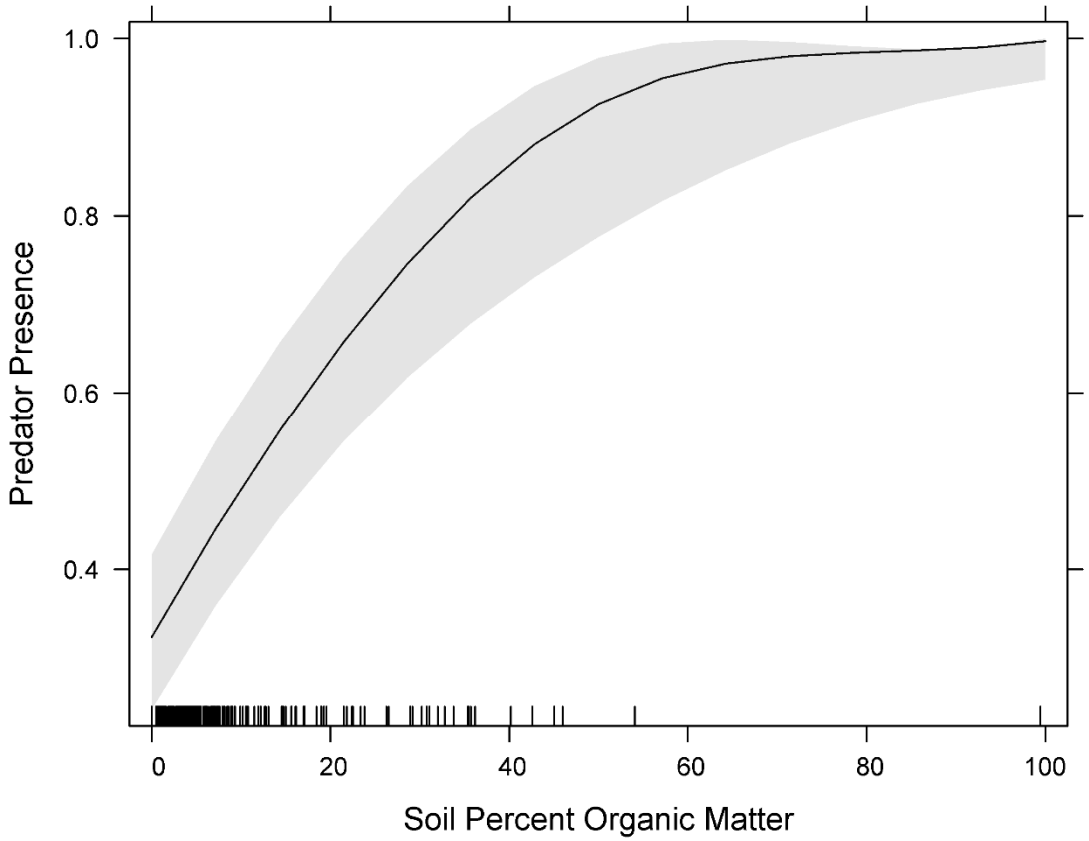

Figure 2.10: The probability of predator presence as a function of soil organic matter. Shaded area represents 95% confidence intervals. Ticks above x-axis represent soil percent organic matter data points.

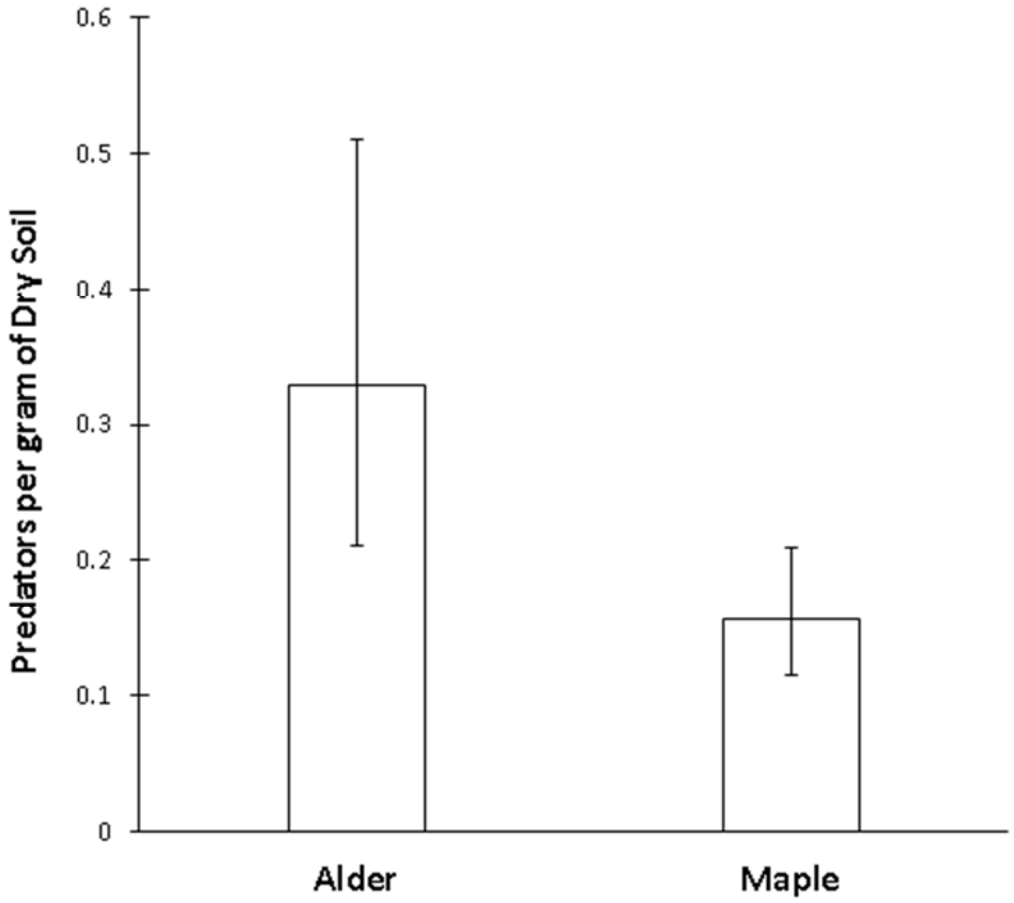

Figure 2.11: Predator abundance as a function of vegetation type. Bars represent 95% confidence intervals.

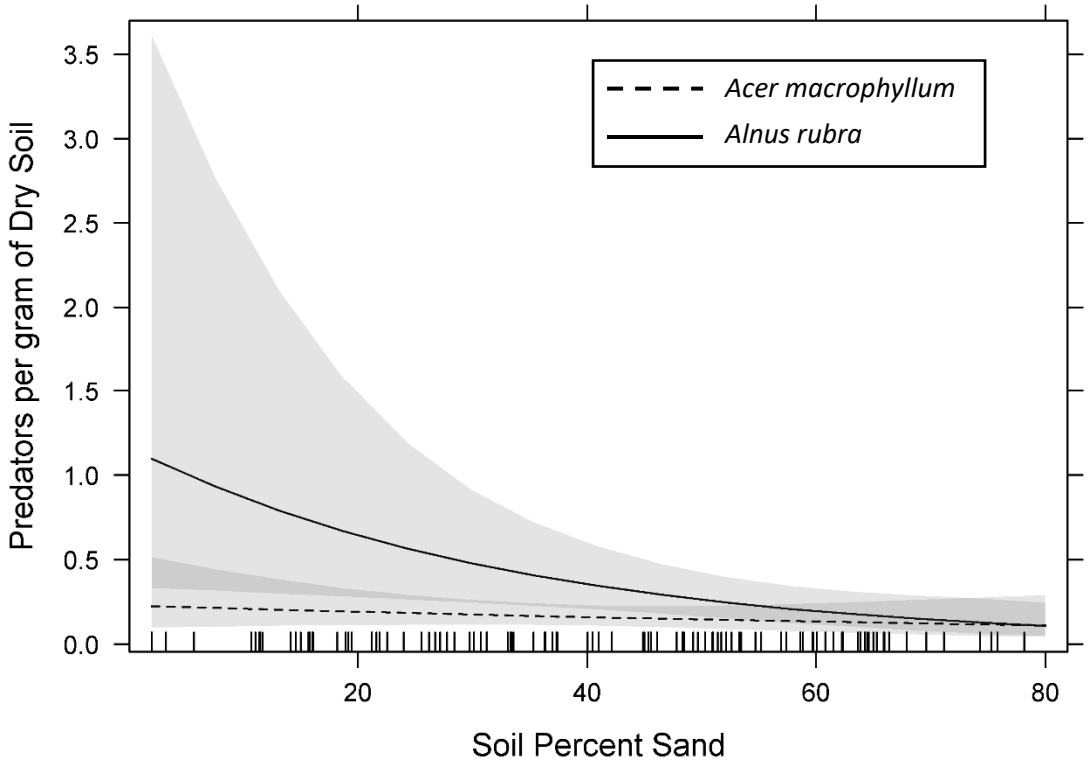

Figure 2.12: Predator abundance, when present, as a function of the interaction of vegetation type and proportion of sand in the soil. Shaded areas represent 95% confidence intervals. Ticks above x-axis represent soil percent sand data points.

Appendix 1: Soil Abiotic Characteristics

Soil Moisture

```
Type III Analysis of Variance Table with Satterthwaite's method
         Sum Sq Mean Sq NumDF DenDF F value   Pr(>F)
site     695.94  347.97     2    39  5.7552 0.006454 **
loc       75.55   75.55     1    39  1.2495 0.270481
site:loc 255.41  127.70     2    39  2.1121 0.134610
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Soil Organic Matter

```
Type III Analysis of Variance Table with Satterthwaite's method
          Sum Sq Mean Sq NumDF DenDF F value   Pr(>F)
site     1135.42  567.71     2    39  9.3400 0.000485 ***
loc        27.43   27.43     1    39  0.4513 0.505698
site:loc  233.30  116.65     2    39  1.9191 0.160337
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Soil Organic Matter

```
Analysis of Variance Table

Response: soilpercentN
             Df Sum Sq Mean Sq F value    Pr(>F)
vegtype       2 0.0658 0.03289  0.2206 0.8031403
site          2 3.1098 1.55489 10.4277 0.0002676 ***
vegtype:site  4 0.7956 0.19889  1.3338 0.2762075
Residuals    36 5.3680 0.14911
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Soil Sand

```
Type III Analysis of Variance Table with Satterthwaite's method
          Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)
loc      2448.35 2448.35     1    39 19.2360 8.497e-05 ***
site      188.17   94.08     2    39  0.7392 0.4840734
loc:site 2467.28 1233.64     2    39  9.6924 0.0003828 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Soil Silt

```
Type III Analysis of Variance Table with Satterthwaite's method
          Sum Sq Mean Sq NumDF DenDF F value   Pr(>F)
site     1135.42  567.71     2    39  9.3400 0.000485 ***
loc        27.43   27.43     1    39  0.4513 0.505698
site:loc  233.30  116.65     2    39  1.9191 0.160337
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Soil Clay

```
Type III Analysis of Variance Table with Satterthwaite's method
          Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)
loc       682.32  682.32     1 38.207  7.7597 0.0082702 **
site      470.90  235.45     2 38.196  2.6777 0.0816075 .
loc:site 1606.42  803.21     2 38.196  9.1345 0.0005727 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Appendix 3: Nematode models

Total nematode abundance

```
Type III Analysis of Variance Table with Satterthwaite's method
              Sum Sq Mean Sq NumDF  DenDF F value   Pr(>F)
soilmoist      3.9278  3.9278     1 217.83  3.8680 0.050486 .
loc            4.1892  4.1892     1 159.85  4.1254 0.043901 *
perc_SOM       4.5081  4.5081     1 204.14  4.4394 0.036340 *
soilmoist:loc 10.2044 10.2044     1 219.82 10.0490 0.001742 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Bacterivore abundance

```
Type III Analysis of Variance Table with Satterthwaite's method
              Sum Sq Mean Sq NumDF  DenDF F value   Pr(>F)
soilmoist     10.1821 10.1821     1 218.22  6.0527 0.014662 *
loc            9.5035  9.5035     1 163.28  5.6493 0.018620 *
soilmoist:loc 18.1735 18.1735     1 218.22 10.8032 0.001181 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Tylenchus abundance

```
Type III Analysis of Variance Table with Satterthwaite's method
              Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)
soilmoist     14.2804 14.2804     1 219.78 14.9753 0.0001436 ***
loc            0.6993  0.6993     1 151.24  0.7333 0.3931584
soilmoist:loc  6.2055  6.2055     1 219.78  6.5075 0.0114218 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Omnivore Abundance

```
Type III Analysis of Variance Table with Satterthwaite's method
       Sum Sq Mean Sq NumDF  DenDF F value  Pr(>F)
X.sand 4.7981  4.7981     1 150.63  5.7144 0.01806 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Parasite presence

```
Mixed Model Anova Table (Type 3 tests, LRT-method)

Model: nonzeropp ~ loc * SOM + (1 | stand)
Data: nem
Df full model: 5
        Df  Chisq Chi Df Pr(>Chisq)
loc      4 6.9212      1   0.008518 **
SOM      4 7.0391      1   0.007975 **
loc:SOM  4 2.1688      1   0.140832
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Parasite abundance when present

```
Mixed Model Anova Table (Type 3 tests, LRT-method)

Model: adj_pp ~ moist * vegtype + (1 | stand)
Data: non0pp
Df full model: 6
              Df   Chisq Chi Df Pr(>Chisq)
moist          5  0.8392      1  0.3596289
vegtype        5  1.7447      1  0.1865448
moist:vegtype  5 10.8531      1  0.0009863 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Fungivore presence

```
Mixed Model Anova Table (Type 3 tests, LRT-method)

Model: nonzerofv ~ SOM + (1 | stand)
Data: nem
Df full model: 3
    Df  Chisq Chi Df Pr(>Chisq)
SOM  2 14.358      1  0.0001511 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Fungivore abundance when present

```
Mixed Model Anova Table (Type 3 tests, LRT-method)

Model: adj_fv ~ SOM + (1 | stand)
Data: non0fv
Df full model: 4
    Df  Chisq Chi Df Pr(>Chisq)
SOM  3 1.3703      1     0.2418
```

Predator presence

```
Mixed Model Anova Table (Type 3 tests, LRT-method)

Model: nonzeropr ~ SOM + sand + (1 | stand)
Data: nem
Df full model: 4
     Df  Chisq Chi Df Pr(>Chisq)
SOM   3 9.7394      1   0.001804 **
sand  3 4.8179      1   0.028166 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Predator abundance when present

```
Mixed Model Anova Table (Type 3 tests, LRT-method)

Model: adj_pr ~ vegtype * sand + (1 | stand)
Data: non0pr
Df full model: 6
             Df   Chisq Chi Df Pr(>Chisq)
vegtype       5  6.7522      1   0.009363 **
sand          5 15.1737      1  9.806e-05 ***
vegtype:sand  5  4.5046      1   0.033803 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Appendix 2: Nitrogen

Foliar d15N

```
Analysis of Variance Table

Response: Fold15N
             Df  Sum Sq Mean Sq F value   Pr(>F)
vegtype       2  7.3671  3.6836  5.9766 0.005738 **
site          2  2.5551  1.2776  2.0728 0.140588
vegtype:site  4  2.8862  0.7216  1.1707 0.339972
Residuals    36 22.1880  0.6163
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Fern d15N_all

```
Analysis of Variance Table

Response: fernd15N
             Df Sum Sq Mean Sq F value  Pr(>F)
site          2  6.107  3.0537  2.6538 0.08451 .
vegtype       2  4.494  2.2471  1.9529 0.15702
site:vegtype  4  6.872  1.7179  1.4930 0.22543
Residuals    35 40.274  1.1507
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Fern d15N_stream

```
Analysis of Variance Table

Response: fernd15N
             Df  Sum Sq Mean Sq F value  Pr(>F)
site          2  8.5147  4.2573  4.1154 0.02906 *
vegtype       1  1.0083  1.0083  0.9747 0.33336
site:vegtype  2  3.3627  1.6813  1.6253 0.21779
Residuals    24 24.8280  1.0345
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Soil d15N

```
Analysis of Variance Table

Response: soild15N
             Df  Sum Sq Mean Sq F value Pr(>F)
site          2  1.2191 0.60956  0.9696 0.3889
vegtype       2  0.1098 0.05489  0.0873 0.9166
site:vegtype  4  1.1956 0.29889  0.4754 0.7535
Residuals    36 22.6320 0.62867
```

Soil moist

```
Analysis of Variance Table

Response: soild15N
          Df  Sum Sq Mean Sq F value  Pr(>F)
soilmoist  1  2.3187 2.31872  4.3658 0.04262 *
Residuals 43 22.8377 0.53111
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Soil Total Nitrogen

```
Analysis of Variance Table

Response: soilpercentN
             Df Sum Sq Mean Sq F value    Pr(>F)
site          2 3.1098 1.55489 10.4277 0.0002676 ***
vegtype       2 0.0658 0.03289  0.2206 0.8031403
site:vegtype  4 0.7956 0.19889  1.3338 0.2762075
Residuals    36 5.3680 0.14911
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Indiscriminate litter d15N

```
Analysis of Variance Table

Response: Genlitterd15N
             Df  Sum Sq Mean Sq F value  Pr(>F)
site          2  3.6954  1.8477  1.9388 0.16093
vegtype       2  7.0110  3.5055  3.6783 0.03686 *
site:vegtype  4  9.1143  2.2786  2.3909 0.07209 .
Residuals    31 29.5433  0.9530
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Indiscriminate total nitrogen

```
Analysis of Variance Table

Response: GenlitterpercN
             Df  Sum Sq Mean Sq F value    Pr(>F)
site          2 0.38578 0.19289  2.9828   0.06329 .
vegtype       2 1.83244 0.91622 14.1684 2.892e-05 ***
site:vegtype  4 0.53022 0.13256  2.0498   0.10799
Residuals    36 2.32800 0.06467
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Specific litter d15N

```
Analysis of Variance Table

Response: Splitterd15N
             Df Sum Sq Mean Sq F value   Pr(>F)
vegtype       2  9.604  4.8020  8.4147 0.001004 **
site          2  0.268  0.1340  0.2348 0.791920
vegtype:site  4  4.616  1.1540  2.0222 0.112008
Residuals    36 20.544  0.5707
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Specific litter total nitrogen

```
Analysis of Variance Table

Response: SplitterpercN
             Df Sum Sq Mean Sq F value    Pr(>F)
vegtype       2 6.2364 3.11822 51.9704 2.435e-11 ***
site          2 0.5658 0.28289  4.7148   0.01518 *
vegtype:site  4 0.6689 0.16722  2.7870   0.04090 *
Residuals    36 2.1600 0.06000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

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