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**Soil conditions and arbuscular mycorrhizal fungal spore abundance and distribution in dewatered reservoir sediments after dam removal**

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## **Abstract**

Many dams in the U.S. are reaching the ends of their lifespans and dam removal as a form of river ecosystem restoration is becoming more prevalent. Revegetation of newly exposed sediments is an important aspect of ecosystem recovery after dam removal. Understanding the soil environment left behind after dam removal is important for understanding revegetation and ecosystem recovery trends. Physical soil properties and soil biota communities help to determine the success of plants colonizing exposed sediments after reservoirs are drained. I investigated soil properties and biota after dam removal by looking at the Elwha Dam Removal in Olympic National Park, WA. I asked three main questions about how soil conditions and symbiotic arbuscular mycorrhizal fungi (AMF) impact the success of revegetation after dam removal. 1) What is the potential for AMF colonization of plant roots in sediments after dam removal? To investigate this question I conducted an inoculum potential experiment in 2013 using live soil from the Elwha. No significant trends with treatment or sample location were found and there were overall low levels of AMF colonization in plant roots. Appendix I contains a summary of this study. 2) Do soil properties of exposed sediments differ from those of the surrounding environment? I measured soil pH, organic matter, mineral oxides, texture and counted spores in Elwha sediments and compared measurements to those of the mature forest in 2014 and 2015. The sediments had lower levels of organic matter and a finer texture. Appendix II contains a summary of this study. 3) What is the abundance of AMF spores and what is the spatial distribution of spores in exposed sediments? In 2016 I conducted spore counts to see if there was a trend of decreasing spore abundance as the distance from the mature forest increases. Spores were extracted from soil samples collected from the Elwha in 2013 and counted under the microscope. Spore counts indicated a decreasing trend of spore abundance with increased

distance from the adjacent forest. In conclusion, reservoir sediments are fine-textured and have a low abundance of AMF spores that decrease in abundance with distance from the forest.

Understanding soil conditions and the spatial distribution of AMF spores in soil after dam removal may help to understand the potential for plant success and distribution patterns in the process of ecosystem recovery.

## **Introduction**

From 2013 to 2014 the largest dam removal in U.S. history was completed in the Olympic National Park in Washington State. Two dams, the Glines Canyon Dam and the Elwha Dam, were removed after obstructing the Elwha River for about 100 years. These dams were removed in order to comply with the Elwha River Ecosystem and Fisheries Restoration Act passed by Congress in 1992. This act called for the full restoration of the Elwha River ecosystem and the historic salmon populations that once inhabited it (Duda et al. 2008). Dam construction results in drastic changes to marine ecosystems, rivers, and surrounding landscapes. The river becomes segmented and disconnected from marine ecosystems leading to hydrologic changes such as sedimentation and channelization (Hall et al. 2011). Dams also block the passage of migratory species such as anadromous salmon (Hall et al. 2011). Dams are prevalent in the United States (Graf 1999); however, dams have a finite lifetime and many of them are reaching the point where they must be either restored or removed.

Removing a dam introduces challenges as the path of the river shifts drastically and former reservoir beds become dewatered. Part of returning a river to a functioning ecosystem involves the revegetation of newly exposed sediments by native plants. While salmon can potentially return to the river directly after dam removal, riparian vegetation usually takes decades to recover (Doyle et al. 2005) and reservoir sediments are vulnerable to rapid

colonization by aggressive invasive plant species (Orr and Stanley 2006). Establishment by riparian plants is crucial for the functioning of ecosystem processes such as providing habitat, nutrient inputs, streambank stabilization, stream temperature control and more (Tabbacchi et al. 1998). Gaining understanding of the soil environment left behind in the reservoir after dam removal is an important aspect of predicting the recovery of vegetation and the ecosystem as a whole.

Arbuscular mycorrhizal fungi (AMF) may play an important role in successful revegetation of the Elwha. Exposed sediments are essentially a new soil which will likely have low levels of microbial and fungal activity. This study aims to assess AMF spore abundance and distribution in the dewatered sediments. The characteristics of reservoir sediments likely make it a stressful environment for seedling establishment and plant growth: sediments are in direct sunlight, likely have few biota, and few organic matter inputs. AMF may be able to mitigate these stressors. Symbioses between plants and AMF are widespread and nearly ubiquitous (Smith and Read 2008). These partnerships can increase a plants stress-tolerance, growth rate, water, and nutrient uptake (Rodriguez 2004; Fracchia et al. 2011). AMF provide these functions for plants while receiving sugars in return (Smith and Read 2008). AMF can also be important for seedling establishment and plant survival, especially in arid and semi-arid environments (Fracchia et al. 2011). Fungal symbionts can even ameliorate abiotic stressors such as drought and increased temperature (Kivlin et al. 2013). AMF spores were used as an indicator of potential mycorrhizal propagation as spores are the dormant stage of AMF and can begin colonization of new areas (Abbott and Robson 1984). AMF are obligate symbionts and require a live host plant to grow. Spores are the only AMF structure that can survive in the soil without a host plant and propagate new fungal growth. Spore abundance may be a particularly relevant

indicator of AMF abundance in sediments after dam removal where the soil is largely unvegetated and there is low availability of host plants.

The mature forest is likely the main source of spores being distributed onto reservoir sediments and an initial survey indicated that spore abundance decreased sharply with distance from the forest along a single transect (Cortese and Bunn in press). This study aims to determine if this trend holds across a larger spatial scale. Gaining a more complete understanding of the soil environment and the potential role of AMF in facilitating plant colonization of sediments will be important in the early years of revegetation of the Elwha. In this study I asked if spore abundance decreased with distance from the forest and estimated spatial variability in abundance.

## **Methods**

Soil samples were collected from the drained Lake Mills in June 2013 from six sampling plots; five plots in exposed sediment and one in the mature forest adjacent to the drained lake. The five plots in the sediment were chosen by revegetation treatments and one control plot with no revegetation treatment was chosen. Samples from the mature forest were taken for comparison purposes. Ten soil samples were collected from each plot for a total of 60 samples. For each sample about 500 mL of soil was collected from 8 cm wide by 10 cm deep holes. The distance from the corner of each sample plot from the mature forest was recorded. Samples were air-dried in paper bags for approximately two and a half years before being processed. Samples for this analysis were selected to get soil samples with an even distribution of distances from the mature forest. Samples ranged from 0 to 87 m from the mature forest. Six groups of ten samples were designated by increasing distance from the mature forest. Seven samples were randomly selected from each distance section for a total of 42 samples of the 60 collected.

Spores were extracted from 10 g of each soil sample through a process of sieving, centrifuging, and vacuum filtration as described in Allen et al. 1979 and Ianson and Allen 1986. For each spore extraction I weighed out 10 g from a soil sample. I added the soil to a pitcher and spray agitated with water. After 15 seconds I poured the water off through 250  $\mu\text{m}$  and 25  $\mu\text{m}$  sieves without pouring out the heavier sediments. I spray agitated the material two more times for a total of three pours. I washed the material through the 250  $\mu\text{m}$  sieve to push any last spores through. I then washed the material caught by the 25  $\mu\text{m}$  sieve into a 50 mL centrifuge tube. I then centrifuged at 2500 rpm for 3 minutes. I poured off the water leaving the soil material and spores behind. I added 2 M sucrose solution to the 45 mL mark of the centrifuge tube and stirred. I then centrifuged samples again at 2500 rpm for 1.5 minutes. I poured off the sucrose solution containing spores onto the 25  $\mu\text{m}$  sieve. I washed the sucrose through the sieve and then washed remaining material on the sieve into a beaker. The contents were captured on filter paper (nylon, 47 mm diameter, 20.0  $\mu\text{m}$  pore size).

I counted spores under a dissecting microscope at 40x magnification. Spores were identified by a spherical shape and clear, yellow, or white coloring (West Virginia University 2014). Parasitized or broken spores, identified by their black color, were not counted so that counts consisted of viable spores only. The petri dish containing a filter paper was moved back and forth along straight lines under the microscope to cover the entire area of the filter paper as spores were counted.

I also compiled data from five other studies that used AMF spore counts to analyze mycorrhizal distributions and compared their means and standard deviations to those of this study. The studies all made comparisons of AMF spore counts: among different coffee production systems in Mexico (Arias et al. 2011), between grazed and non-grazed grasslands in

Yellowstone National Park (Murray et al. 2010), between salt marshes and desert brush ecosystems in Portugal (Carvahlo et al. 2003), among different agricultural methods (Oehl et al., 2004), and seasonal differences among soil colonized by plants with different metabolic pathways (Lugo and Cabello 2002). Means and standard deviations were compiled from each study from either results reported in-text, results tables, or estimations from figures. Estimating statistics from figures introduced some error as these estimations were not exact.

## **Results**

The results of regression analysis indicate a statistically significant relationship of decreasing spore abundance as the distance from the mature forest increases ( $R^2 = 0.37$ ,  $F_{1,39} = 22.60$ ,  $p\text{-value} < 0.001$ , Figure 1). Analyses were based on 41 of the 42 selected soil samples. One sample was left out of analyses due to a high rate of parasitized spores indicating that the sample was perhaps no longer valid after sitting in storage for three years.



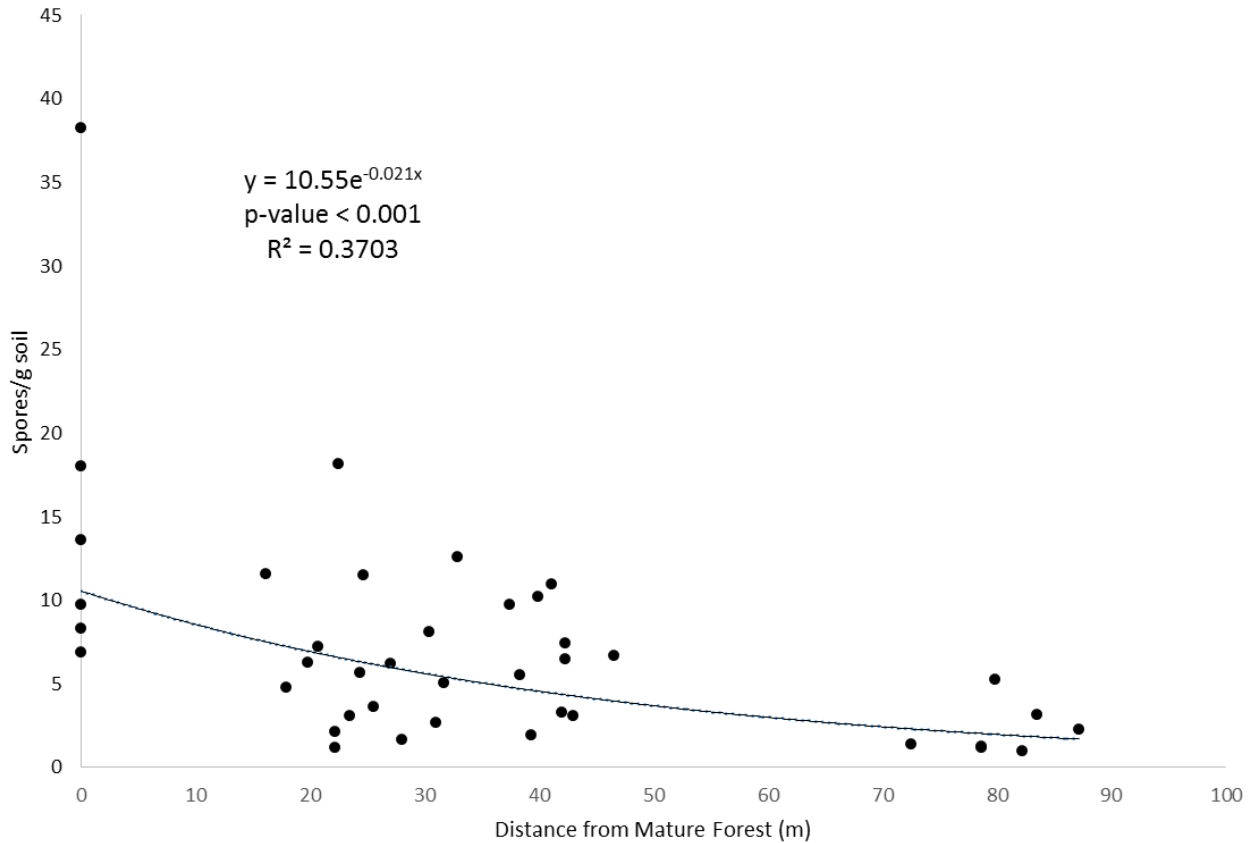


Figure 1. Spore counts (spores/g soil) plotted against distance from the mature forest. Fit line is an exponential decay function with equation and  $R^2$  displayed

The mean spore count from this study (7.07 spore/gram soil) was comparable to spore counts from other studies of AMF spore distributions (Figure 2). The standard deviation from this study (6.66 spores/gram soil) was one of the lower standard deviations of the studies compared (Figure 2).

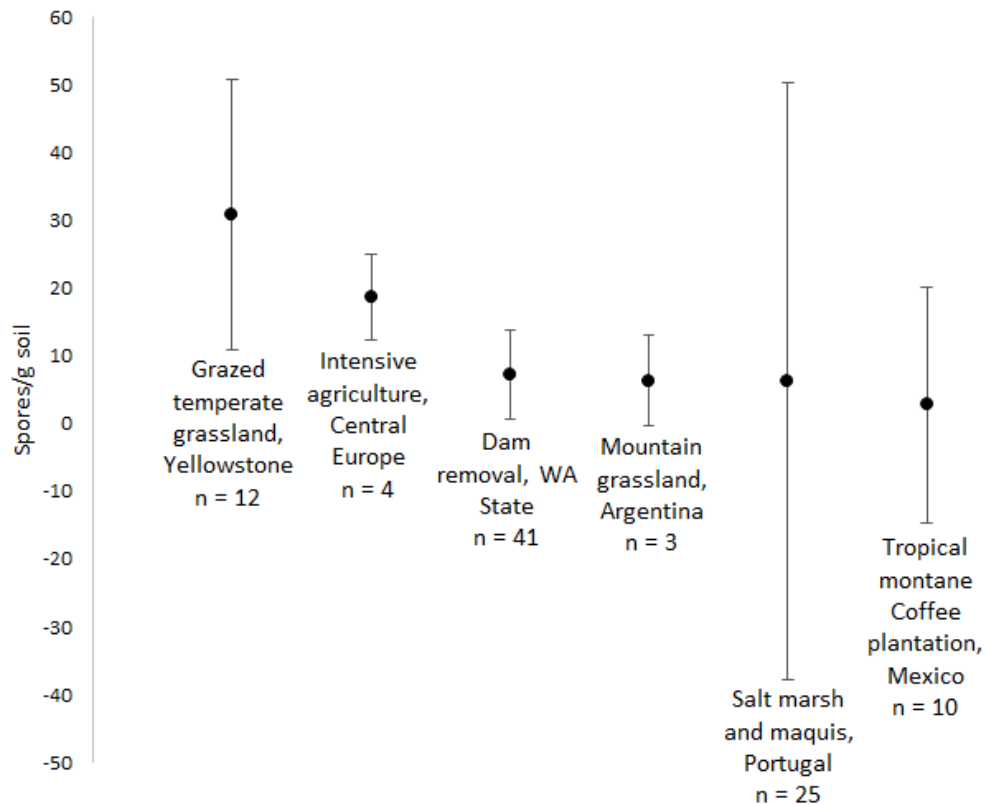


Figure 2. Comparison of spore counts (spores/g soil) of various AMF spore abundance studies in different ecosystems and locations including our results (Dam removal, WA State). Results are mean spore counts with error bars representing SD. Mean  $\pm$  SD is reported as well as sample size (n)

## Discussion

This study confirms a trend of decreasing AMF spore abundance with increasing distance from the mature forest. Decreasing spore counts with distance from the forest found along one transect in 2012 (Cortese and Bunn in press) are confirmed on a larger spatial scale by this study. This pattern may be due to the mature forest being the source for spores being distributed onto reservoir sediments. Although the average spore count of this study is low compared to most natural systems, it is comparable to spore densities found by other studies of disturbed sites. Analysis of spore abundance from other studies of disturbed sites revealed mean spore counts

similar to that of this study (7 spores per gram of soil, Figure 2) and standard deviations generally similar to or larger than those of spore counts in Elwha sediments.

Reservoir sediments are likely a stressful environment for colonizing plants due to direct sun exposure, low nutrient availability, and a minimal microbial community. The fine texture of reservoir sediments compared to mature forest soils also introduces plant stressors (Appendix I). Fine-textured soils have smaller pore spaces and therefore restricted oxygen, water, and microbial movement. This leads to depleted oxygen and water availability for plants as well as decreased rates of nutrient cycling. AMF may be especially important for plants in sediments due to these stressors.

The soil environment is an important aspect of ecosystem recovery after dam removal. The establishment of a diverse community of native plants is crucial for ecosystem functioning. Successful revegetation by plants attempting to establish in exposed reservoir sediments depends greatly on the soil environment of the sediments. Availability of mycorrhizal fungi in this unique and potentially stressful soil environment will likely impact the trajectory and success of revegetation. Abundance of mycorrhizal fungi will likely influence the plant species that will successfully colonize reservoir sediments as certain plant species are more dependent on mycorrhizal associations than others (Klironomos and Moutoglis 1999). As the frequency of dam removal continues to increase in the future, gaining a clearer picture of the soil environment left behind will be crucial for understanding revegetation and ecosystem restoration trends.

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## Appendix I – Inoculum Potential

What is the potential for colonization of plant roots by arbuscular mycorrhizal fungi in exposed reservoir sediments?

\*This study was presented at the 2013 WWU Scholars Week poster session  
Poster Title: “Mycorrhizal Fungi in the Revegetation of the Elwha: Infectivity of Existing Inoculum”

### Methods

Samples were prepared with inoculum from fourteen different sites in the Elwha river bed that varied in distance from the forest edge. One pot was prepared with inoculum from each of the sites except for sites B007, P171, and U376, where five replicated pots were prepared from each sample. Five sterile pots were also prepared to ensure the inoculum was the only source of microbial growth. All pots and soil mixtures were sterilized prior to planting to ensure Elwha inoculum was the only source of microbial growth. Seeds were also sterilized using vapor-phase sterilization. Approximately 30 mL of *Elymus glaucus* seeds were sterilized.

All pots were filled to approximately equal levels (less than half full) with sterilized 5-way soil mix. About 25 mL of inoculum was taken from each site sample, flattened out, and added to the pots. Five replicates were made from sites B007, P171, and U376. About 25 mL of inoculum was also taken from each site sample and set aside to be used in a microbial wash. No inoculum was added to the sterile treatment. All pots were then topped to approximately equal levels with a sterilized soil-sand mixture.

A microbial wash was prepared from the approximate 25 mL of inoculum set aside from each site sample in an attempt to give plants a similar microbial community to that of their natural habitat. Approximately 300 mL of total inoculum was mixed with 1 L of water. The soil-water mixture was poured through a series of sieves of varying sizes. The soil-water mixture was

then run through an 11  $\mu\text{m}$  filter in a Buckner funnel. When the filter became clogged it was removed and replaced with a new 11 $\mu\text{m}$  filter. This was repeated until all of the microbial wash made it through one filter without clogging. Nearly 1 L of microbial wash was obtained.

The native grass species *Elymus glaucus* was chosen due to its prominence in the Elwha riverbed. Before planting, about 50 mL sterilized vermiculite was added to each pot. About 25 mL of the microbial wash was then added to all of the pots except for the sterile treatment. Approximately 10 *Elymus glaucus* seeds were planted in each pot. All of the pots were then topped off with the sterilized soil-sand mix. The planted pots were watered and set in the greenhouse to germinate. After about two weeks the plants were thinned down to three plants per pot. Plants grew for approximately three months and were harvested over a two-week period. Roots were cleaned and the three shoots were cut off. Five root samples totaling about 0.6 g were taken from each root system. The wet-weight of each sample as well as that of the remaining root ball was recorded. Remaining root balls were placed in envelopes and dried in an oven at 60°C along with the shoots. After 48 hours dry weights for the roots and shoots were recorded. The root samples were then cleared and stained using 2.5% KOH, 3.0% HCl and 0.05% Tyran blue. Microscope slides were made from the stained roots.

Slides were read under a compound microscope at 200x magnification. Cross-section views perpendicular to the root length were read. AMF structures were counted if they crossed the cross-section marker. I counted AMF vesicle, arbuscule, and hyphal structures.

## **Results**

There were no significant differences in total biomass between *Elymus glaucus* plants grown in sterile soil or with inoculum collected at any distance from the mature forest (Figure

A1). A very low colonization rate was found in *Elymus glaucus* roots with an average of 5.9% colonization by AMF (Figure A2). No significant trend between AMF colonization and distance from the mature forest was found.

Note: Because of this low colonization rate, AMF spore abundance was assessed as an indicator of AMF presence in Elwha soils. AMF is an obligate symbiont and requires a live host plant to grow. Spores are the only AMF structure that can survive in the soil without a host plant and propagate new fungal growth. Spore abundance may be particularly relevant in sediments after dam removal where the soil is largely unvegetated and there is low availability of host plants at least initially.

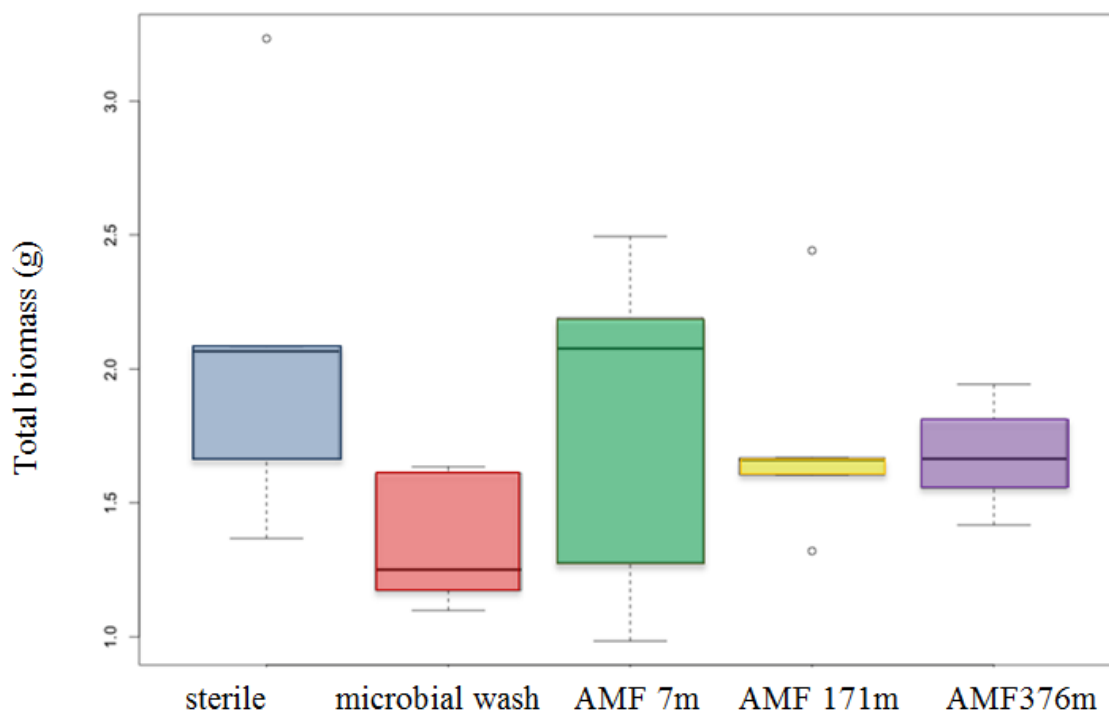


Figure A1. Total biomass of harvested *Elymus glaucus* grown in sterile soil, microbial wash only, and inoculum collected 7, 171, and 376 meters from the mature forest



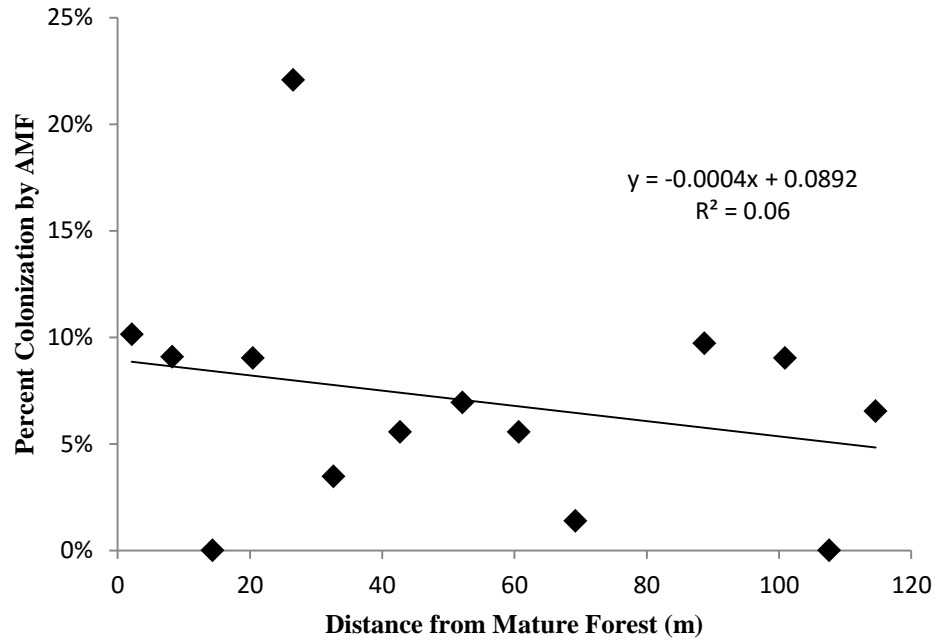


Figure A2. Percent AMF colonization of *Elymus glaucus* roots against distance from the mature forest

## **Appendix II – Soil Properties**

How do soil properties of reservoir sediments differ from those of the adjacent mature forest?

\*This study was presented at the 2014 WWU Scholars Week poster session

Poster title: “Characterizing the soil environment of the Elwha basin and potential effects on plants”

### **Methods**

Soil properties were assessed based on a subsample of soil samples collected in 2013 from the former Lake Mills. Three samples were randomly chosen from each of the six sites for a total of 18 samples. I measured the pH of each soil sample by adding 10 g of soil to 20 mL of  $\text{CaCl}_2$  and measuring with a pH probe. The organic matter content of soil was quantified by loss on ignition. Soil samples were first placed in an oven at  $105^\circ\text{C}$  to remove any moisture. Oven-dried soil was then placed in crucibles and incinerated in an oven at  $500^\circ\text{C}$  for 2 hours. The soil was then weighed again so that the mass lost represented organic matter content. Mineral content of was also assessed using EDX (SEM, VEGA TS 5136 150 mm w/ EDAX, TESCAN). Soil texture was qualitatively compared also using a scanning electron microscope. Soil texture was also assessed using the hydrometer method (Gee and Bauder 1986). Soil for the hydrometer test was passed through a 2.36 mm sieve. 50-100 g of sieved soil, distilled water, and 5 mL of hexametaphosphate were added to a cylindrical tube. A blank cylinder was also made with water and hexametaphosphate. Hydrometer readings and temperatures were taken after 40 seconds and after 2 hours. Particle sizes were then calculated from differences between the cylinder with soil and the blank cylinder. Spore extraction was done using the same method as described above.

### **Results**

Results of soil property analyses showed some differences between the mature forest and reservoir soils. There was no significant difference in pH between the mature forest soil and

reservoir sediments ( $p = 0.667$ , Figure A3). The results of loss on ignition showed higher % organic matter in the mature forest soil than the reservoir sediment ( $p = 0.046$ , Figure A4). There were no significant differences in mineral oxide content between the two soil types and both had high levels of iron and silicates (Table A1). SEM images qualitatively showed larger and more variable particle sizes in mature forest soil than reservoir sediments. Results of hydrometer tests showed differences in soil textures between the two soil types: mature forest soil was classified as having sandy loam texture while reservoir sediments ranged from loam to silt loam in texture based on the proportion of sand, clay, and silt particles (Table A2). I found a low abundance of AMF spores with no significant trend with distance from the mature forest (Figure A5).

Table A1. Percent by weight of mineral oxides in mature forest soil and reservoir sediments. Data are means  $\pm$  standard deviations,  $n = 3$

<b>Mineral Oxide</b>	<b>Mature Forest</b>	<b>Reservoir Sediment</b>
Al <sub>2</sub> O <sub>3</sub>	10.01 $\pm$ 2.27	8.51 $\pm$ 1.02
SiO <sub>2</sub>	43.20 $\pm$ 7.02	53.02 $\pm$ 5.93
K <sub>2</sub> O	5.80 $\pm$ 0.85	6.25 $\pm$ 0.82
CaO	9.97 $\pm$ 4.98	2.72 $\pm$ 2.38
TiO <sub>2</sub>	5.06 $\pm$ 0.77	2.92 $\pm$ 0.61
Fe <sub>2</sub> O <sub>3</sub>	25.33 $\pm$ 3.60	26.56 $\pm$ 5.62

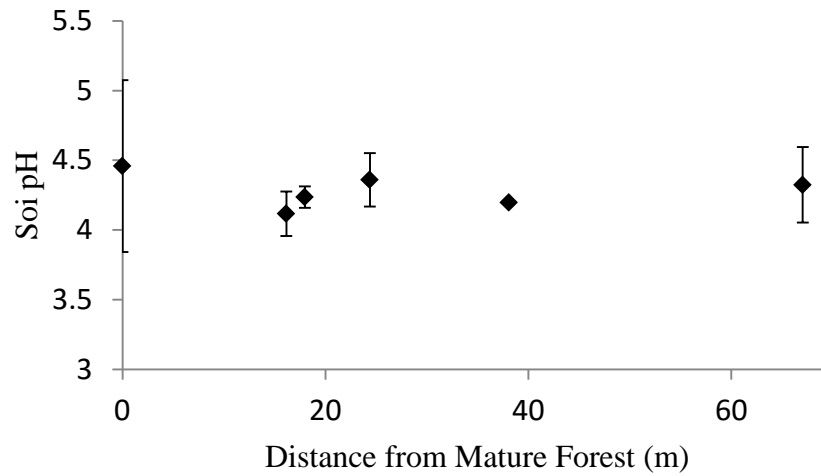


Figure A3. Soil pH from each site measured in 2013 plotted against distance from the mature forest. Data are presented as means  $\pm$  standard deviations,  $n = 3$

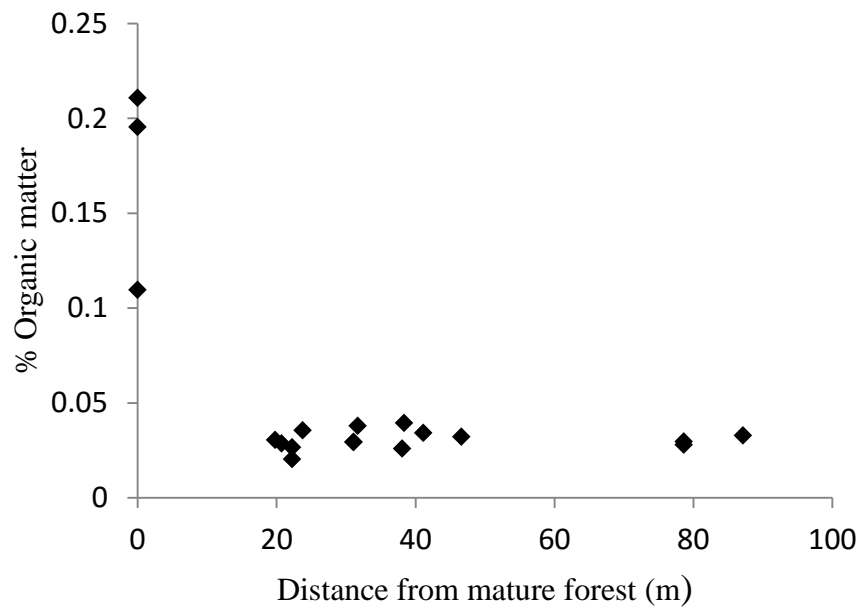


Figure A4. Percent organic matter for each site measured in 2013 plotted by distance from mature forest. Data are presented as means,  $n = 3$

Table A2. Percent sand, clay, and silt of mature forest and reservoir soils measured by the hydrometer method

Particle Size Class	Mature Forest	Reservoir Sediment
% Sand	62.5 ± 3.5	25.0 ± 11.5
% Clay	15.3 ± 1.7	17.8 ± 1.3
% Silt	22.2 ± 2.3	57.2 ± 10.3

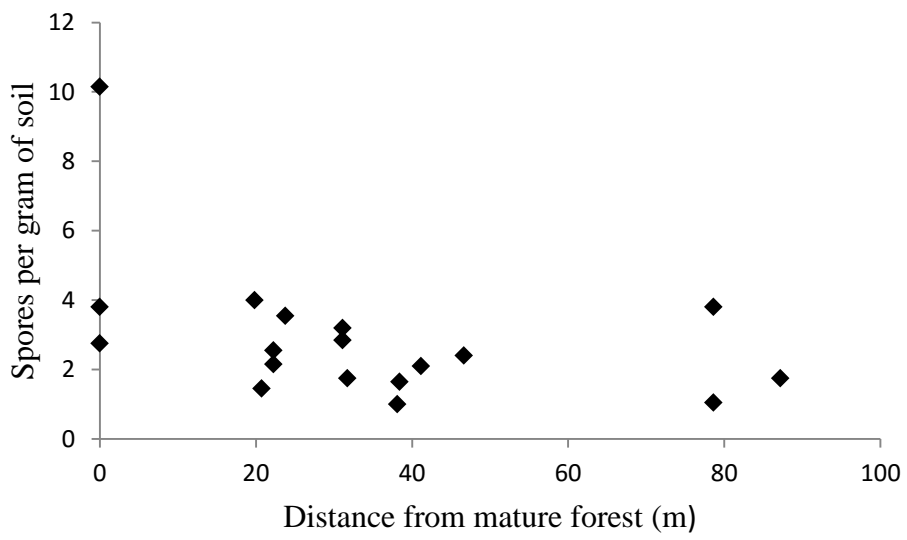


Figure A5. Spore counts per gram of soil plotted against distance from the mature forest (m)