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Forest restoration of the exposed Lake Mills bed: assessing vegetation, ectomycorrhizae, and nitrogen relative to riverbank lupine (Lupinus rivularis)

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Forest restoration of the exposed Lake Mills bed: assessing vegetation, ectomycorrhizae, and nitrogen relative to riverbank lupine (*Lupinus rivularis*)

By

James Kardouni

Accepted in Partial Completion
of the Requirements for the Degree
Master of Science

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Master's Thesis

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Forest restoration of the exposed Lake Mills bed: assessing vegetation, ectomycorrhizae, and nitrogen relative to riverbank lupine (*Lupinus rivularis*)

A Thesis
Presented to
The Faculty of
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Abstract

This thesis investigated the managed revegetation outcomes of the exposed Lake Mills reservoir bed was investigated following the Glines Canyon dam removal on the Elwha River located in the Pacific Northwest, United States. During the following four years of restoration, one seeded species, riverbank lupine (*Lupinus rivularis*), quickly established on the coarse textured terraces that also had low organic matter (OM) and low soil nitrogen (N) levels. Nitrogen-fixing lupines may facilitate plant recruitment and conifer establishment, while demonstrating a relationship with ectomycorrhizal (ECM) communities which perform essential forest ecosystem functions. The purpose of this study was to investigate lupine's influence on forest recovery, measured under three levels of abundance: sparse, medium, and dense. Each plot was assessed for nitrogen bioavailability, tree growth, plant community structure, soil N and OM development, and ECM abundance. My study demonstrates that lupine abundance has a positive relationship with restoration conifer growth and foliar N concentrations. In addition, lupine abundance corresponds with the surrounding plant species richness and diversity. Finally, lupine abundance has an inverse relationship with conifer root ECM colonization, thereby demonstrating that certain ecological conditions driven by the presence of lupine may dictate the symbiotic strategies between trees and fungi. Though the data illustrate lupine's ability to influence N uptake in neighboring conifers, I did not see differences in soil N or differences in soil OM during this early phase of succession. I provide supportive evidence of lupine's ability to aid in forest restoration in highly disturbed novel landscapes following dam removal is demonstrated. These data will be synthesized into best management practices for similar revegetation projects where dam removal, coupled with seeding and planting, restores forests that are linked to the greater riverine ecosystems.

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I am honored to acknowledge my thesis chair Dr. Jenise Bauman for her consistent dedication to this study. Her skill set, constructive feedback, creativity, and ability keep me focused after long meetings of problem solving was paramount. Jenise was also a great field partner and her enthusiasm was contagious. My thesis committee presented a welcoming environment for me to grow as an ecological scientist as I learned from them. Dr. Robin Matthews and Dr. Jim Helfield provided much needed feedback on study design, statistical analysis, and draft revisions. Joshua Chenoweth whom lead the forest restoration project in the Elwha River basin provided intimate knowledge of the system and restoration methods. Joshua also offered very useful and efficient field sampling methodologies. I'm grateful for my thesis committee and couldn't have done this without them. I would also like to thank Rose Kawczynski, Ed Weber, and Ingrid Patrick for their hard work keeping me on track and providing necessary resources. Dr. Rebecca Bunn provided important insight and feedback especially on the topics of soil-fungi-plant interactions. Finally, grant funds from WWU through the Research and Sponsored Programs and Ross Travel Grants were necessary, as well as external funding provided by the Stuntz Foundation. Big thanks to these grant funding organizations and WWU for offering teaching assistantships.

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Introduction

The environmental impacts of dams on the forest-river processes have been well documented (Hall et al. 2011; Reidy Liermann et al. 2012) and include alterations to aquatic animal populations, riparian vegetation, hydrology, and river deltas (Aparicio et al. 2000; Friedman & Auble 1999; Jansson et al. 2000; Li et al. 1987; Perry et al. 2016; Sharma 2001). Damming rivers in the Pacific Northwest (PNW) obstructs many historic anadromous fish migration routes, leading to major species declines (Duda et al. 2008). Riparian and upland forests are inundated through the creation of reservoirs that impede downstream flow and sediment exchange, which cause deeply channelized riverbeds that become disconnected from floodplains (Rood & Mahoney 2000; Shafroth 1999). Further, dams decrease the delivery of large woody debris to riverine systems that negatively affects floodplain, delta, and instream habitat complexity (Gregory et al. 2002). Dams block the access of anadromous fish that is tied to overall riparian health in terms of the nutrient availability for mutualistic animal-to-plant interactions. For example, connections between marine-derived nutrients (MDN) and riparian productivity has been demonstrated (Drake et al. 2002, Helfield & Niaman 2001), indicating that access to MDN is important to the health of PNW forests.

Until recently, much of the Elwha River Basin was inaccessible to sea-run fish due to the Elwha and Glines Canyon Dams (Andersson et al. 2000; Duda et al. 2011; Jansson et al. 2000; Morita et al. 2000), which blocked access to 90% of the spawning habitat in the watershed and degraded stream habitat (Pess et al. 2008). The deconstruction of both dams and lake draw-down was completed by 2014, which restored access to the upper river. This marked the largest dam removal project to date in the United States (Gregory et al. 2002). During draw-down, coarse-grained terraces approximately 3.3 to 7 meters thick formed along the Lake Mills bed above the Glines Canyon dam. These terraces, composed of unconsolidated sand, gravel, and cobble, resulted in novel landforms perched above the water table and situated adjacent to the riparian corridors along the Elwha River (Chenoweth et al. 2011)

Directed seeding and planting of the exposed reservoir beds was required for site amelioration and the development of a community structure that will restore primary productivity, species interactions, food webs, and nutrient cycling (Walker & del Moral 2009). The functional diversification of plant material generates variations in canopy structure and rooting depths. These variations are needed for slowing water movement, controlling erosion, buffering temperatures, and deterring invasion of exotic plant species, all of which are essential for restoring PNW rivers and forests (Bauman & Kardouni 2018; Naiman et al. 2005; Lake et al. 2007; Naiman & Laterell 2005; Pess et al. 2008). Because PNW forests require centuries to grow into old-growth, guiding successional trajectories to the formation of native pioneer plant communities in the early years of restoration is paramount. Restoration managers must design the best approach to restore damaged ecosystems, which will involve the incorporation of scientific protocols and peer-reviewed publications to support successful successional outcomes (Walker & del Moral 2009).

The objective of the Elwha River and Ecosystem Restoration Project is to minimize presence of non-native species, restore ecosystem processes, and establish native forest communities (DOI 2015). Early seral species provide mechanisms that assist the development of old-growth forest communities by building up soil organic matter that provides nutrient cycling essential for later seral forest communities. Planting a variety of PNW forest species will accelerate succession by revitalizing the exposed reservoir bed with the initial resources necessary for the eventual generation of old-growth forests that are dominated by conifer species. Pacific Northwest old-growth forests are unique ecosystems with complex heterogeneous aboveground structure and variations of rooting depths in well-developed soils. They provide essential habitat for wildlife, fungi, microbes, and supporting vegetation with functions that sequester carbon, regulate hydrology, regulate atmospheric gas exchange, buffer temperature, and maintain soil and nutrient processes (Edmunds & Murray 2002; Norse 1990; NRC 2000; Van Pelt & North 1996). Restoration will also minimize the presence of non-native species and establish native forest communities that reduce the threat of exotic plant invasion (DOI 2015; Newcomb 2012). Specifically, PNW conifer dominated forests become more effective than deciduous forests at reducing some exotic plant species through canopy closure (Naiman et al. 2000).

Mid to latter successional conifers such as Douglas-fir (*Pseudotsuga menziesii*), grand fir (*Abies grandis*), and western white pine (*Pinus monticola*) were incorporated into the Elwha revegetation planting plan and are considered important indicators of forest recovery, as well as iconic species to the PNW forests (Franklin 1988; Shafroth et al. 2002). Conifers play an important role in the soil building processes and river-forest dynamics. Downed trees and litter fall contribute to forest floors and to nearby river systems. For example, large woody debris contributions augment river systems by increasing fluvial dynamics that generate a variety of fish habitats and improve hyporheic exchange that is important for thermal regulation (Bennett & Simon 2004; Mitsch et al. 2001). In the PNW, legacy hardwood species are depleted from the channel at a faster rate than conifers (Naiman et al. 2000). Therefore, old-growth forests dominated by conifer species may be more effective at the creation of instream habitat than younger mixed canopies. Successful restoration will ultimately generate old-growth forest ecosystems that are necessary for long-term soil building processes and contribute to fluvial systems including the Elwha River and its tributaries.

The revegetation initiative has been met with varying results. Along the valley walls, directed plantings and natural pioneering species were successful partially due to the existence of fine sediments, organic material, and soil moisture availability immediately after reservoir draw-down (J. Chenoweth per comm). Conversely, restoration on coarse sediments has been much slower due to reduced water-holding capacity, nutrient availability, and lack of soil organic matter (Chenoweth et al. 2011). Also, the dewatered reservoir has been disconnected from intact forests and their biologic legacies needed for forest succession post disturbance such as seeds, vegetative propagules, pollinators and seed dispersers (Chenoweth et al. 2011; Walker & del Moral 2003). Additionally, the lack of crucial microbial and fungal organisms have negative impacts on successful vegetation establishment (Cortese & Bunn 2017).

Ectomycorrhizae ecology

Ectomycorrhizal fungi (ECM) are another key component to PNW old-growth forests essential for tree establishment and survival (Nehls et al. 2007; Smith & Read 2008). Ectomycorrhizal fungi are the result of symbiosis between plants and fungi, which form a complex organ of plant roots colonized intracellularly by fungi forming a fungal sheath (Strullu-Derrien et al. 2016). Plants hosts often regulate carbon allocation to fungi, while fungi facilitate nutrient and water access for the plants, which enhance photosynthesis. The active association among plants and ECM promote fungal to growth and, in many cases reproduce (Jones & Smith 2004) while the fungi protects plant hosts against adversities such a parasites, drought, and toxic compounds (Balestrini et al. 2016). Trees are capable of supplying up to 30% of their photosynthate to the ECM (Nehls et al. 2007; van der Heijden et al. 2015), and ECM are dependent on this steady supply of carbon from the tree during the growing season (Smith &

Reed 2008). In return, up to 70% of the tree's nutrients may be received from the ECM's hyphal network (Daguerre 2016).

Plants that form ECM associations are important for uptake of nitrogen, phosphorus, and other nutrients in reservoir soils post dam removal (Cortese & Bunn 2017). Conifers used in the revegetation plan (Douglas-fir, grand fir, and western white pine) are known to interact with a variety of ectomycorrhizal fungi (Kranabetter et al. 2015). Through symbiotic interaction, both plant and fungal species play a role in determining the characteristics of the other (Balestrini et al. 2016; Smith & Reed 2008). For example, the acquisition and allocation of resources such as carbon and nitrogen are known to be driving factors which influence fungal-to-tree relationships and their physiological response to one another (Corrêa et al. 2008; Corrêa et al. 2012; Ingestad et al. 1986; Hobbie 2006; Högberg et al. 2003). This improved acquisition of resources allows both species to extend their growing and reproductive capabilities that would otherwise be limited without such symbiotic relations. Mature soils typically promote the support of diverse and abundant fungal communities that are in-turn essential for tree establishment and greater forest health (Balestrini et al. 2016; Smith & Read 2008). Therefore, the large-scale disturbance leading to poor soil conditions may not be conducive to ECM symbioses that is required for native tree establishment after dam removal (Cortese & Bunn 2017).

Lupine ecology

Despite the difficult growing conditions created by the coarse substrate, native riverbank lupine (*Lupinus rivularis*) that was seeded upon Elwha vegetation restoration thrived in many locations along the Lake Mills terraces. Riverbank lupine is a 30 to 150 cm tall pioneer species adapted to well drained sandy or gravelly soils and may grow as an annual, biennial, or short-lived perennial (Darris & Young-Mathews 2012). The two to three-year life cycle of riverbank lupine starts with a vegetative phase followed by a seeding phase and finally senescence with subsequent grow-back periods. As lupine densities gradually decrease, their legacy facilitates soil development to promote plant community recovery (Bishop 2002; del Moral 2007), where lupine may ultimately accelerate succession (Vitousek et al. 1987).

As a legume, riverbank lupine assimilates nitrogen (N) through the N-fixing bacterium *Rhizobium* that forms an endosymbiotic association in root nodules (Staniewski 1970). Myrold and Huss-Danell (2002) showed that N-fixing species such as lupine can enhance N availability in nutrient limited soils. Additionally, Oldřich et al. (2013) demonstrated that lupine positively affected conifer seedling growth parameters including height and root collar diameter, and increased foliar-N within four years of restoration on a site that experienced whole-area forest floor removal by dozer. This is important because N is often the most limiting nutrient in PNW forests (Harrison et al. 2011) especially during early successional conditions (Vitousek 1999) and dewatering events where soils have limited nutrient availability from the effects of impoundment (Lafrenz et al. 2013).

Riverbank lupine may also facilitate the initial establishment of successional species by demonstrating a “nurse plant” effect that is shown to promote neighboring plant colonization (Bertness & Callaway 1994; Niering et al. 1963). Lupine growing on the coarse terraces may improve conditions for neighboring species by moderating desiccating winds and evapotranspiration rates, while adding soil-N and organic matter (OM) to the system. Del Moral and Rozzell (2005) showed that lupine can promote the abundance and diversity of plant communities while improving micro-site conditions. Lupine is considered to be a driver in altering successional trajectories by enhancing soil fertility and reducing water stress that ameliorates the adverse effects of harsh site conditions. Improved site conditions can foster complex structure while accelerating succession even decades faster than continued application of inorganic N fertilizer (del Moral 2007). Future seres (successional stages) may continue to benefit as lupine fulfills its ecological niche as a pioneering perennial N-fixing species.

Lupine was the first colonizing plant after the Mount Saint Helen's eruption. Halpern et al. (1983), and Morris and Woods (1987) observed the patchy rapid increase of lupine on the Pumice Plain of Mount St. Helens followed by subsequent vigorous seedling recruitment. However, lupine was shown to have both a facultative and inhibitory effect on invading plant species, depending on its lifecycle stage (Morris & Woods 1989). Similarly, Walker et al. (2003) and Chapin et al. (1994) demonstrated both inhibitory and facilitative responses of subsequent species relative to N-fixing shrubs with studies conducted on a volcano in New Zealand and a glacial moraine in Alaska, respectively. The initial temporary impediment to germination and establishment of neighboring vegetation was demonstrated. However, chronosequence showed a fostering effect originating from elevated organic matter inputs from lupine detritus which in turn enhanced the growth of later successional species. Therefore, developing our understanding of lupine during primary succession may be an important component to forest restoration projects given its adaptability, ability to facilitate the establishment of neighboring plants, and N-fixing capability (Bishop 2002). Successional interactions related to how surrounding plant species respond to N-fixers depend on their historical role in the community, their population density, and life cycle of the N-fixer, which have impacts on seed dispersal, microclimate, and light availability (Walker & del Moral 2003). Early-established primary successional vegetation may create microclimates that foster late-successional tree species (Richards et al. 2009; Sánchez-Gómez et al. 2006) by moderating soil temperatures, increasing water and nutrient availability, stimulating microbial interactions, and increasing soil aeration (Flores & Jurado

2003; Raffaele & Veblen 1998). Additionally, reforestation projects have demonstrated a successional facilitation where shrubs increased ECM colonization and microbial biomass to neighboring plants (Allen 1993; Bai et al. 2009; Bauman et al. 2013).

Research Purpose and Hypotheses

Riverbank lupine is capable of growing in high densities that may be responsible for two types of sequential vegetative outcomes. On one hand, lupine may hinder facilitation through competition and negatively affect native seedling survival resulting in an undesirable outcome (Bruno et al. 2000; Dickie et al. 2002). Alternatively, lupine may be capable of facilitating tree establishment while reducing the threat of exotic-invasive species (Bishop 2002). Therefore, it is possible that lupine acts as an early-established primary successional species integral to forest restoration given its ability to thrive in harsh environments. Furthermore, lupine physiology as a N-fixing species along with its life cycle may also show benefits to forest restoration.

The objective of my study is to examine the effects of lupine on conifer growth, ECM root tip colonization, plant species composition, and soil development under certain levels of ground cover. I hypothesize that greater lupine densities will exert an effect on; 1) planted conifer tree growth and conifer foliar N concentrations, 2) soil OM and N accumulation, 3) the community structure of the nearby vegetation, and 4) ECM root colonization suggesting an ecologically driven preference of symbiotic strategy between the planted conifers and fungi. The overall goal of this study was to examine species interactions that can accelerate succession by aiding in conifer establishment and soil development on the exposed Lake Mills terraces along the Elwha River. Results will help guide forest restoration efforts that use riverbank lupine because of lupine’s possible facilitation and acceleration of conifer establishment during the early years of succession in heavily disturbed systems.

Methods

The Elwha River is located on Washington State's Olympic Peninsula and flows north from its headwaters into the Strait of Juan de Fuca near Port Angeles (Figure 1). The river basin comprises 161 km of channels which drain 833 km^2 of mountainous and forested terrain from an elevation of 1372 m to sea level. Over the past 104 years, the US Geological Service station (ID # 12045500) near Port Angeles has recorded Elwha River discharge, which ranged from 12.4 to 33.4 $m^3 s^{-1}$ (1st and 3rd quartiles respectively) with a median of 18.5 and mean of 29.7 $m^3 s^{-1}$ (USGS 2017). The installation of two dams between 1912 and 1923; the Elwha (32 meters high) and Glines Canyon dam (64 meters high) were located 8 and 22 km from the river mouth, respectively. The Elwha dam created Lake Aldwell covering a surface area of 108 ha and the Glines Canyon dam created Lake Mills covering a surface area of 168 ha (Gregory 2002, USDA 2017). However, this development came at a cost for the Lower Elwha Klallam Tribe who lived in that region for 1000s of years. The Elwha River valley was a provisionary system for the tribe, notorious for plentiful fish runs. Culturally, the river was the place of creation for the Elwha Klallam Tribe and harbored their iconic spirit, the Thunderbird. After the completion of the two dams, the environmental impact was evident. Historical runs of 500,000 salmon were dramatically reduced to 5,500 fish restricted below the lower Elwha dam (Pess et al. 2008).

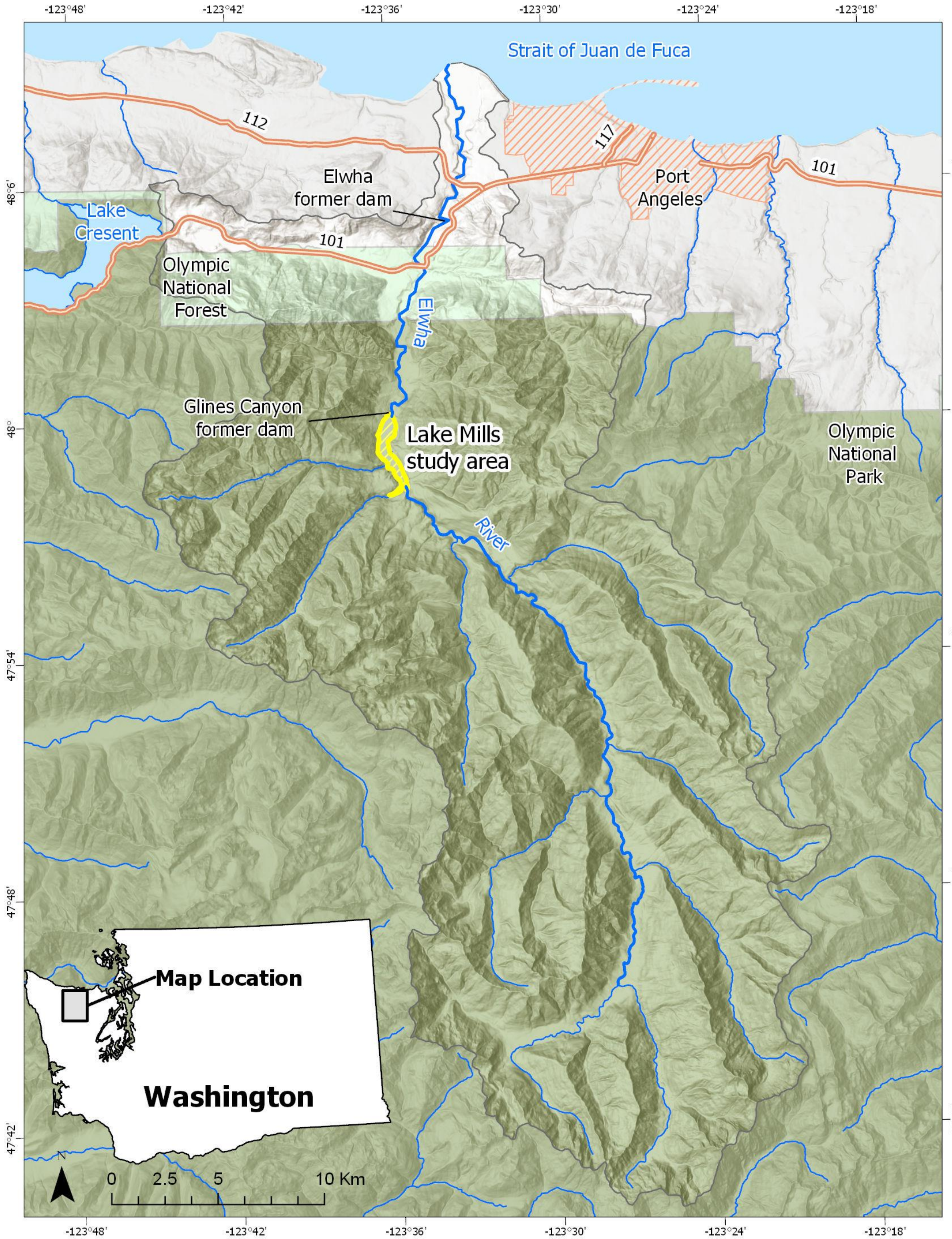

Figure 1. The Elwha River watershed and Lake Mills study area is located on Washington State's Olympic Peninsula.

Biologists at Olympic National Park and their partners implemented the Elwha River and Ecosystem Restoration Project with goals to create an early establishment of native plants to accelerate forest succession to regain functions such as erosion control, water temperature regulation, and habitat creation, while managing against exotic plant invasion. (DOI 2015; Newcomb 2012). From 2013 through 2015, 320,000 native trees and shrubs, and 3,000 kilograms of pure-live-seed of forbs and graminoids were introduced, all harvested from regional seed source and locally greenhouse grown (Chenoweth et al. 2011; Appendix A; Table 1.A). Areas not targeted for woody species plantings received 861 pure live seed per square meter, while areas planted with wood species received half this density, amounting to 430 pure live seed per square meter. From pure live seed, lupine was one of the few species that grew vigorously and spread to dominate many restoration areas.

Study plots were selected using the transitional zones where lupine is abundant in high to low densities along the western terraces of the Lake Mills reservoir bed (Appendix A; Figure 1.A and Table 2.A). Plots (8 m × 8 m) were assigned to one of three treatments; 1) sparse lupine cover, 2) medium lupine cover, and 3) dense lupine cover for a total of 24 plot divided equally among treatments (Figure 2). Our vegetation survey results confirmed significant differences among treatments with mean lupine cover as follows; dense = 76 ± 0.04%, medium = 58 ± 0.04%, and sparse = 15 ± 0.02% (Appendix A; Table 3.A). Each plot included three randomly selected conifers that were planted during restoration for a total of 72 sampling points. Three species of conifer were identified as Douglas fir (*Pseudotsuga menziesii,* n = 33), grand fir (*Abies grandis,* n = 18), and Western white pine (*Pinus monticola,* n = 12) and were assessed for their growth, foliar N and ECM colonization. Conifer saplings were grown in the greenhouse for a few years and planted on the restoration site, growing in place for approximate three years and roughly from the same cohort age class. Using the planted conifer as the center of the sampling point, a 2 m^2 circular area around each tree was measured and sampled for vegetation assemblage, soil particle size, soil organic matter, and C:N content (detailed below).

Figure 2. Examples of study plots along the coarse terraces of the Lake Mills reservoir showing the three lupine treatment groups: 1) sparse lupine cover; 2) medium lupine cover; and, 3) dense lupine cover.

Conifer Tree Assessment

Height and basal stem diameter were recorded for each randomly selected restoration conifer which included Douglas fir, grand fir, and Western white pine. Tree height was measured from the ground to the top of the main-stem of the tree and recorded to the nearest centimeter using a meter stick. Basal stem diameter was measured to the nearest millimeter approximately two centimeters above the soil using mechanical calipers. Tree growth was also quantified into one metric using a ratio of tree height (cm) divided by basal stem diameter (cm) thereby producing a height:diameter ratio (Courbaud 2000).

Small foliar samples (3 – 5 cm) were taken by clipping the seasonal new growth branch tips for C:N analysis. Foliage samples (n = 72) were collected from each conifer sampling point during October 2018, targeting the beginning of the dormant season (Harrison et al. 2011). In the PNW, conifers typically cease putting on new growth mid-autumn. During this time nutrient allocation to the needles have settled providing the best window for sampling without the potential confounding issue of temporally high nutrient flux that occurs during the growing season. Foliar samples were oven dried for one week at approximately 45°C, followed by grinding to a powder using a mortar and pestle. Samples were placed in a desiccant chamber to ensure a controlled environment. Foliar N concentrations were measured at the WWU, Bellingham campus using the Thermo Scientific Flash EA1112 Elemental Analyzer.

Root tips were excavated by trenching approximately 50 cm from tree center and at an 18 cm depth to expose conifer feeder roots. Approximately 10 cm root segments were removed, stored in a plastic bag with soil, and returned to the lab for further analysis of the associated ECM community. Soil samples were also collected during this time for N analysis using a spade to a depth of roughly 18 cm toward the roots of each randomly selected restoration conifer (n = 72).

Conifers were visually inspected for tree health and placed into a categorical scale (0 – 4) based on needle color, needle density, and tree growth patterns. The presence or absence of herbivory on each tree was also noted as factor of tree health. Category four represented optimal health by showing dark green needles along with thick healthy growth. Category three showed less chlorophyll-*a* pigmentation in the needles along with a reduction in needles density and healthy branching. Category two and one had yellow or rusty-brown needles, while category zero was a dead tree. Only two of the randomly sampled trees, one Douglas fir and one grand fir, were dead upon the close of the field data collection; these two trees were not used when comparing statistical differences in tree health among tree species.

Soil Texture Using Substrate Particle Size Assessments

Substrate particle size distributions were assessed to test the significance of possible interactions with lupine treatment. Particle size class data were collected at 10 cm intervals as blind samples along two 1.6 m perpendicular transects within the circular areas used for vegetation assessments, resulting in approximately 36 counts per 2 m^2 quadrat sample point. The

Wentworth scale was used to classify each randomly selected particle by measuring the length of its intermediate axis (Wentworth 1929; Appendix A; Table 4.A). The median particle size for each plot was determined and assigned as a categorical variable from the Wentworth scale.

Fair comparisons between sampling locations can be done by comparing the medians of each normally distributed sample population (Bunte & Abt 2001). The average length of each Wentworth size class was determined and assigned to the median of the particle count as a numeric value. Small particle sizes such as sands and silt were roughly estimated where 1 mm to 0.13 mm was classified as sand, while all sizes less than 0.13 mm comprised silt. Sands and silt were not directly measured using the meterstick, however they were categorized based on visual assessments and texture by rubbing the selected soil particles between the finger and thumb where sands were smoother to the touch than silts.

Vegetation Community Assemblage

Vegetation surveys were conducted within the 2 m^2 circular area around each randomly selected conifer tree (n = 72). All plant species were identified to the lowest practical taxonomic level, usually species, and placed into the following cover-class categories based on visual estimation: 1) < 1%, 2) 1 to 10%, 3) 11 to 25%, 4) 26 to 50%, 5) 51 to 75%, 6) 76 to 90%, and 7) > 90%. Bare ground and animal scat cover-classes were also recorded at this time. Vegetation species indices were calculated for each plot, including Shannon-Wiener H’ diversity, Menhinick richness, and Pielou’s J evenness, where *S* is the number of taxa and *n* is the number of individuals in a sample.

Shannon’s *H’* Index: $$H' = -\sum_{i=1}^{S}\left[\left(\frac{n_i}{n} \times ln\left(\frac{n_i}{n}\right)\right)\right]$$

Menhinick Index: $$R2 = \frac{s}{\sqrt{n}}$$

Pielou’s *J* Index: $E1(J) = \frac{H'}{lnS}$

Soil Organic Matter and Nitrogen Assessment

Soil collection for OM content was done by taking the first 3 cm of sediment in 4 locations per sampling point (n = 72) for a total of approximately 250 g. The soil samples were dried by placing them in a drying oven at 45°C for one week and soil moisture content was measured. Soil OM was measured using weight-loss-on ignition. To do this, dried samples were weighed then heated at 540°C for five hours in a Nanbei muffle furnace. After ashing, each sample was weighed to calculate the difference between dry and burned composites indicating the OM (g) content of the soil sample. Soils for N analysis were collected from the conifer root interface and composited (n = 24) reducing the number of soil N sampling points from 72 to 24. These soil samples were then ground to powder using the Spex Mill grinder and N content was measured using the Thermo Scientific Flash EA1112 Elemental Analyzer.

Ectomycorrhizae Quantification and Species Identification

In the laboratory, root tips were washed and placed into a Petri dish in autoclaved, distilled water. Roots were cut into 3 cm segments, randomly selected, and scored for ECM by the presence of a fungal sheath. The ECM fungal species identification was assessed using root tip morphology followed by DNA sequencing of the internal transcribed spacer (ITS) region of the fungal DNA (Bauman et al. 2013). To accomplish this, a 3-mm section of root representing each unique fungal morphotype was transferred to a micro-centrifuge tube for DNA isolation. The ITS region was amplified using PCR primers and analyzed at WWU labs in Poulsbo, WA using electrophoresis. ITS DNA sequence results were compared with those in the GenBank using the BLAST search for ECM identification (Altschul et al. 1997).

Statistical Approach

All data were examined for normality and homoscedasticity Shapiro-Wilks and Levene's tests, respectively, to satisfy parametric test assumptions. Transformations were done on lupine percent cover, the soil particle size numeric value (ψ), and ECM abundance, which was necessary to achieve equal variance. The square root transformation was used for ECM colonization and lupine cover satisfied homoscedasticity assumptions; median particle size ψ was transformed by squaring their values to achieve equal variance. Particle size gradation has a logarithmic scale ($\log_2$) since it doubles between each size class when expressed metrically as a length. Therefore, $\log_2$ was applied to the mean value for each particle bin level to produce ψ. All statistical analyses were done using R Core Team (2013).

Lupine cover was examined as a predictor for each response variable using a linear mixed effects model (Zuur et al. 2007). Lupine treatment was the fixed factor while the median soil particle size class and tree species comprised the random factors. The dependent factors included each response variable such as, foliar and soil C:N, foliar and soil N, tree growth, soil organic matter, bare ground, and plant species diversity, richness, and evenness indices. In its simplest form the linear mixed effects model can be written as follows:

$$Y_{ijkl} = \mu + L_i + S_j + T_{(i)k} + \varepsilon_{(ijk)l}$$

where Y is the response variable such as tree growth, L is the fixed treatment of lupine such as dense, medium, or sparse, S is the random factor of median soil particle size class using the Wentworth scale, T is the random factor of tree species which is nested in treatment L, μ is the true value of the response variable averaged over all basic experimental units, and ε is residual error. Although tree species performance was accounted for in the mixed effects model, a nonparametric Chi-squared test followed by a Games-Howell post-hoc test was conducted to detect differences in health scores (1 – 4) among the three conifer species, Doug fir, grand fir, and white pine.

Evaluation of mixed-effect model terms was done using ANOVA tests and posterior predictive simulation was done to make informed judgement on model fit (Bates 2015; Gelman and Hill 2006). The ANOVA tested for significance of the fixed and random effects on each response variable ($\alpha = 0.05$). The Welch-Satterthwaite equation was used to calculate the approximate degrees of freedom. Significant ANOVA products were evaluated for differences among lupine treatments using Fisher's least significant difference (LSD) pairwise comparisons. Fisher’s LSD procedure tends to have a minimal risk of false positive judgements only when the number of planned comparisons is small, such as with the three lupine treatment groups in my study (Gamst et al. 2008; Keppel 1991; Marcus et al. 1976; Meier 2006). The posterior predictive simulation (predictive power %) of the model was examined by generating an ensemble of simulations (n = 1000) and observing how often the model predictions fell within the inner quartile range of the observed data to examine model fitness (Gelman and Hill 2006).

Bivariate analysis was done using Pearson’s *r* correlation tests for linear relationships comparing each descriptive variable to one another. Multivariate assessments were conducted using principal component analysis (PCA) of the descriptive variables followed by hierarchical cluster analysis on the loading scores. PCA was applied separately to the descriptive variables and vegetation assemblage. Lupine cover was not included in the PCA in order to examine the datasets without bias from the predetermined lupine abundance treatments.

Divisive hierarchical cluster analysis on the PC scores was done using Euclidean distances with Ward’s minimum variance. Clustering on PCs 1 through 4 produced the most stable groupings while accounting for the majority of sample variability. Contingency tables were used to compare observed vs. expected groups, where PC cluster group represented the observed groups and lupine treatment categories represented the expected groups. Significant proportional differences of observed vs. expected were examined using a Fisher's exact test of independence ($\alpha = 0.05$). Clustering accuracies were assessed using the weighted harmonic mean or F-measure which balances precision and recall of cluster assignment.

Results

Lupine treatment as a fixed factor had a significant effect on tree growth, foliar N, ECM root colonization, and plant species richness according an ANOVA the mixed effect model terms ($\alpha = 0.05$) (Figure 3). Random factors of the linear mixed effect model did not show significant effects on the dependent variable. Fisher's LSD pairwise comparisons indicated that tree growth was greater in medium lupine cover (40.3 ± 2.1) when compared to sparse treatments while sparse (30.0 ± 1.6) and dense treatments (35.2 ± 2.8) had similar tree growth with a predictive power of roughly 46% ($P < 0.01$; Figure 3A). Foliar N concentrations were higher in dense lupine cover (1.8 ± 0.12) when compared to sparse treatments (1.3 ± 0.08) while sparse and medium treatments (1.6 ± 0.09) had similar foliar N with a predictive power of 85% ($P <0.001$; Figure 3B). ECM abundance was greater in plots with sparse lupine (0.31 ± 0.08) when compared with dense (0.12 ± 0.05) and medium coverage (0.12 ± 0.04) with a predictive power of 95% ($P = 0.03$; Figure 3C). Finally, plant species richness was greater in plots with sparse lupine cover (28.5 ± 1.37) when compared with medium coverage (24.3 ± 0.89) while dense (25.2 ± 0.99) was similar to both medium and sparse ($P = 0.04$; Figure 3D). The predictive power of the model for species richness was 3%. Soil conditions such as particle size, OM, and N as well as the vegetative species diversity and evenness were not significantly different among treatments according to an ANOVA of the mixed effects model (Appendix B; Figure 2.B; A-D).

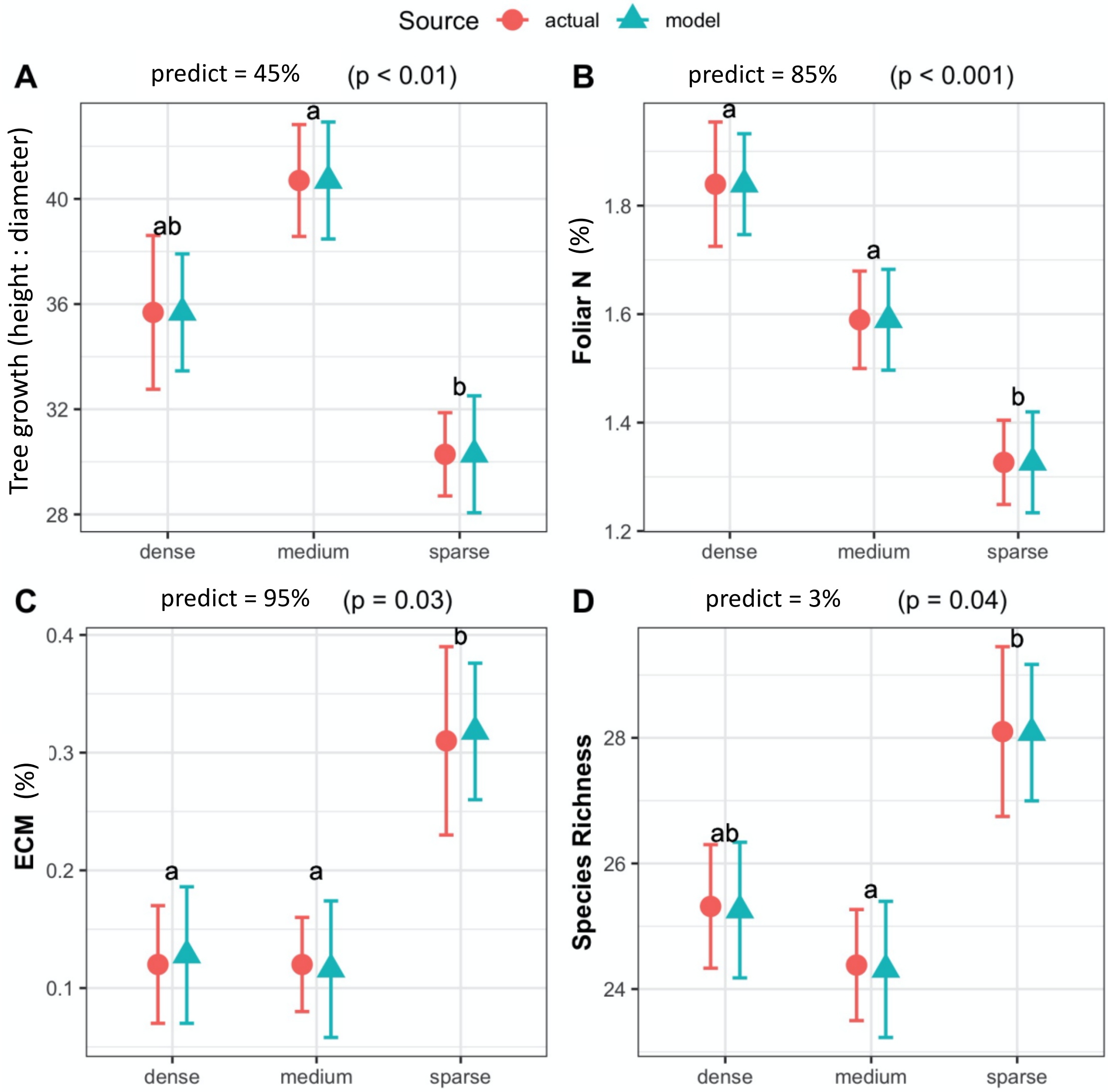

Figure 3. ANOVAs of the mixed effects model output indicated the fixed factor of lupine treatment was a significant predictor for: (A) tree growth, (B) foliar N, (C) ECM, and (D) plant species richness. Plots show observed (actual) and predicted (model) values along with standard error.

ECM fungi species

Eight fungal species were sequenced and reported in Table 1. The ECM colonization and species assemblages consisted of primarily of *Thelephora terrestris and Wilcoxina mikolae;* with *Suillus luteus*, *Tuber pacificum*, and *Hebeloma velutipes* also present, but less common. Each of these species share attributes of early stage, stress tolerating (S-selected) mycorrhizal fungi. Rare species, which are characterize as root endophytes, included *Phialocephala* sp. and one unknown species from the order Helotiales (Table 1). Lastly, a *Leptosphaeria* sp. was also sequenced, but it is not a mycorrhizal species. *Rhizopogon* was not found, which is noteworthy due to its known association with Douglas fir.

Table 2. List of fungal taxa sampled from the three conifer types in the dewatered terraces of Lake Mills in the Elwha River Valley. Nine species are reported with their percent abundance as documented from root tip morphological counts. Species reported were verified using DNA sequencing of the ITS region of the fungal genome and compared to know sequences in GenBank.

ECM Taxa	% Abundance	Function
Thelephora terrestris	0.56	ECM
Wilcoxina mikolae	0.16	ECM
Suillus luteus	0.05	ECM
Tuber pacificum	0.04	ECM
Hebeloma velutipes	0.03	ECM
Phialocephala sp.	0.01	Pathogen
Leptosphaeria sp.	0.01	Endophyte
Helotiales	0.01	Endophyte

No significant difference was detected among the planted conifer tree health scores (1 - 4) when comparing lupine treatment effects using a Chi-squared test. However, tree species did show an overall difference among health scores regardless of lupine treatment, as confirmed by a Chi-squared test (Chi-squared = 12.92, P = 0.04) (Figure 4). Post-hoc analysis using a Games-Howell test indicated that white pine had a significantly higher health score than Douglas fir (P < 0.001) while grand fir had an intermediate score.

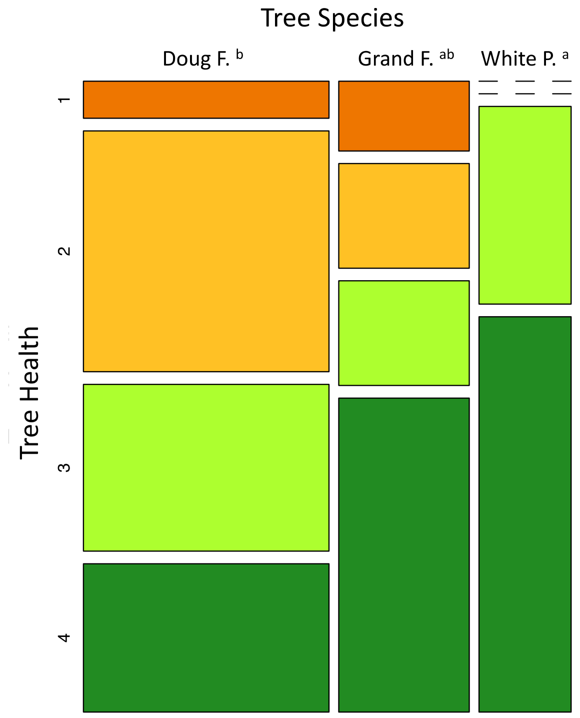

Figure 4. Mosaic plot showing tree health scores and population size (*n*) where Douglas fir = 33, grand fir = 18, and white pine = 12 in the Lake Mills basin. Chi-squared test (X^2 = 12.92, P = 0.04) indicated a significant difference in tree health among species. Tree species that do not share the same letter are significantly different (α = 0.05) using the Games-Howell post-hoc comparisons. The categorical health scale (1 – 4) is based on needle color, needle density, and tree growth patterns. Category four represented optimal health by showing dark green needles along with thick healthy growth. Each subsequent category showed less chlorophyll-*a* pigmentation in the needles along with a reduction in needles density and healthy branching. Category two and one had yellow or rusty-brown needles, while category zero included dead trees that were not used in this particular analysis. Only one Douglas fir and one grand fir were placed in the zero category (mortality) not shown here.

Correlations

Parametric Pearson's *r* correlation statistics were calculated for each descriptive variable assuming linear relationships for all possible comparisons (Table 2). Lupine cover had a significant positive correlation with foliar N and tree growth (r = 0.43 and 0.34, respectively), while showing a negative relationship with ECM (-0.27), species richness (-0.27), tree diameter (-0.28) and bare ground (-0.87). Tree diameter was also positively correlated with ECM (0.26) and tree height (0.45) while being negatively correlated with foliar N (-0.33) and tree growth (-

0.26). Lupine cover was positively correlated with above ground tree growth (0.34) (height:root collar diameter). Tree height was positively correlated with tree growth (0.73) and root collar (0.45). Bare ground was positively correlated with species richness (0.31) and ECM (0.34), while being negatively correlated with foliar N (-0.36), tree growth (-0.32), and OM (-0.29). Species diversity was negatively correlated with richness (-0.66).

Table 2. Bivariate correlations were conducted using Pearson product moment correlation coefficients for all variables of the dewatered Lake Mills reservoir. Bold font indicates significant correlations ($P < 0.05$).

	Foliar N	ECM	Tree Growth	Tree Height	Tree Diameter	Species Richness	Shannon Diversity	Substrate Size	Organic Matter	Bare Ground
ECM	-0.19									
Tree Growth	0.06	-0.14								
Tree Height	-0.16	0.08	**0.73**							
Tree Diameter	**-0.33**	**0.26**	**-0.26**	**0.45**						
Species Richness	-0.13	0.18	-0.14	-0.17	0.01					
Shannon Diversity	-0.03	-0.06	0.15	0.18	-0.02	**-0.66**				
Substrate Size	-0.17	-0.08	-0.02	-0.02	0.02	-0.13	0.04			
Organic Matter	-0.01	-0.03	0.18	-0.01	-0.20	0.03	0.12	-0.04		
Bare Ground	**-0.36**	**0.34**	**-0.32**	-0.14	0.24	**0.31**	-0.23	0.09	**-0.29**	
Lupine Cover	**0.43**	**-0.27**	**0.34**	0.12	**-0.28**	**-0.27**	0.22	0.12	0.16	**-0.87**

Principal Components Analysis

According to F-measure results, the highest overall accuracy for the descriptive variables was achieved when clustering on PC 1 – 4 when compared to clustering on other consecutive PC combinations. The highest overall accuracy for vegetation was achieved by clustering on PC 1 – 10. Contingency tables (3 x 3) of the three different cluster groups versus the three different lupine treatments were compared for proportional independence using the Fisher's exact test. The vegetative community did not form significant proportional groups where clustering on PCs 1 – 10 showed 93% of the sampling point formed one group, while the remaining two groups included 4% and 3% of the sites. The descriptive variables did form significant cluster groups ($P < 0.001$) when using PCs 1 – 4 which accounted for 72% of sample variability. The contingency table comparing treatment by PC cluster group showed 59% of the total formed one group, followed by 25% and 16% in the remaining two groups. The Goodman and Kruskal lambda ($\lambda = 0.43$), which is similar to the coefficient of determination, suggested that cluster performance explained approximately 43% of the variability per treatment group. The weighted harmonic mean, or F-measure ($F1 = 0.62$) suggested an overall information recall accuracy of 62%.

Cluster group 1 ($n = 37$) included 17 medium (46% of the group total), followed by 12 dense (32%), and 8 sparse (22%) which ordinated with species diversity, substrate, foliar N, and tree growth (Figure 5). Cluster group 2 ($n = 10$) generally ordinated with foliar N and species diversity which included 9 dense (90%), followed by 1 medium (10%), and 0 sparse. Cluster group 3 ($n = 16$) generally ordinated with bare ground, ECM, and species richness which included 13 sparse (81%) treatments, followed by 3 medium (19%), and 0 dense.

Principal component 1 accounted for 27.6% of the sample variability and PC 2 accounted for 17.5% of the sample variability. Bare ground had the highest variable loading score for PC 1, followed by species richness, species diversity, tree growth, ECM, foliar N, OM, and substrate. Species diversity had the highest variable loading score for PC 2, followed by foliar N, substrate, species richness, bare ground, OM, ECM, and tree growth. Species diversity and richness loading scores had cluster groups that ordinated in opposite directions. Bare ground and ECM loading scores ordinated groups in the opposite direction of foliar N and tree growth.

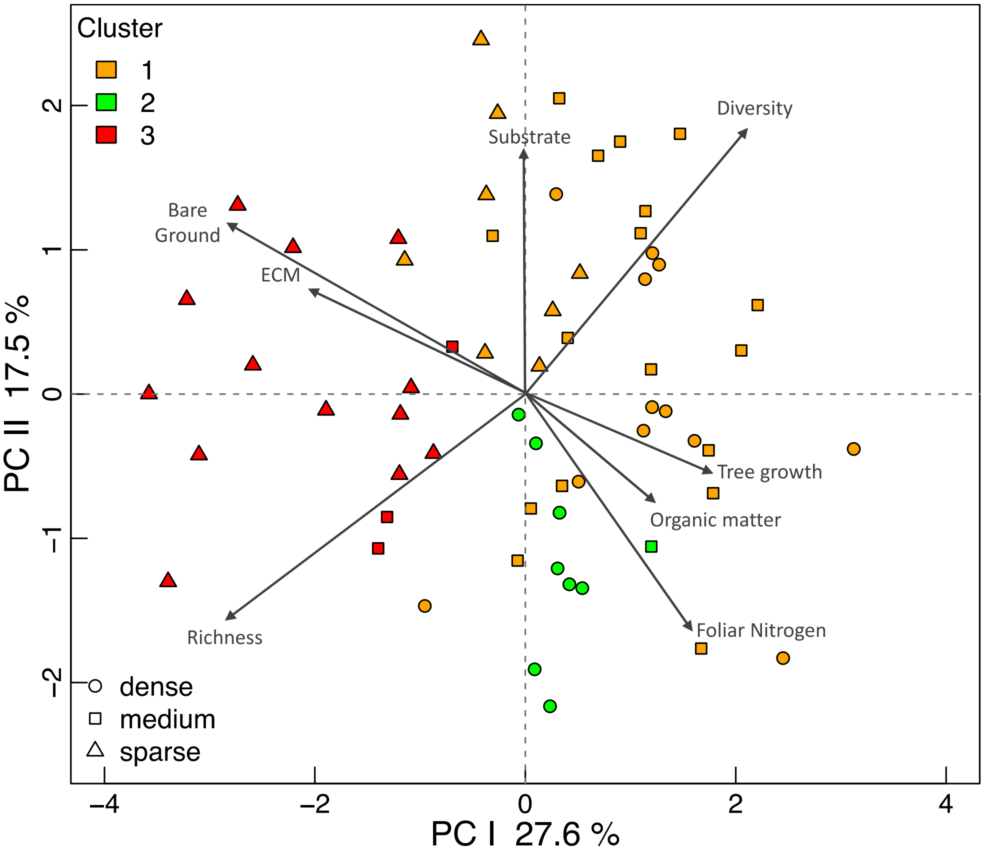

Figure 5. Principal component (PC) analysis of descriptive variables not including lupine were plotted along the axes of PCs 1 and 2 that accounted for 45% of the total sample variability. Cluster groups indicate similarities among individual study plots along with their ordination relative to each descriptive variable loading influence.

Vegetation Species Assemblage

A total of 144 m^2 were surveyed where 48 different plant species were identified, including 10 taxa of graminoids, 15 taxa of forbs, 14 taxa of shrubs, 8 taxa of trees, and 1 bryophyte (Table 3). An additional eight species were not identified and tallied as unknown taxa. *Lupinus rivularis* comprised the majority of total cover with 36%, followed by invasive *Aira* spp. at 12%, *Populus balsamifera* spp. *Trichocarpa* and *Achillea millefolium* each at 4%, followed by *Elymus* spp. and

Eriophyllum lanatum at 3%. *Artemisia* spp. and *Sphagnum* spp. comprised 2% cover each, and *Pseudotsuga menziesii* covered 1%. Twenty species covered between 0.9 and 0.6% each while an additional 20 species were considered rare, each covering < 0.01%.

Table 3. Vegetation taxa list identified and quantified ocularly for total cover in the dewatered Lake Mills reservoir during August, 2018. Seventy-two vegetation surveys were conducted within a 2 m^2 circular area for a total of 144 m^2 of area surveyed. *Nativity (i = introduced, n = native) Site Found (d = dense, m = medium, s = sparse)

Taxa Name	Common Name	Nativity	Total Cover (%)	Site Found
Graminoid				
Agrostis spp.	bentgrass	n	0.03	d, s
Aira spp.	hairgrass	i	12	s
Brome spp.	brome grass		0.2	d, m, s
Dactylis glomerata	orchard grass	i	< 0.01	m
Deschampsia cespitosa	tufted hairgrass	n	0.06	m
Deschampsia elongata	slender hairgrass	n	0.7	d, m, s
Elymus glaucus	blue wildrye	n	0.2	d, m, s
Elymus spp.	wildrye	n, i	3	d, m, s
Fescue spp.	blue fescue	?	0.06	d, s
Holcus spp.	velvetgrass	i	0.13	d, m, s
Forb				
Achillea millefolium	common yarrow	n	4	d, m, s
Anaphalis margaritacea	pearly-everlasting	n	0.06	m
Artemisia spp.	wormwood	n	2	d, m, s
Chamerion angustifolium	fireweed	n	< 0.01	d
Cirsium spp.	thistle	n, i	< 0.01	m
Epilobium spp.	willowherb	n	0.4	d, m, s

Taxa Name	Common Name	Nativity	Total Cover (%)	Site Found
Eriophyllum lanatum	Oregon sunshine	n	3	d, m, s
Fragaria spp.	strawberry	n, i	< 0.01	d
Hieracium spp.	hawkweed	n, i	0.2	d, m, s
Lupinus rivularis	riverbank lupine	n	36	d, m, s
Mycelis spp.	wall lettuce	i	< 0.01	s
Myosotis spp.	forget-me-not	n, i	< 0.01	m
Rumex acetosella	common sheep sorel	i	< 0.01	s
Unknown	exploding star	NA	0.08	m
Senicio spp.	ragwort	n, i	0.4	d, m, s
Solidago canadensis	Canada goldenrod	n	< 0.01	d
Shrub				
Acer glabrum	Douglas maple	n	< 0.01	d, s
Amelanchier alnifolia	western serviceberry	n	< 0.01	d
Holodiscus discolor	Oceanspray	n	0.06	d, m
Physocarpus capitatus	ninebark	n	< 0.01	d
Prunus emarginata	bitter cherry	n	0.08	s
Rosa gymnocarpa	baldhip rose	n	0.2	d, m, s
Rosa nutkana	Nootka rose	n	< 0.01	m, s
Rubus laciniatus	evergreen blackberry	i	0.08	s
Rubus parviflorus	thimbleberry	n	0.03	d, m, s
Rubus spectabilis	salmonberry	n	< 0.01	m, s
Rubus ursinus	Pacific backberry	n	0.06	m
Salix hookeriana	Hooker’s willow	n	< 0.01	s
Salix sitchensis	Sitka willow	n	0.4	d, m, s
Spiraea spp.	spirea	n, i	< 0.01	m

Taxa Name	Common Name	Nativity	Total Cover (%)	Site Found
Trees				
Abies grandis	grand fir	n	< 0.01	d, m, s
Alnus rubra	red alder	n	< 0.01	s
Crataegus douglasii	black hawthorn	n	< 0.01	m
Malus fusca	western crab apple	n	< 0.01	s
Pinus monticola	western white pine	n	0.3	d, m, s
Pseudotsuga menziesii	Douglas fir	n	1	d, m, s
Populus balsamifera ssp. *trichocarpa*	black cottonwood	n	4	d, m, s
Thuja plicata	western red cedar	n	< 0.01	s
Bryophyte				
Sphagnum spp.	true moss	n	2	d, m, s

Discussion

This study demonstrated that lupine abundance had a positive relationship with restoration conifer growth and foliar N concentrations, suggesting its ecological benefit as a restoration plant. In addition, lupine abundance also negatively correlated with the surrounding plant species richness. Finally, lupine abundance showed an inverse relationship with root ECM colonization supporting the hypothesis that certain ecological conditions driven by the presence of lupine may dictate the symbiotic strategies between trees and fungi. These confirmed hypotheses provide supportive evidence of lupine's ability to aid in forest restoration in highly disturbed novel landscapes following dam removal. I did not observe differences in soil OM among lupine treatments during this early phase of succession. However, we do consider lupine litter fall to be an important source of OM which was noted as duff on the soil surface (personal observation, data not shown). Though I illustrated lupine's ability to influence bioavailable N to neighboring conifers, we did not see differences in soil N, presumably due to immediate bio-assimilation or leaching of N in low OM soils.

Conifer Growth

Lupine appeared to optimize ecological conditions for above ground conifer growth by serving as a nitrogen source for nearby trees, as evident by the increase in foliar N in restoration conifers. I observed elevated foliar N levels in restoration seedlings among the high and medium lupine plots, along with a positive correlation between lupine cover and foliar N. Increased foliar N levels improves photosynthetic efficiency (Prietzel et al. 2008) that leads to an increase in tree growth. When compared to existing literature of PNW conifer foliar N concentrations, I found that this study indicated adequate N levels in the dense and medium treatments (foliar N > 1.25 %) while trees among sparse lupine were experiencing N deficiencies (Walker & Gessel 1990, Van den Dreissche 1979, Radwan & Brix 1986, Cross & Perakis 2010). Other macronutrients such as phosphorus (P) are important to conifer growth but were not included in my study. Long-term P availability (Cavaliere & Homann 2012) along the Lake Mills terraces combined with adequate soil moisture immediately after draw-down seemed to be sufficient for robust lupine growth resulting in the generation of important N pools (Harrison et al. 2011, Vitousek 1999). However, other pioneering species may not be proficient at extracting P from the dewatered soils, which is possibly due to the lack of arbuscular (AM) mycorrhizal fungal colonization that limits the associated benefits of extracting recalcitrant mineral-bound P from soils (Cortese & Bunn 2017). Riverbank lupine's capability to thrive in difficult conditions may be partially due to its ability to extract mineralized P which is driven by its AM mycorrhizal associations (O'Dell & Trappe 1992). Examining AM mycorrhizal colonization on lupine roots may determine the one mechanism that is responsible for the successful pioneering growth of lupine on the exposed terraces.

Lupine can contribute inputs to soil N as sites develop with extended periods of occupation (Halvorson and Smith 2005, Prietzel et al. 2008). Studies have also shown that N-fixing species such as lupine can enhance N availability in nutrient limited forest soils (Myrold and Huss-Danell 2002) often driving the increase of foliar N concentrations such as those found in pine seedlings growing in sandy podzol soils in Victoria, Australia (Smethurst et al. 1986). In another study, Scots pines also showed an increase in foliar N levels during the first decade of growth when lupine was present (Prietzel et al. 2008). My study showed increased above ground tree growth expressed by the height:root collar ratio was observed among medium lupine treatments when compared to sparse treatments. Most often the lupine plant height exceeded that of the planted restoration conifers. Medium lupine abundance may provide optimal conditions, or a “Goldilocks effect” for increased tree growth by being not too dense nor too sparse, thereby allowing sufficient sunlight to reach the planted restoration trees that is necessary for growth and survival. Additionally, the height:root collar ratio was positively correlated to lupine cover, while bare ground showed a negative correlation. As discussed above, bioavailable nitrogen is an important component for enhancing tree growth in the early years of conifer establishment by increasing photosynthetic efficiency. Components of tree size are commonly quantified using height and stem diameter, which may be related to individual growth (Hulshof et al. 2015; Simard et al. 1997; Sumida et al. 2013; Waring & Schlesinger 1985; Wonn & O’Hara 2001). The height:diameter ratio standardized the tree growth metric for fair comparisons by accounting for the autocorrelation between the height and diameter of each individual tree.

Long-term (40 years) studies using Scots pine also demonstrated accelerated stand growth in response to the introduction of lupine within the first 2 decades, while the subsequent 20 years did not show a significant difference in stand growth between the control and treatment groups (Prietzel et al. 2008). In another similar study, there was an increase in forb biomass and their probability of flowering when compared to areas void of lupine (Morris and Woods 1989, Gill et al. 2006). Based on these studies, I predicted initial elevated growth rates of restoration conifers in the Lake Mills basin among the presence of lupine as evident from my data analysis. Therefore, by modifying soil conditions, lupine appears to kick-start the natural successional trajectory that leads to the timely establishment of conifers that will eventually shade the herbaceous species of the understory. The light limiting canopy of native conifers disrupts the high energetic cost of N-fixing species, such as lupine, resulting in their decline and a shift from N-fixing plants to those obligatory to ECM for N aquisition (Bauman et al. 2013). Forest succession should exhibit the dominance of Douglas-fir in both establishing and mature forests west of the Cascade Crest (Franklin 1988; Franklin & Spies 1991) with grand fir and western white pine well-established given their common occurrence in the PNW.

I suggest that variations in lupine density imposed a physiological feedback demonstrated by an increase in height:root collar metric under competition for light, thus generating a response in tree growth strategies. Trees may allocate resources into growing tall when competition for light is paramount, which may result in the cost of a reduced carbon allocation to belowground growth (Cremer et al. 1982). On the other hand, a tree may increase its root collar relative to its height as the root system develops when water or nutrients are scarce, or to develop structural integrity under the susceptibility of wind or snow damage (Cremer et al. 1982, Nykänen et al. 1997). Root collar diameter is strongly correlated to root growth and can be a predictor for belowground growth (Clark et al. 2012). My study indicated that root collar diameter generally showed a negative, though weak, relationship with lupine cover, specifically, root collar increased as lupine density decreased. Therefore, I considered competition for sunlight as an influence on tree height where excessive lupine can block incoming solar radiation causing the conifer to allocate carbon resources to aboveground growth, and as a tradeoff, showed no increase in root collar diameter. The observed increase in root collar diameter as lupine abundance decreased suggests that lupine may enhance belowground resources in the forms of bioavailable N or improved water relations. Future studies that measure soil moisture, relative humidity, and transpiration rates of lupine neighbors is required to reconcile.

ECM on Conifer Roots

My study illustrated low ECM root colonization (30%) along with low species richness on novel terraces, agreeing with Cortese and Bunn (2017), who found that the recently dewatered Elwha River reservoirs may not have the ability to form beneficial mycorrhizae at that time. Marx et al. (1982) suggests that root colonization must exceed 50% in order to invoke a positive host response. The overall low ECM production and recovery may be driven by edaphic factors, edge effects, legacy resources, the magnitude of disturbance, and the brevity of the successional timeline (Allen et al. 1992, Molina et al. 1992, Gehring et al. 1998, Kernaghan and Harper 2001, Wallenda and Kottke 1998). In addition, soil OM will be very important for mycorrhizal symbiosis and at this time is very limited in these soils. Forest system studies have shown mycorrhizal infection responding to changes in available OM (Baar & deVries, 1995) that increase with organic amendment (Lunt & Hedger 2003).

My study also demonstrated an increase in ECM root colonization in sparse plots, as well as a decrease in foliar N, which can be a limiting nutrient in PNW forest soils (Harrison et al.

2011). In contrast, lupine augmented bioavailable N in both high and medium plots, as described above. Further, there was a reduction of ECM colonization on the conifer roots in the medium and dense lupine plots. The observed ECM colonization can be explained by the following hypotheses: 1) increase of carbon flow to roots in sparse treatment, or 2) the plant is controlling its carbohydrate loss to the ECM fungi in the medium and dense lupine plots (Nehls et al. 2007). My first hypothesis is that the trees among sparse lupine may be allocating more carbon to the root system, as indicated by a larger root collar diameter, due to the detection of a limiting resource(s). Therefore, belowground resource allocation may also attract and consent to ECM colonization, which has been found to increase the overall photosynthetic efficiency of the plant, when symbiosis is functional (Vodnik & Gogala 1994). Or conversely, the plant is controlling its carbon allocation to the fungi when N is not a limiting factor (Corrêa et al. 2008), supporting other research that illustrates the supply of carbon from the host plant to the fungus is largely dependent on the nitrogen status of the tree (Nilsson & Wallander 2003). ECM colonization on roots can be lower in soils with higher nutrient availability (Nilsson et al. 2005) however, the mycorrhizal relationship can range from mutualistic to parasitic, depending on soil nutrient status (Johnson 1993; Johnson et al. 1997). Further studies are required to determine the regulation of carbon flow from the plant to fungus by examining (i) sucrose export into the apoplast, (ii) plant-derived sucrose enzyme activity, and (iii) competition for hexose from the apoplast between root cells and fungal hyphae (Nehls et al. 2007).

Multiple host fungi in mixed communities of ECM plants are frequently dominated by a few species with rare encounters of most others (Dahlberg 2001; Horton et al. 2005). Our study did find *Thelephora terrestris* to be the most abundant fungal taxon which is found in the environment and greenhouses, and develops a symbiotic ECM with grand fir, Douglas fir, and

Western white pine (Marx et al., 1984; Menkis and Vasaitis, 2011, Massicotte et al. 1999, Horton & Bruns 1998). The second most abundant taxon was species *Wilcoxina mikolae* that occurs naturally in North America, common in greenhouses, and forms a symbiont with Pinaceae which includes all three tree species in our study (Barroetavena et al. 2010, Ivory and Pearce 1991, Walbert et al. 2010). Presumably, both *T. terrestris* and *W. mikolae* came in on the tree seedlings upon planting. Rare species included *Suillus luteus* and *Tuber pacificum*, where both are known form ECM symbionts with Pinaceae (Molina et al. 1992, Trappe et al. 2009). These two fungi may represent early successional ECM species that were either present in the sediments or disseminated from the forests. *Rhizopogon* was not observed in my study, which has been reported to be the most abundant ECM species in PNW forests (Dahlberg 2001).

I did not observe edge effects of ECM colonization since the sampling points were greater than 80 m from the intact forest edge and showed not spatial correlation. However, other studies have shown that fungal communities of the exposed Lake Mills have demonstrated edge effects where greater species richness and mycorrhizal activity occurred near the forest edge than in areas farther away from such legacy resources (Cortese & Bunn 2017). Similarly, Dickie and Reich (2005) found high mycorrhizal abundance near the forest edge followed by a rapid decline at around 15 m from the base of trees along with lower species diversity. Seeds along the forest edge were also ectomycorrhizal, but seeds farther away, at 16 m, did not have ECM inoculation after one to two years. Edge effects caused slower mycorrhizal activity at greater distances from intact forest, which also influenced the corresponding plant succession and establishment.

ECM associations as an ecological strategy appears later in succession to enhance nutrient acquisition, particularly under nutrient limiting conditions (Hermans et al. 2006; Smith & Read 2008). In restoration projects using mid to later ECM host plant successional species, such as Douglas fir, grand fir, and western white pine, fungal inoculum has been documented to buffer transplant shock and ameliorate difficult soil conditions but becomes replaced by species that are better adapted to the environmental conditions of the site (Bauman et al. 2013). Therefore, an additional ECM inoculation using native ECM species from local forest soils may be required for functional mycorrhizae in the reservoir field sites during plant establishment (Amaranthus & Perry 1987; Cortese & Bunn 2017). Follow up studies of fungal inoculum and lupine can determine whether lupine plays a role in reversing ECM consent, or ECM plays a role in stockpiling available soil nutrients.

Vegetation

With regard to the vegetation community, my study showed that medium lupine treatments had fewer plant species as indicated by a decrease in species richness when compared to sparse treatments. I did note an increase in species richness in the sparse treatments driven by rare species appearing as singletons in our data set. Also noteworthy, was the presence of invasive hairgrass (*Aira* spp.), which only occurred in the sparse plots and was the most abundant species which may have contributed to the decrease in species evenness and diversity measured. Though compromising species richness, abundant lupine demonstrated competitive exclusion of the non-native *Aira* grass species suggesting lupine's potential role as a beneficial "cover crop" used in forest restoration that hinders the development and establishment of exotic and ruderal species. Factors that promote exotic and ruderal species include lack of canopy closure, sediment texture composition, exposure to extreme temperatures, and an increased likelihood of desiccation (Hood & Naiman 2000; Michel et al. 2011; Tabacchi et al. 2005). Therefore, the newly exposed lakebed sediments are especially prone to opportunistic-exotic species (Orr & Stanley 2006; Shafroth et al. 2002). Even small amounts of exotic species can negatively affect microbial and vegetative communities (Batten et al. 2007; Bellingham et al. 2005; D’Antonio & Vitousek 1992; Fierke & Kauffman 2006; Peltzer et al. 2009; Walker & Vitousek 1991) which can alter forest succession (Urgenson et al. 2009, D’Antonio & Vitousek 1992) and in some cases result in unwanted irreversible effects (Coblentz 1990).

In the Lake Mills basin, I expect that future seres will show an increase in species richness among abundant lupine as well as sustained species diversity and abundance until the system stabilizes through the phases of hierarchical dominance. Similarly, the Morris and Woods (1989) investigations of lupine ecology post Mount Saint Helens eruption demonstrated that Pacific lupine (*L. lepidus*) inhibited immediate colonization of forbs such as pearly everlasting and willowherb through natural succession. Despite this initial inhibition, further studies showed that the plant community structure changed over time demonstrated by an increase in species richness and cover that was accelerated by the presence of lupine (del Moral and Rozzell 2005). Lupine colonies 12 to 20 years in age showed an increase in species richness and diversity when compared to younger lupine colonies or to study plots void of lupine (del Moral 2007). This study demonstrated that lupine facilitated the arrival of other species within a couple of decades despite the harsh growing conditions.

When species require a particular resource for survival, such resource levels should be examined as well as the mechanism of acquisition (Pickett et al. 1987; Tillman 1987). Understanding the mechanisms that produce available resources may be used to make separate, testable observations to infer the influential natural patterns of succession. For example, resources such as soil nutrients, OM, and moisture were limited along the coarse terraces where lupine may provide several mechanisms that ameliorate environmental stressors by increasing resource availability. We suggest that one mechanism of lupine is its ability to tolerate resource limited systems and serve as an early seral species functional type such as a pioneering species. Furthermore, the N-fixing mechanism of lupine will enhance nearby vegetation by providing the essential resource of bioavailable N. Measuring bio-assimilated N in conifer foliage is one way to characterize the influence of lupine on the neighboring vegetation, thereby providing an examination of resource level processes. In addition, OM contributions from riverbank lupine are also important for successional trajectories given its perennial life cycle and ability to tolerate resource limited soils. The physiology and behavior of lupine is the mechanism that contributes to cyclical OM resource inputs, thereby providing a mulching effect.

Future trajectory

This project documents lupine's ability to thrive in highly disturbed landscapes while facilitating conifer establishment, relaxing the need for a mycorrhizal symbiont in soils where fungi may have not yet recolonized, and excluding invasive grass species. I did not detect a critical density threshold in which lupine cover negatively affects species diversity through over-crowding. Greater densities (58-76% lupine cover) contributed greater conifer foliar N concentration, with medium densities (58% lupine cover) facilitating larger trees when compared to sparse plots. Based on our findings and corroborative literature, we recommend the inclusion of native lupine in highly disturbed, N-limiting soils to serve as a facilitator of important N pools during this early successional phase.

My study indicated that the coarse terraces along Lake Mills had little to no soil OM. Many soil functions are strongly influenced by the availability of soil OM including biological, chemical, and physical properties which affect ecosystem functioning (Baldock & Broos 2012). Seven years of ecological succession at Mount St. Helens showed significant organic matter production near the lupine's (*L. lepidus* and *latifolius*) rhizosphere as well as increased soil N on the novel landscapes formed after eruption (Halvorson et al. 1991; Halvorson et al. 1992; Halvorson & Smith 1995). Although we didn't find differences in OM among treatments, we hypothesize that lupine will contribute important carbon pools to the forest soil building process. Further, the development of OM in soils may increase ECM fungal colonization and improve the functional mycorrhizal symbiosis (>50% colonization). Future field studies of fungal succession may also reveal the emergence of *Rhizopogon* fungi as ECM species diversify along with greater conifer species recruitment. Changes in ECM colonization and hyphal growth should happen alongside of the above ground successional trajectory, given how the microbial community often respond to disturbance.

I speculate that future permanent plot studies will show elevated soil OM inputs and enhanced soil moisture retention as lupine acts as a cover crop for forest regeneration and exotic weed control by providing a mulching effect. The damming and the subsequent dewatering of Lake Mills created a phenomenal disturbance that resulted in a novel landscape that lacked an ecological legacy. Similar to the eruption of Mount St. Helen, seeded lupine in the Elwha River Valley was able to establish under harsh conditions without impeding the success of nearby plant recruitment and restoration conifer plantings. Further, lupine's life cycle promotes the reinvigoration of nearby vegetation which ultimately accelerates forest succession. This study documented the benefits of including a locally native N-fixing species (riverbank lupine) in the Elwha River Restoration project and can inform future practitioners when considering strategies of revegetation, post dam removal.

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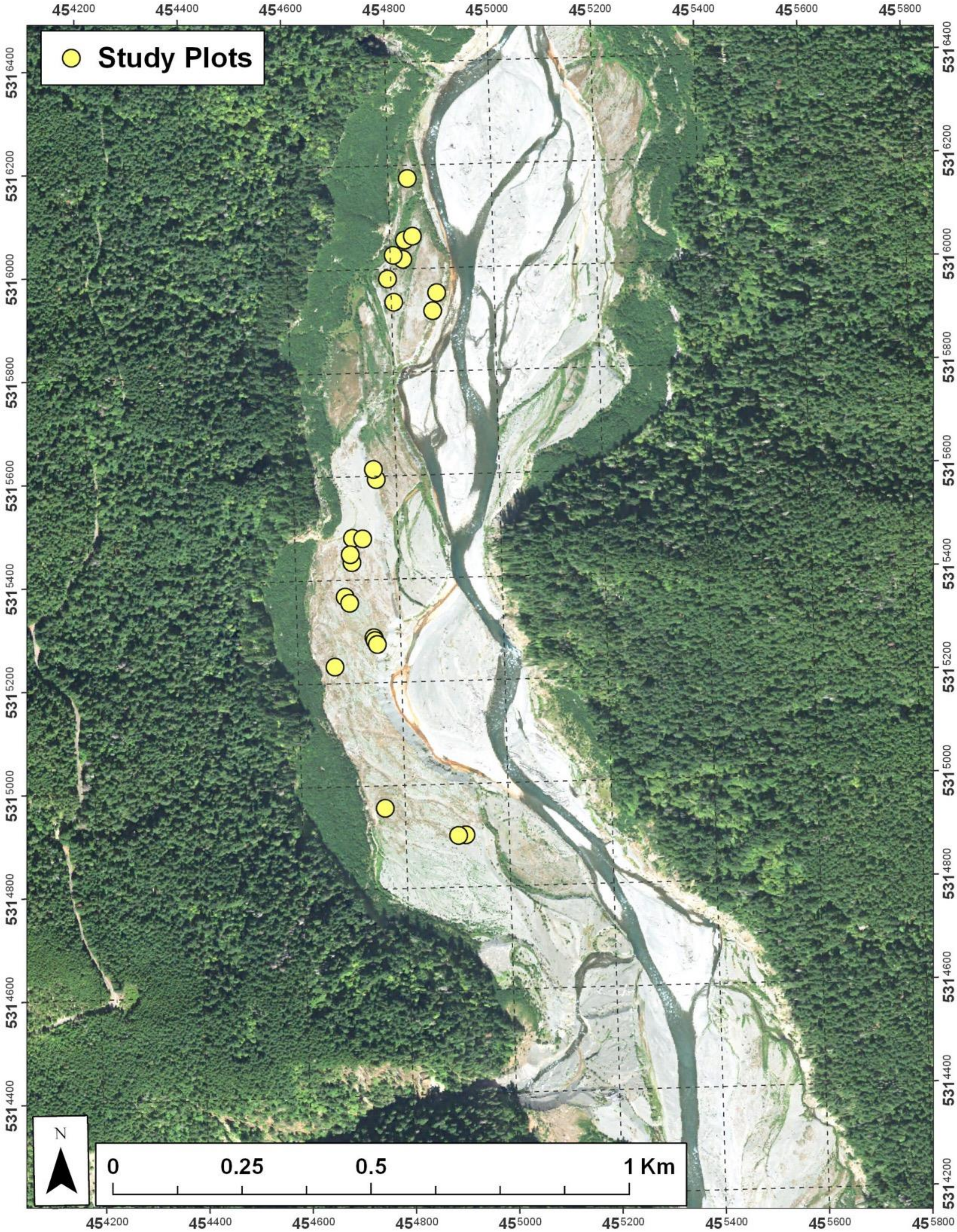
Appendix A. Methods

Appendix A; Table 1.A. Vegetative species planted for restoration along the Elwha River post dam removal.

Species Name	Common Name
Graminoid	
Agrostis exarata	spike bentgrass
Agrostis humilis	Thurber’s bentgrass
Bromus carinatus	California brome
Bromus complex	brome sp.
Carex deweyana var. *deweyana*	Dewey’s sedge
Carex pachystachya	thick-edge sedge
Deschampsia cespitosa	tufted hairgrass
Deschampsia elongata	slender hairgrass
Elymus glaucus	blue wildrye
Luzula comosa	Pacific woodrush
Scirpus microcorpus	small-flowered bulrush
Forb	
Achillea millefolium	common yarrow
Anaphalis margaritacea	pearly-everlasting
Aquilegia formosa	Sitka columbine
Artemisia suksdorfii	Suksdorf’s wormwood
Aruncus dioicus	goatsbeard

Species Name	Common Name
Chamerion angustifolium	fireweed
Circaea alpina	enchanter’s nightshade
Claytonia sibirica	Siberian springbeauty
Erigeron philadelphicus	Philadelphia fleabane
Eriophyllum lanatum	common woolly sunflower
Fragaria vesca	wood strawberry
Fragaria virginiana	Virginia strawberry
Galium aparine	cleavers
Geum macrophyllum	large-leaved avens
Heracleum lanatum	cow parsnip
Lathyrus polyphyllus	leafy peavine
Lupinus polyphyllus	big-leaf lupine
Lupinus rivularis	riverbank lupine
Mycelis spp.	wall lettuce
Myosotis spp.	forget-me-not
Petasites frigidus	sweet coltsfoot
Polystichum munitum	sword fern
Rumex acetosella	common sheep sorel
Senicio spp.	ragwort
Solidago canadensis	Canada goldenrod
Stachys chamissonis	Cooley’s hedge-nettle
Shrub	
Acer circinatum	vine maple
Acer glabrum	Douglas maple
Aluns viridis	Sitka alder
Amelanchier alnifolia	western serviceberry
Ceanothus sanguines	red stem ceanothus
Clinopodium douglasii	Yerba buena
Gaultheria shallon	salad
Holodiscus discolor	Oceanspray
Lonicera involucrata	black twin berry
Mahonia aquifolium	tall Oregon-grape

Species Name	Common Name
Mahonia nervosa	Oregon-grape
Oemleria cerasiformis	Indian plum
Philadelphus lewisii	mock orange
Physocarpus capitatus	ninebark
Prunus emarginata	bitter cherry
Ribes divaricatum	spreading gooseberry
Ribes lacustre	prickly currant
Ribes lobbii	gummy gooseberry
Ribes sanguineum	red-flowering currant
Rosa nutkana	Nutka rose
Rubus parviflorus	thimbleberry
Rubus spectabilis	salmonberry
Rubus ursinus	Pacific backberry
Salix lucida	Pacific willow
Salix scouleriana	Scouler willow
Salix sitchensis	Sitka willow
Sambucus cerulea	blue elderberry
Sambucus racemosa	red elderberry
Spiraea douglasii	Douglas' spirea
Symphoricarpos albus	common snowberry
Vaccinium parvifolium	red huckleberry
Trees	
Abies grandis	grand fir
Acer macrophyllum	big-leaf maple
Alnus rubra	red alder
Malus fusca	western crab apple
Pinus monticola	western white pine
Pseudotsuga menziesii	Douglas fir
Populus balsamifera spp. trichocarpa	black cottonwood
Prunus emarginata var. *mollis*	bitter cherry
Thuja plicata	western red cedar



Appendix A; Figure 1.A. Restoration study plots (n = 24) in 2018 along the terraces of Lake Mill reservoir post Glines Canyon dam removal of the Elwha River, WA.

Appendix A; Table 2.A. UTM coordinates of the restoration study plots in the Lake Mills reservoir

Plot ID	Easting	Northing	Terrace
D1	454915	5314902	3
D3	454693	5315368	3
D8	454878	5315918	2
D10	454823	5316019	2
D11	454841	5316173	2
D12	454749	5315282	2
D13	454760	5314957	3
D14	454765	5314963	3
M5	454702	5315356	3
M6	454712	5315436	3
M7	454760	5315594	2
M8	454887	5315953	2
M9	454805	5316027	2
M10	454828	5316056	2
M11	454836	5316175	2
M13	454753	5315275	3
S5	454670	5315233	3
S7	454708	5315434	3
S8	454706	5315450	3
S9	454755	5315614	2
S10	454793	5315982	2
S11	454803	5315936	2
S12	454843	5316063	2
S15	454753	5315275	3

UTM zone 10N, NAD 83

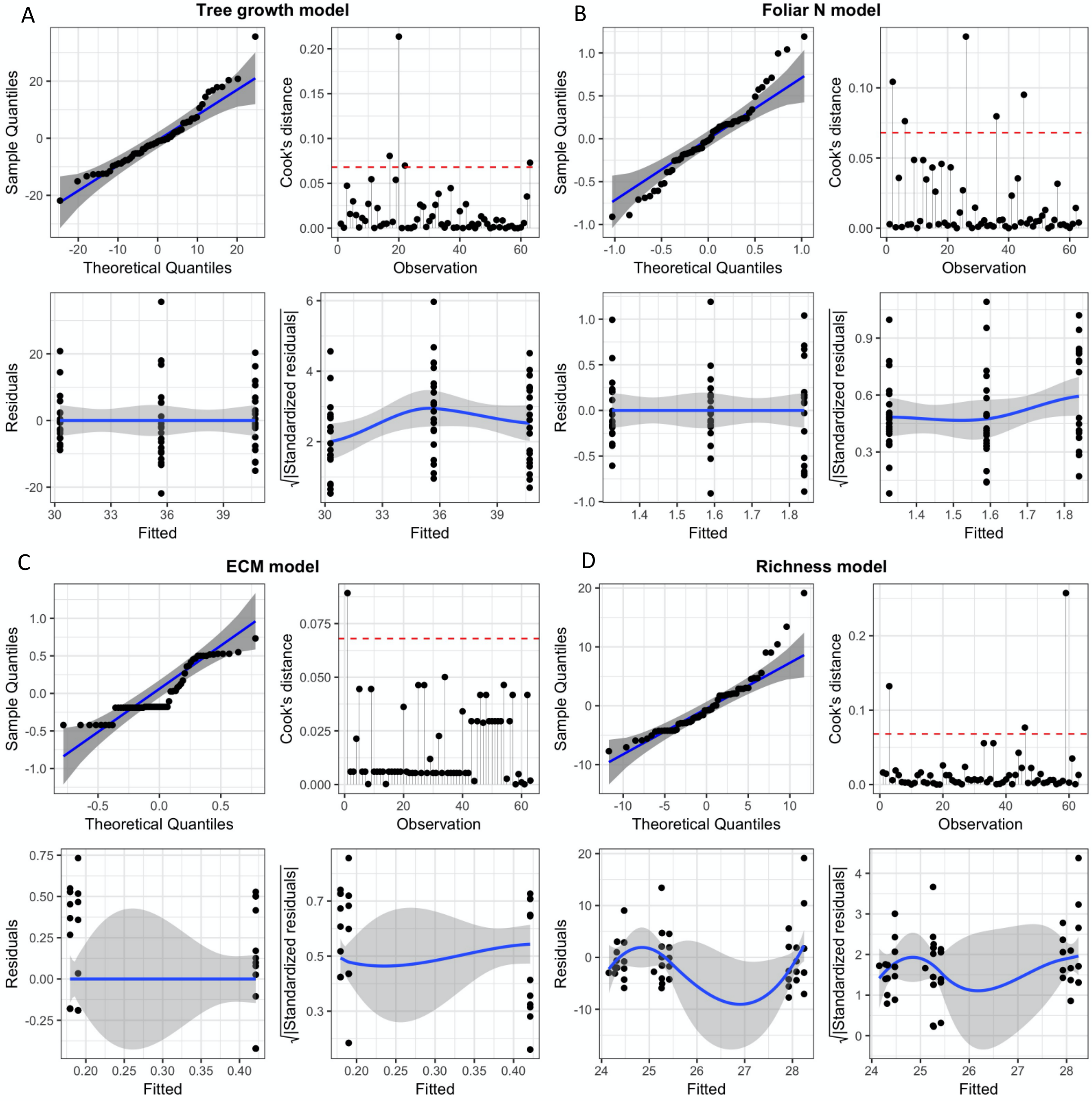
Appendix A; Table 3.A. Sample means and standard error for all measured variables. Treatment that do not share the same letter are significantly different when presented ($\alpha = 0.05$).

	Treatment		
Variable	Dense	Medium	Sparse
Lupine Cover (%)	76 ± 0.04^{a}	58 ± 0.03^{b}	15 ± 0.04^{c}
Foliar C:N	32.1 ± 2.3^{a}	34.8 ± 2.3^{a}	40.8 ± 2.4^{b}
Foliar Nitrogen (%)	1.8 ± 0.12^{a}	1.6 ± 0.09^{a}	1.3 ± 0.08^{b}
Soil C:N	4.9 ± 0.6	5.4 ± 0.8	3.9 ± 0.4
Soil Nitrogen (%)	0.12 ± 0.01	0.12 ± 0.02	0.11 ± 0.01
Tree Growth	35.2 ± 2.8^{ab}	40.3 ± 2.1^{a}	30.0 ± 1.6^{b}
ECM	0.12 ± 0.05^{a}	0.12 ± 0.04^{a}	0.31 ± 0.08^{b}
Shannon Diversity (H')	1.8 ± 0.08	1.9 ± 0.07	1.7 ± 0.1
Richness	25.2 ± 0.99^{ab}	24.3 ± 0.89^{a}	28.5 ± 1.37^{b}
Pielou Evenness (J)	0.44 ± 0.02	0.46 ± 0.02	0.40 ± 0.02
Organic Matter (g/g)	0.02 ± 0	0.02 ± 0	0.02 ± 0
Particle Size (mm)	4.2 ± 1.8	7.4 ± 2.4	6.5 ± 1.9
Bare Ground (%)	19 ± 5^{a}	33 ± 4^{b}	80 ± 3^{c}

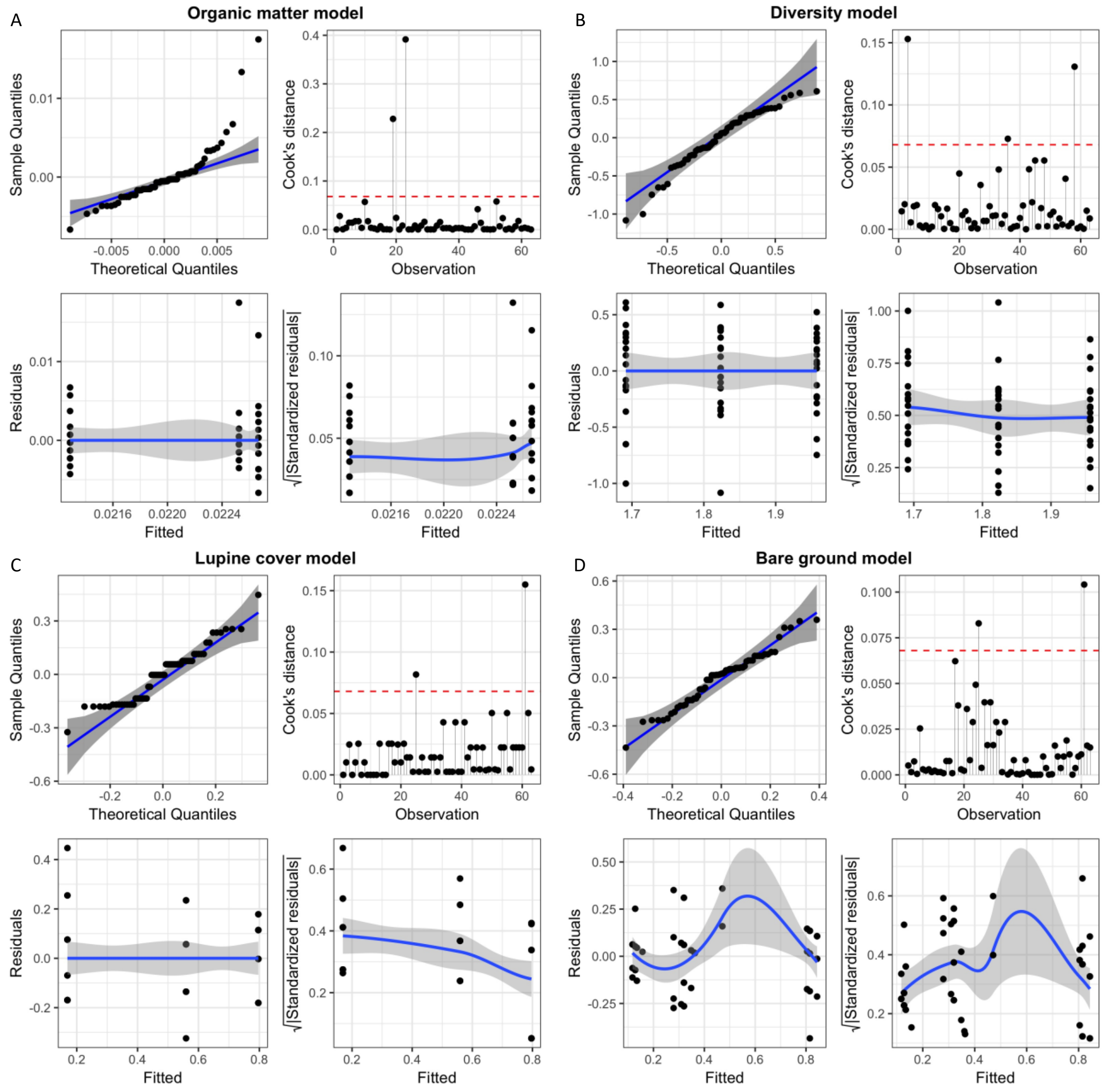
Appendix A; Table 4.A. Slightly modified Wentworth soil texture size class (Wentworth 1929)

Size range (mm)	Wentworth class	$\psi = \log_2$
> 256	Boulder	10.0
64 - 256	Cobble	7.0
32 - 64	Very coarse gravel	5.5
16 - 32	Coarse gravel	4.5
8 - 16	Medium gravel	3.5
4 - 8	Fine gravel	2.5
2 - 4	Very fine gravel	1.5
1 - 0.13	Sand	-1.5
< 0.13	Silt	-6.0

Appendix B. Results



Appendix B; Figure 1.B. Mixed effects model diagnostic plots showing theoretical quantiles (–) and 95% confidence interval (▨), Cook's distances with high leverage above the dotted red line (---), residual, and standardized residuals (–) along with their confidence interval (▨) in gray. An ANOVA of the model indicated that lupine treatment as the fixed factor has a significant effect on the response variable ($P < 0.05$, $\alpha = 0.05$). A) shows diagnostics for tree growth, B) shows foliar nitrogen, C) shows ectomycorrhizal colonization, and D) shows species richness.



Appendix B; Figure 2.B. Mixed effects model diagnostic plots showing theoretical quantiles (−) and 95% confidence interval (▨), Cook's distances with high leverage above the dotted red line (---), residual, and standardized residuals (−) along with their confidence interval (▨) in gray. A) shows diagnostics for organic matter, B) shows species diversity, C) shows lupine cover, and D) shows bare ground. An ANOVA of the model failed to indicate that lupine treatment as the fixed factor had a significant effect on organic matter (A) and species diversity (B) ($P > 0.05$, $\alpha = 0.05$). However, the ANOVA did indicate that lupine treatment had a significant effect on lupine cover (C) and bare ground (D) ($\alpha = 0.05$).